LEUPHANA
UNIVERSITÄT LÜNEBURG

Neurologika in der aquatischen Umwelt:
Identifizierung relevanter Arzneistoffe,
ihr Verbleib und ihr Verhalten am Beispiel von Gabapentin und Quetiapin

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Manuel Herrmann
geboren am 28. Februar 1984 in Lahr/Schwarzwald

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Betreuer und Erstgutachter: Prof. Dr. Klaus Kümmerer
           (Leuphana Universität Lüneburg)

Zweitgutachter: Prof. Dr. Ralf Ebinghaus
           (Helmholtz-Zentrum Geesthacht)

Drittgutachter: Prof. Dr. Daniel Jonas
           (Universitätsklinikum Freiburg)

VORWORT

Die Pharmazie vereint grundlegende naturwissenschaftliche Bereiche der Chemie, Biologie und Physik. Aufbauend darauf finden medizinische und analytische Chemie, pharmazeutische Biologie, pharmazeutische Technologie und Biopharmazie, Pharmakologie und Toxikologie sowie Klinische Pharmazie Eingang in die heilberufliche Ausbildung. Dabei wird auch der größte Teil des Lebenslaufs von Arzneimitteln von der Entwicklung von Arzneistoffen bis hin zur Wirkung und Ausscheidung abgedeckt. Das weitere Schicksal von Arzneistoffen nach der Ausscheidung wird jedoch bisher nur wenig beleuchtet. Mittlerweile ist jedoch umfassend bekannt, dass die vom Menschen nicht aufgenommenen und nicht metabolisierten Anteile an Arzneistoffen über Ausscheidungen in das Abwasser gelangen können. Zudem werden Arzneistoffe bei der Abwasserreinigung in Kläranlagen oft nicht vollständig entfernt. Dies hat zur Folge, dass diese Stoffe in die Umwelt gelangen. Über den maßgeblichen Eintrag und Verbleib von Arzneistoffen und dessen Metabolite und Transformationsprodukte und die toxikologischen Wirkungen auf die Umwelt sind nur teilweise Informationen verfügbar. Daher sollen in dieser Arbeit zusätzliche Informationen zu dem Umweltverbleib und -verhalten von bisher wenig untersuchten Arzneistoffen, die auf das menschliche Nervensystem wirken (Neurologika), bereitgestellt werden. Zudem sollen mit dieser Arbeit Angehörige von Heilberufen für das Thema „Arzneimittel in der Umwelt" sensibilisiert werden, damit dies zunehmend Eingang in die heilberufliche Ausbildung findet.

Die dieser Arbeit zu Grunde liegenden Tätigkeiten wurden im Zeitraum von Juli 2012 bis Dezember 2015 am Institut für Nachhaltige Chemie und Umweltchemie in Zusammenarbeit mit den Institutionen des Ortenau Klinikums Offenburg-Gengenbach, der Zentralapotheke und der Arbeitssicherheit/Ökologie durchgeführt. Die kumulative Dissertationsschrift stellt im Wesentlichen Forschungsergebnisse in einem Gesamtzusammenhang dar, die in drei Artikeln (Artikel 1, 2 und 3) internationaler Fachzeitschriften aus den Bereichen Ressource Wasser und Umwelt veröffentlicht wurden:

1. Manuel Herrmann, Oliver Olsson, Rainer Fiehn, Markus Herrel, Klaus Kümmerer (2015): The Significance of Different Health Institutions and Their Respective Contributions of Active Pharmaceutical Ingredients to Wastewater. *Environment International* 85:61-76. DOI: 10.1016/j.envint.2015.07.020.

2. Manuel Herrmann, Jakob Menz, Oliver Olsson, Klaus Kümmerer (2015): Identification of Phototransformation Products of the Antiepileptic Drug Gabapentin: Biodegradability and Initial Assessment of Toxicity. *Water Research* 85:11-21. DOI: 10.1016/j.watres.2015.08.004.

3. Manuel Herrmann, Jakob Menz, Matthias Gassmann, Oliver Olsson, Klaus Kümmerer (2016): Experimental and *in silico* Assessment of Fate and Effects of the Antipsychotic Drug Quetiapine and Its Bio- and Phototransformation Products in Aquatic Environments. *Environmental Pollution* 218:66-76. DOI: 10.1016/j.envpol.2016.08.040.

ZUSAMMENFASSUNG

Verbräuche von Arzneistoffen, die auf das menschliche Nervensystem wirken (Neurologika), unterliegen aufgrund der auf dem Markt befindlichen Arzneistoffvielfalt einem ständigen Wandel. Zudem waren die Haupteintragspfade für Neurologika in die aquatische Umwelt bisher nicht eindeutig geklärt. Haushalte (diffuser Eintrag) und Einrichtungen des Gesundheitswesens (punktueller Eintrag), wie psychiatrische Fachkliniken oder Pflegeheime, wurden als maßgebliche Eintragspfade diskutiert. Ziel dieser Arbeit war es deshalb, Arzneimittelverbräuche und damit verbundene Arzneistoffemissionen durch Haushalte und Einrichtungen des Gesundheitswesens mit Hilfe einer neu entwickelten Methode abzuschätzen. Bei dieser Methode wurde das jeweilige Ausmaß der Emissionen durch die Kalkulation von Abwasserkonzentrationen und den Vergleich von Verbrauchsmengen an Arzneistoffen bestimmt. Im Ergebnis konnte gezeigt werden, dass sich Arzneimittelverbrauchsmuster in psychiatrischen Fachkliniken und Pflegeheimen von denen in allgemeinen Krankenhäusern und Haushalten unterscheiden. Außerdem konnte mit dieser Methode deren jeweiliger Beitrag am gesamten Arzneistoffeintrag in das kommunale Abwasser eingeschätzt und in hohen Mengen in das Abwasser eingetragene Arzneistoffe identifiziert werden. Durch Haushalte wurde das hinsichtlich des Umweltverbleibs und -verhaltens wenig untersuchte Antiepileptikum Gabapentin in hohen Mengen in das Abwasser eingetragen. Die Bedeutung von Einrichtungen des Gesundheitswesens am Arzneimitteleintrag in das kommunale Abwasser konnte für alle untersuchten Einrichtungstypen im Vergleich zu Haushalten als gering eingestuft werden. Bestimmte einrichtungstypische Arzneistoffe, insbesondere Neurologika, können bei regionaler Betrachtung jedoch eine größere Rolle spielen. Insbesondere Quetiapin wurde in psychiatrischen Fachkliniken und Pflegeheimen als Substanz mit hohen Verbrauchsmengen und hohem Emissionspotential identifiziert.

Ausgehend von diesen Erkenntnissen wurden Gabapentin und Quetiapin tiefergehend hinsichtlich ihres Verbleibs und ihres Verhaltens in der aquatischen Umwelt charakterisiert. Beide Arzneistoffe wurden bei verschiedenen Startkonzentrationen zur Simulation eines technischen Behandlungsverfahrens mit UV-Licht bestrahlt. Im weiteren Verlauf wurden Gabapentin und Quetiapin und die jeweilige Muttersubstanz im Gemisch mit gebildeten Phototransformationsprodukten hinsichtlich biologischer Abbaubarkeit im Closed Bottle Test und im Manometrischen Respirationstest nach OECD-Richtlinien und hinsichtlich toxischer Eigenschaften im Leuchtbakterientest und im Umu-Test beurteilt. Die Strukturaufklärung von Photo- und Biotransformationsprodukten erfolgte mittels hochauflösender Massenspektrometrie. Im Ergebnis konnten weder Gabapentin noch Quetiapin bei hohen Startkonzentrationen durch Photolyse über 128 min mineralisiert oder vollständig eliminiert werden. Identische Phototransformationsprodukte wurden bei unterschiedlichen Startkonzentrationen für die UV-Behandlung gebildet. Die Arzneistoffe Gabapentin und Quetiapin waren nach OECD-Richtlinien im Closed Bottle Test nicht leicht biologisch abbaubar. Die photolytischen Gemische von Gabapentin sind nicht besser als Gabapentin selbst abbaubar und die Phototransformationsprodukte wurden im Closed Bottle Test ebenfalls nicht eliminiert. Auch das photolytische Gemisch von Quetiapin im Closed Bottle Test war nicht besser biologisch abbaubar als Quetiapin selbst. Die Phototransformationsprodukte von Quetiapin und Quetiapin selbst unterlagen beim Closed Bottle Test und im Manometrischen Respirationstest verschiedenen biologischen Transformationsprozessen und führten zur Bildung von verschiedenen

Biotransformationprodukten. Das in biologischen Abbautests von Quetiapin maßgeblich gebildete Biotransformationprodukt BTP 398 konnte in diversen Flusswasserproben nachgewiesen werden. Dies lässt sich höchstwahrscheinlich damit erklären, dass BTP 398 unter anderem auch beim humanen Metabolismus gebildet wird. Die Langzeit-Leuchthemmung und die Zellvermehrungshemmung im Leuchtbakterientest stiegen im Verlauf der Photolyse von Gabapentin durch Bildung von Phototransformationsprodukten. Dies deutet auf eine erhöhte Toxizität der Phototransformationsprodukte im Vergleich zu Gabapentin hin. Bei Quetiapin war unter Photolyse keine Abnahme der schon vorhandenen Toxizität beim Leuchtbakterientest zu erkennen. Gabapentin, Quetiapin und deren Phototransformationsprodukte wiesen im Umu-Test keine Genotoxizität auf.

In das kommunale Abwasser eingetragene Arzneistoffe sollten mit Hilfe von Verbrauchsbilanzierungen regelmäßig identifiziert werden, da der Arzneimittelmarkt einem ständigen Wandel unterliegt. Mit Hilfe der hier empfohlenen Methode kann abgeschätzt werden, welche Arzneistoffe aus diffusen und punktuellen Eintragsquellen später sehr wahrscheinlich auch in der Umwelt vorkommen. Für diese und auch andere Arzneistoffe unter Einbezug der Metaboliten und Transformationsprodukte sollten umfassende Umweltcharakterisierungen vorgenommen werden, da die gesetzlich vorgeschriebene Umweltrisikobewertung keine ausreichenden Bewertungskriterien liefert und die Ergebnisse zudem selten frei verfügbar sind. Aufgrund der hier erhaltenen Ergebnisse sollte die UV-Behandlung verschiedener Wassermatrices als technologische Minderungsmaßnahme zumindest kritisch beurteilt werden, da eine Elimination aller Arzneistoffe nicht immer ausreichend möglich ist und zudem sehr wahrscheinlich Phototransformationsprodukte mit unbekannten Eigenschaften gebildet werden. Vielmehr wäre es wichtig, Angehörige von Heilberufen, Wissenschaftlerinnen und Wissenschaftler und Zuständige für die Abwasserentsorgung hinsichtlich potentieller Auswirkungen von Arzneistoffen auf die Umwelt aufzuklären. Darauf aufbauend könnten entsprechende Strategien zum nachhaltigen Umgang mit Arzneimitteln entwickelt werden.

ABSTRACT

The pharmaceutical consumption of drugs acting on the nervous system (neurological drugs) keeps changing due to the broad variety of active pharmaceutical ingredients on the market. In addition, the main pathways of these drugs to the aquatic environment have also not been clearly understood. Relevant pathways via households (diffuse sources) or via health institutions (punctual sources) have been discussed in literature. The objective of this study was to develop a new method for determining the pharmaceutical consumption and emissions in households and health institutions. In this method, the respective extent of emissions was determined by calculating wastewater concentrations and comparing consumption data of active pharmaceutical ingredients. As a result, the pharmaceutical consumption patterns in psychiatric hospitals and nursing homes were different from households and general hospitals. Moreover, with the provided method, the contribution of health institutions and households to the total discharge of neurological drugs to wastewater could be determined. Likewise, active pharmaceutical ingredients that can be found in high amounts in wastewater could be identified. Gabapentin was discharged in high amounts via households. The significance of health institutions and their contributions of pharmaceuticals to wastewater was very low for all types of institutions. However, some active pharmaceutical ingredients, especially neurological drugs, may have a greater impact in some regional catchment areas. Quetiapine, for instance, was discharged in greater amounts at psychiatric hospitals and nursing homes than by households.

Based on these findings, gabapentin and quetiapine were investigated regarding their fate and effects in the aquatic environment. Both active pharmaceutical ingredients were exposed to UV light at different initial concentrations to simulate a water treatment method. In addition, gabapentin, quetiapine and their photolytic mixtures were assessed regarding their biodegradability in the Closed Bottle Test and the Manometric Respirometry Test according to OECD guidelines. The luminescent bacteria test and the umu-test were also performed to give information about the toxicological properties. The structures of photo- and biotransformation products were elucidated by means of high-resolution mass spectrometry. As a result, for gabapentin and quetiapine no mineralization or elimination occurred after 128 min of photolysis. Phototransformation products were mostly identical at different initial concentrations. Gabapentin and quetiapine were not readily biodegradable in the Closed Bottle Test according to OECD guidelines. Photolytic mixtures of gabapentin were not better biodegradable than gabapentin and phototransformation products were not eliminated in the Closed Bottle Test. The photolytic mixture of quetiapine was also not better biodegradable in Closed Bottle Test compared quetiapine. In the Closed Bottle Test and the Manometric Respirometry Test, different biotransformation products were formed from quetiapine and its phototransformation products. The main biotransformation product BTP 398 was detected in several river water samples. BTP 398 is also known as a human metabolite. Long-term luminescence and growth inhibition in the luminescent bacteria test increased during UV treatment of gabapentin due to the formation of phototransformation products. It can, therefore, be assumed that phototransformation products are more toxic than gabapentin. In contrast, the cytotoxicity of quetiapine, which was shown to be cytotoxic, was not affected by UV treatment. For gabapentin, quetiapine and their photransformation products, no genotoxicity in the umu-test was observed.

As the pharmaceutical market is constantly changing, it is necessary to regularly check for active pharmaceutical ingredients in wastewater by means of a consumption-based mass balance. Using the method developed and recommended in this study, the kind and the amount of pharmaceuticals emitted from diffuse and from punctual sources could be determined and predicted. Active pharmaceutical ingredients, including those examined here, metabolites, and transformation products should be examined carefully because the commonly required environmental risk assessment studies do not provide sufficient evaluation criteria. Moreover, these studies are not always readily accessible. Technological mitigation measures such as UV treatment should be considered critically, since the elimination of active pharmaceutical ingredients is not always sufficient and as phototransformation products with unknown properties are very likely to be formed. Instead, health professionals, scientists, and professionals dealing with the disposal of effluents need to become aware of the potential impact of active pharmaceutical ingredients on environment. These groups will also need possible strategies for a more sustainable consumption of these pharmaceuticals and for the treatment of the wastewater containing them.

INHALTSVERZEICHNIS

ABBILDUNGSVERZEICHNIS

TABELLENVERZEICHNIS

ABKÜRZUNGSVERZEICHNIS

AMG	Arzneimittelgesetz
API	Active pharmaceutical ingredient (Arzneistoff)
ATC Code	Anatomical Therapeutic Chemical Classification System (Anatomisch-therapeutisch-chemisches Klassifikationssystem)
AVR	Arzneiverordnungs-Report
BOD	Biochemical oxygen demand (Biologischer Sauerstoffbedarf)
BTP	Biotransformationsprodukt
CBT	Closed Bottle Test
EMA	European Medicines Agency
ERA	Environmental Risk Assessment (Umweltrisikobewertung)
f_{nm}	Nicht metabolisierter Anteil
GAB	Gabapentin
HI	Health institution (Einrichtung des Gesundheitswesens)
HOSP	Untersuchtes allgemeines Krankenhaus
I_{EP}	Emissionspotential
k	Geschwindigkeitskonstante
LBT	Leuchtbakterientest
LC-HRMS	Hochleistungsflüssigkeitschromatographie gekoppelt mit hochauflösender Massenspektrometrie
LC-ITMS	Hochleistungsflüssigkeitschromatographie gekoppelt mit Ion Trap-Massenspektrometrie
LOD	Limit of detection (Nachweisgrenze)
MEC	Measured environmental concentration (gemessene Umweltkonzentration)
MRM	Multiple Reaction Monitoring
MRT	Manometrischer Respirationstest
MWWC	Measured wastewater concentration (gemessene Abwasserkonzentration)
NH	Untersuchtes Pflegeheim
NPOC	Non-purgeable organic carbon (nicht ausblasbarer organischer Kohlenstoff)
OECD	Organisation for Economic Co-Operation and Development
PBT	Persistent/bioakkumulierend/toxisch
PEC	Predicted environmental concentration (vorhergesagte Umweltkonzentration)

PIC   Predicted influent concentration
                 (vorhergesagte Kläranlagenzulaufkonzentration)

PWWC   Predicted wastewater concentration (berechnete Abwasserkonzentration)

PTP   Phototransformationsprodukt

PSY   Untersuchte psychiatrische Fachklinik

(Q)SAR   (Quantitative) structure-activity relationship

QUT   Quetiapin

ROS   Reactive oxygen species (reaktive Sauerstoffspezies)

STP   Sewage treatment plant (Kläranlage)

$t_{1/2}$ Halbwertszeit

ThOD   Theoretical oxygen demand (theoretischer Sauerstoffbedarf)

UV   Ultraviolett

WHO   World Health Organization (Weltgesundheitsorganisation)

1   EINLEITUNG

1.1   Verbrauchsentwicklung und Umweltvorkommen von Arzneistoffen

Arzneimittel sind laut Arzneimittelgesetz[1] (AMG) „zur Heilung oder Linderung oder zur Verhütung" von Krankheiten oder krankhaften Beschwerden bestimmt (§2 Abs. 1 AMG). Damit nehmen sie im Gesundheitswesen eine wesentliche Bedeutung ein. Weltweit lagen die Gesamtausgaben für Arzneimittel im Jahr 2013 bei 989 Milliarden Euro. Laut IMS Health (2015) ist ein Anstieg von 30 % bis im Jahr 2018 zu erwarten. Die hauptsächlichen Zuwächse sind dabei in Asien zu erwarten. In Deutschland haben sich in den letzten 20 Jahren die Arzneimittelausgaben der gesetzlichen Krankenversicherung fast verdoppelt. Allerdings hielten sich die Ausgaben in den letzten fünf Jahren auf einem konstanten Mittel von 30 Milliarden Euro (Schwabe und Paffrath 2010, 2011, 2012, 2013, 2014).

Im Jahr 2012 wurden etwa 8 100 Tonnen von 1 200 potentiell umweltrelevanten Arzneistoffen in Deutschland verwendet (Ebert et al. 2014). Als umweltrelevant werden in diesem Zusammenhang Arzneistoffe im chemisch kleinmolekularen Bereich (< 900 Da[2]) angesehen. In Abbildung 1 sind die meist verwendeten Arzneistoffe der Jahre 1999 und 2012 zu finden. Es ist zu erkennen, dass in beiden Jahren über zwei Drittel der verbrauchten Arzneistoffe von 16 Substanzen repräsentiert wurden. Allerdings haben sich im Verlauf der Jahre die Verhältnisse der Verbrauchsmengen der Arzneistoffe zueinander verändert. So haben beispielsweise Acetylsalicylsäure und Paracetamol an Bedeutung verloren, wohingegen Ibuprofen und Metformin an Bedeutung hinzugewonnen haben. Arzneistoffe wie die Antiepileptika Gabapentin (GAB), Levetiracetam oder Valproinsäure treten neu auf. Der Verbrauch von Carbamazepin aus der gleichen Arzneistoffklasse hingegen nahm ab.

Mit Hilfe der Daten über Verbrauchsmengen von Arzneimitteln lassen sich allerdings keine direkten Rückschlüsse auf das Vorkommen in der Umwelt ziehen. Nach der Einnahme eines Arzneimittels wird ein stoffspezifischer Anteil des enthaltenen Arzneistoffs unverändert ausgeschieden. Andere Teile werden biochemisch verändert und als Metaboliten[3] ausgeschieden. Die unveränderten Anteile des Arzneistoffs und die Metaboliten werden dann über die Toilette in das Abwasser eingetragen und im weiteren Verlauf der kommunalen Kläranlage (STP) zugeführt. In der STP können die Muttersubstanzen[4] oder Metaboliten biotischen und abiotischen Umbauprozessen unterliegen und dahingehend weiter zu stabilen Transformationsprodukten[5] (TP) umgebaut werden (Kern et al. 2010; Kosma et al. 2015). Da die Bildung von TP vielen verschiedenen Prozessen unterliegt, sind die entstehenden Strukturen nur schwer vorhersehbar und daher bei Nichtkenntnis auch nicht analytisch bestimmbar. Es ist auch möglich, dass Arzneistoffe oder Metaboliten in der STP gänzlich mineralisiert[6] oder aufgrund ihrer Stabilität unverändert bestehen bleiben. Da das geklärte Wasser in der Regel in Oberflächengewässer

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[1] Arzneimittelgesetz in der Fassung der Bekanntmachung vom 12. Dezember 2005 (BGBl. I S. 3394), das durch Artikel 2 des Gesetzes vom 10. Dezember 2015 (BGBl. I S. 2210) geändert worden ist.

[2] Dalton (Da): Atomare Masseneinheit (amu) zur Angabe von Molekülmassen.

[3] Stoffwechselprodukte von Arzneistoffen, die durch meist enzymatische Veränderung im menschlichen Organismus entstehen können.

[4] Der verabreichte Arzneistoff ohne Berücksichtigung gebildeter Metaboliten oder Transformationsprodukte (TP).

[5] Substanzen, die nach Ausscheidung und damit Freigabe in die Umwelt durch Transformationsprozesse aus den Muttersubstanzen entstehen.

[6] Mineralisierung: Abbau organischer Verbindungen zu Kohlendioxid und Wasser.

eingeleitet wird, können dadurch Arzneistoffe, Metaboliten und TP weiter in die Umwelt transportiert werden (Nödler et al. 2013). Auch dort unterliegen die Stoffe weiteren biotischen oder abiotischen Abbau- oder Transformationsprozessen.
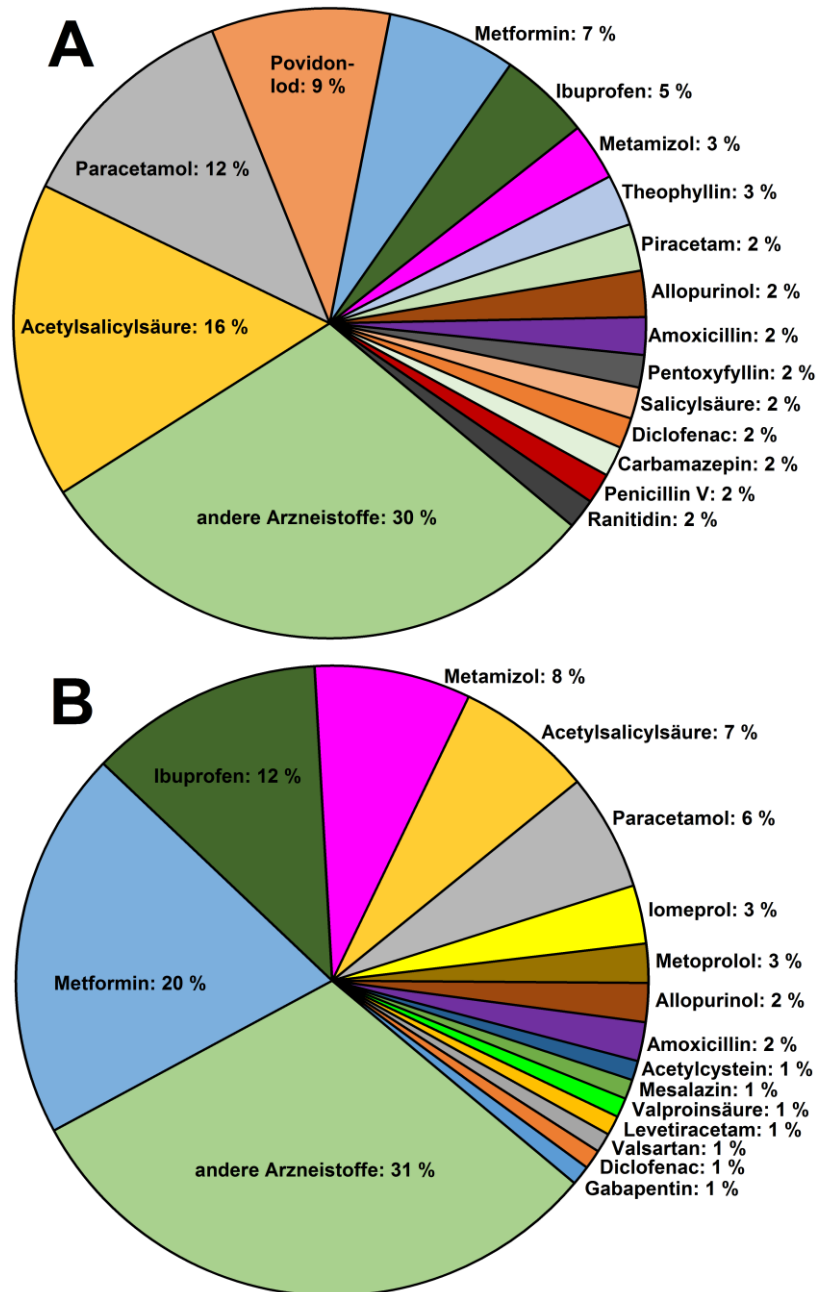
Abbildung 1   Prozentuale Verbrauchsmengenanteile der bundesweit meist verbrauchten Arzneistoffe [A] für das Jahr 1999 (erstellt nach BLAC 2003) und [B] für das Jahr 2012 (nach Ebert et al. 2014).

Etwa 150 Arzneistoffe konnten bundesweit schon in der Umwelt bestimmt werden (Ebert et al. 2014). Während sich in Krankenhausabwässern Konzentrationen von Arzneistoffen von bis zu 150 µg L^{-1} finden (Gómez et al. 2006), führt eine Verdünnung durch kommunales Abwasser zu geringeren Konzentrationen an Kläranlagenzuläufen. Da Arzneistoffe je nach Art dort in unterschiedlichem Umfang eliminiert werden, finden sich an Kläranlagenabläufen

Konzentrationen von bis zu 10 µg L^{-1}. In Einzelfällen können diese Konzentrationen jedoch auch deutlich überschritten werden (BLAC 2003). Eine weitere Verdünnung sowie Abbau- und Transformationsprozesse in Oberflächengewässern oder Meeren führen zu Konzentrationen unter 10 µg L^{-1} - meist jedoch im Bereich zwischen 0,1 und 1 µg L^{-1} (Nödler et al. 2010; Trautwein et al. 2014; Writer et al. 2013). Gemessene Konzentrationen im Trinkwasser können bis zu 1 µg L^{-1} betragen. In der Regel sind sie jedoch weitaus geringer (Capdeville und Budzinski 2011).

Grundsätzlich ist es notwendig, den Eintritt von Arzneimitteln in die Umwelt nach Möglichkeit zu vermindern, da langfristige Folgen auf die Gesundheit des Menschen und Auswirkungen auf die Umwelt teilweise schwierig einzuschätzen sind (Boxall 2004; Santos et al. 2010). Generell ist es möglich, belastete Abwässer mit erweiterten Abwasserbehandlungsmethoden zu klären (Köhler et al. 2012; Lee et al. 2014). Da diese Möglichkeit aber oft nur zu begrenzten Verbesserungen hinsichtlich Eliminationsleistung führt, muss deshalb das zukünftig nachhaltigere Ziel sein, Arzneistoffe zu entwickeln, die leicht biologisch abbaubar sind und daher auch vollständig schon in der STP eliminiert werden (Kümmerer 2007). Weitere, schneller realisierbare Möglichkeiten setzen zur kurzfristigen Verminderung des Eintrags an den Emissionsquellen an, um diesen durch einen geringeren Einsatz von Arzneimitteln herabzusetzen. Hierbei gilt es, über einen verantwortungsvollen Einsatz mit Arzneimitteln zu informieren. Oft werden diese verordnet, obwohl dies nicht zwingend erforderlich ist oder ein Absetzen sogar einen positiven Effekt haben könnte (Iyer et al. 2008). Grundsätzlich ist es aber bei allen Möglichkeiten der Minderung von Arzneistoffeinträgen in die Umwelt notwendig, Eintragsquellen und relevante Arzneistoffe zu identifizieren. Auf diese Weise können entsprechende Emissionsminderungsmaßnahmen eingeleitet werden.

1.2 Relevante Eintragsquellen für Arzneistoffe

Offensichtlich trägt die pharmazeutische Industrie ihren Anteil zum Eintrag von Arzneistoffen in das Abwasser und später in die Umwelt bei. Studien haben bereits belegt, dass Zu- und Abläufe von STP in Asien mit Anschluss an die pharmazeutische Industrie mit Arzneistoffen im µg bis mg L^{-1}-Bereich belastet sind (Cui et al. 2006; Larsson et al. 2007). Jedoch stellen insbesondere in der westlichen Welt die Ausscheidungen des Menschen im Zusammenhang mit falscher Entsorgungspraxis die wesentliche Eintrittsquelle für Arzneistoffe in die aquatische Umwelt dar. Tatsächliche Verbrauchsmengen von Arzneistoffen werden derzeit nur durch Marktforschungsstudien (Bsp. IMS Health, Danbury, USA) oder durch die Veröffentlichung von Forschungsinhalten (Alexy et al. 2006; Schuster et al. 2008) verfügbar gemacht. In Deutschland erscheint jährlich der Arzneiverordnungs-Report (AVR, aktuelle Auflage 2015, Schwabe und Paffrath 2015). Im AVR werden gegebene Tagesdosen (defined daily doses, DDD, WHO 2015a) der verfügbaren Arzneistoffe hinsichtlich ökonomischer Aspekte ausgewertet. Die Daten betreffen allerdings nur die Ausgaben der gesetzlichen Krankenkassen. In Einrichtungen des Gesundheitswesens (HI) verwendete Arzneimittel, Arzneimittel zur Selbstmedikation und zu Lasten der privaten Kassen werden dabei nicht berücksichtigt. Deshalb ist es wichtig, Methoden und Konzepte zu entwickeln, um die Emissionssituation durch Arzneimittel besser darstellen zu können, da sich diese im Verlaufe der Jahre auch durch das Hinzukommen neuer Arzneistoffe verändert (siehe Abbildung 1).

Da Ausscheidungsraten[7] stoffspezifisch sind, können Verbrauchsmengen zur Beurteilung von Emissionsquellen und zur Identifizierung relevanter Arzneistoffe herangezogen werden. Als relevante zu unterscheidende anthropogene Emissionsquellen stehen Haushalte und verschiedene HI wissenschaftlich in der Diskussion. Zu erwarten wäre ein wesentlicher Beitrag am Arzneistoffeintrag durch allgemeine Krankenhäuser als HI. Sie werden als Hauptverursacher für die Verschmutzung der Umwelt durch Arzneistoffe diskutiert (Escher et al. 2011; Gupta et al. 2009). Durch viele Studien wurde allerdings bereits ein gegenteiliges Bild dargestellt. Wesentliche Anteile am Arzneistoffeintrag werden durch die Haushalte beigetragen (Alexy et al. 2006; Le Corre et al. 2012; Ort et al. 2010; Schuster et al. 2008). Unklar ist jedoch weiterhin inwiefern andere HI, wie beispielsweise psychiatrische Kliniken oder Pflegeheime, einen Beitrag am Arzneistoffeintrag leisten. Die Anzahl und die Bedeutung dieser Einrichtungen werden vermutlich in der Zukunft zunehmen, da psychiatrische Erkrankungen immer häufiger erkannt, länger behandelt werden und die Lebenserwartung in der Weltbevölkerung weiterhin zunimmt (Ilyas und Moncrieff 2012; Janssens et al. 2014; Laidlaw und Pachana 2009; United Nations 2015). In Deutschland haben psychiatrische Fälle und das Angebot an Pflegeplätzen in den Jahren 2005 bis 2011 um 13 % zugenommen (Gesundheitsberichterstattung des Bundes 2015a, 2015b). Daher sollten umfassende Informationen hinsichtlich des Beitrags und der Verbrauchsmuster dieser Einrichtungstypen generiert werden.

Zur Bestimmung der Emissionsquellen und zur Identifizierung relevanter Arzneistoffe können im Wesentlichen zwei verschiedene Methoden herangezogen werden. Es ist möglich, Konzentrationen zu modellieren (predicted environmental concentration, PEC) oder Konzentrationen in Umweltkompartimenten analytisch zu bestimmen (measured environmental concentration, MEC) (Johnson et al. 2008; Liebig et al. 2006). In der Studie von Al Aukidy et al. 2012 werden analytische Messungen als brauchbare Methode angesehen, um die tatsächliche Situation in der Umwelt abzubilden. Offensichtlich ist aber auch, dass nur das gemessen werden kann, wofür auch analytische Methoden und entsprechende Probeentnahmestellen zur Verfügung stehen (Le Corre et al. 2012). Außerdem können bei Modellierungen größere zeitliche wie auch räumliche Skalen berücksichtigt werden. Momentaufnahmen bei analytischen Bestimmungen können oft nur die Situation für ein begrenztes Untersuchungsgebiet und einen begrenzten Probeentnahmezeitraum abbilden. Die genannten Vor- und Nachteile der beiden Herangehensweisen wurden bereits in verschiedenen Studien diskutiert (Coetsier et al. 2009; Mullot et al. 2010; Verlicchi et al. 2014). Beispielsweise haben sich Mullot et al. 2010 auf den Vergleich von vorhergesagten und gemessenen Konzentrationen im Krankenhausabwasser fokussiert. In ihrem Fall wurde festgestellt, dass PEC und MEC gut korrelieren.

1.3 Bedeutung von Neurologika: Bewertung des Verbleibs und des Verhaltens und mögliche Maßnahmen zur Minderung des Vorkommens in der Umwelt

Aus regulatorischer Sicht ist in Europa seit 1993 eine Umweltrisikoprüfung vorgesehen, die in Deutschland seit 1998 durch das Umweltbundesamt durchgeführt wird. Seit 2006 zugelassene Arzneimittel unterliegen einer einheitlichen Umweltrisikobewertung (Environmental Risk Assessment, ERA), die allerdings nicht zulassungsentscheidend für ein Arzneimittel ist und damit

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[7]   Nicht metabolisierter (unverändert ausgeschiedener) Anteil f_{nm} an verabreichtem Arzneistoff.

nicht in die Nutzen-Risiko-Analyse einfließt (European Medicines Agency 2006). ERA ist in zwei Phasen gegliedert. In der ersten Phase (grobe Expositionsabschätzung) wird das Aktionslimit und der Übergang in Phase II zur vertieften Umweltprüfung mit Hilfe von PEC-Berechnungen und Bestimmung der PBT-Eigenschaften[8] festgestellt. Die tiefergehende Prüfung umfasst diverse Abbaubarkeits- und Effekttests (für weitere Informationen: European Medicines Agency 2006). Die Ergebnisse dieser Tests sind aber nicht zwingend öffentlich verfügbar und beziehen sich in der Regel nur auf die untersuchte Muttersubstanz.

Da Arzneistoffe teilweise nur insuffizient in STP eliminiert werden können, erreichen sie weitere aquatische Umweltkompartimente, wie Oberflächen-, Grund- oder auch Rohwasser zur Trinkwassergewinnung (siehe Abschnitt 1.1). Daher wurden schon diverse Arzneistoffe verschiedener Gruppen, wie zum Beispiel Analgetika, Antihypertensiva, Antibiotika, Hormone und Zytostatika in der Umwelt gefunden (Capdeville und Budzinski 2011; Guedes-Alonso et al. 2014; Kosjek und Heath 2011; Nödler et al. 2010). Es ist für manche Arzneistoffgruppen naheliegend, dass sie ein potentielles Risiko bei Vorkommen in der Umwelt darstellen. Antibiotika beeinflussen das Bakterienwachstum, Hormone beeinflussen das endokrine System von Wasserorganismen und Zytostatika beeinflussen die Vermehrung von eukaryotischen Zellen. Deshalb wurde das Verhalten dieser Arzneistoffgruppen auch schon in diversen Studien untersucht (Kidd et al. 2007; Kümmerer et al. 2014; Kümmerer und Henninger 2003). Andere Arzneistoffgruppen, wie Neurologika[9], sind hingegen weitaus weniger untersucht. Nichtsdestotrotz wurde diese Arzneistoffklasse schon in diversen aquatischen Wasserkompartimenten nachgewiesen (González Alonso et al. 2010; Petrović et al. 2014; Writer et al. 2013). Sie wurden sogar schon in Trinkwasser gefunden (Huerta-Fontela et al. 2011). Ein direkter umweltbeeinflussender Effekt ist für Neurologika, auch mit Hilfe teilweise verfügbarer ERA-Berichte, schwer einzuschätzen. Allerdings wurde durch Studien bereits belegt, dass auch diese Arzneistoffe negative Effekte auf die Umwelt haben können, auch wenn sie sich nicht direkt von der Wirkung am Menschen auf andere Organismen ableiten lassen (Brodin et al. 2013; Chiffre et al. 2014). Deshalb sollten relevante, in der Umwelt vorkommende Neurologika identifiziert und hinsichtlich ihrer Eigenschaften und ihres Verbleibs in der Umwelt eingehend charakterisiert werden.

Generell scheint die UV-Behandlung von mit Arzneistoffen kontaminiertem Wasser in bestimmten Fällen eine Möglichkeit zur ihrer Elimination zu sein (De la Cruz et al. 2012). Diskutiert wird die UV-Behandlung deshalb auch als technisches Verfahren zur dezentralen Behandlung von Krankenhausabwasser (Kovalova et al. 2013). Bei der Trinkwasserdesinfektion ist die UV-Behandlung bereits ein übliches Verfahren (Canonica et al. 2008; Hijnen et al. 2006). Teilweise sind Arzneistoffe je nach chemischen Eigenschaften jedoch schlecht eliminierbar (De la Cruz et al. 2012). Zudem werden bei Untersuchungen zur Elimination von Arzneistoffen durch den Einsatz der UV-Bestrahlung oft nur die untersuchten Ausgangsstoffe betrachtet (Neamțu et al. 2014). Photochemisch gebildete Phototransformationsprodukte (PTP) werden größtenteils nicht berücksichtigt. PTP können in vielen Fällen gegenüber bestimmten Organismen toxischer als die untersuchte Muttersubstanz selbst sein (Gómez et al. 2008; Wang und Lin 2012), wobei in manchen

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[8]   Eine Substanz, die schlecht abbaubar (**p**ersistent) ist, sich in Organismen anreichert (**b**ioakkumulierend) und **t**oxische Eigenschaften hat.
[9]   Arzneistoffe, die auf das menschliche Nervensystem wirken.

Fällen die Toxizität durch UV-Behandlung auch unter Bildung von PTP abnimmt (Lutterbeck et al. 2015).

Im Falle des Vorkommens in aquatischen Umweltkompartimenten unterliegen die Arzneistoffe auch biotischen Prozessen. Bakterien vermögen in manchen Fällen Arzneistoffe gänzlich abzubauen. Im Regelfall sind diese jedoch nicht abbaubar oder unterliegen Transformationsprozessen (Kümmerer et al. 2000; Mahmoud und Kümmerer 2012). Teilweise werden dabei auch Biotransformationsprodukte (BTP) gebildet, die persistent in der Umwelt verbleiben können (Trautwein et al. 2008; Trautwein et al. 2014).

2 BEITRAG UND ZIELE DER ARBEIT

Arzneistoffverbrauchsmuster unterliegen einem ständigen Wandel (vgl. Abbildung 1). Aus diesem Grund ändert sich auch ihr Vorkommen in der Umwelt ständig. Berechnete und gemessene Konzentrationen am Kläranlagenzulauf korrelieren weitestgehend miteinander (Azuma et al. 2015; Mullot et al. 2010; van Nuijs et al. 2015). Daher war ein Ziel dieser Arbeit, zum ersten Mal den Gesamtarzneimittelverbrauch einer mittelgroßen Stadt in Deutschland zu charakterisieren, um für den Eintrag in das Abwasser relevante Arzneistoffe zu identifizieren. Zu diesem Zwecke wurden eigens erhobene Daten bilanziert und für eine Auswahl an Arzneistoffen die Zulaufkonzentrationen PIC an der STP berechnet. (Olsson et al. 2015)

Allgemeine Krankenhäuser als HI sind hinreichend hinsichtlich ihrer Arzneistoffverbrauchsmuster und ihres Beitrags am Arzneistoffeintrag in das kommunale Abwasser charakterisiert (Le Corre et al. 2012; Ort et al. 2010; Schuster et al. 2008). Adäquate Informationen zu Pflegeheimen und psychiatrischen Fachkliniken hingegen sind nicht vorhanden (Escher et al. 2011). Daher war ein weiteres Ziel dieser Arbeit, eine Methode zu entwickeln, womit eine psychiatrische Fachklinik, ein Pflegeheim und vergleichend dazu ein allgemeines Krankenhaus bilanziert werden können, um den spezifischen Arzneistoffverbrauch beurteilen zu können. Um anhand dieser Erkenntnisse ein mögliches erhöhtes Emissionspotential gegenüber Haushalten zu ermitteln, wurde zusätzlich das Ziel verfolgt, anhand von durchschnittlichen Jahresverbrauchsmengen in Haushalten, Informationen zu regionalen und nationalen Emissionspotentialen der Einrichtungen bereitzustellen. Daraus ableitend sollten für die Einrichtungen relevante Arzneistoffe identifiziert werden. Ein weiteres Ziel war, die Eignung der bilanzierenden Methodik zur Bestimmung von Arzneistoffverbrauchsmustern in Kombination mit berechneten Arzneistoffkonzentrationen im Abwasser zu beweisen, da diese Methodik eine kostengünstigere Alternative zu aufwändigen Monitoringprogrammen darstellt. Dies erfolgte mit Hilfe eines Vergleichs mit analytisch bestimmten Abwasserkonzentrationen ausgewählter Arzneistoffe. (Artikel 1)

Das Antiepileptikum Gabapentin (GAB) wird bei vielen medizinischen Indikationen angewendet. Es unterliegt keinem humanen Metabolismus und wird damit unverändert ausgeschieden (PFIZER PHARMA GmbH 2014). Daher entsprechen Verbrauchsmengen von GAB weitestgehend den Mengen, die im Abwasser ankommen (O'Brien et al. 2014). Da GAB vergleichsweise hohe Verbrauchsmengen aufweist, sind auch die Zulaufkonzentrationen PIC an STP unter den Neurologika am höchsten (Olsson et al. 2015). Durch eine zudem schlechte Elimination von GAB bei der Wasserbehandlung (De la Cruz et al. 2012; Jekel et al. 2015; Jekel 2015; Sacher 2015; Ternes 2015) resultiert folglich hieraus auch das Vorkommen in allen denkbaren aquatischen Umweltkompartimenten: Kläranlagenabläufe (Hollender et al. 2009; Reungoat et al. 2010), Oberflächengewässer (Kasprzyk-Hordern et al. 2008) und Trinkwasser (Morasch et al. 2010). Aus diesem Grunde war ein zusätzliches Ziel dieser Arbeit, das Stoffverhalten von GAB unter Verwendung eines ausgewählten technischen Behandlungsverfahrens (UV-Behandlung) zu untersuchen. Außerdem sollten weitere Informationen zum Verhalten und Verbleib von GAB in der aquatischen Umwelt bereitgestellt werden. Dieses beinhaltete Strukturaufklärung gebildeter PTP, die Bildung von PTP bei verschiedenen Startkonzentration unter Photolyse, biologische Abbaubarkeit von GAB und PTP sowie die initiale Bewertung der Toxizität im Verlaufe der

Photolyse. Diese neuen Erkenntnisse sollten eine erste Bewertung zu möglichen Risiken infolge der Bildung von TP ermöglichen. (Artikel 2)

Das atypische Neuroleptikum Quetiapin (QUT) konnte trotz eines extensiven humanen Metabolismus (AstraZeneca Pharmaceuticals 2013) schon in Abläufen von STP nachgewiesen werden (Oliveira et al. 2015; Yuan et al. 2013). QUT ist zudem nicht leicht biologisch abbaubar (Food and Drug Administration 2007). Es wurde auch schon im Labormaßstab einer UV-Behandlung unterzogen (Skibiński 2012). Ausreichende Informationen über mögliche biologische Transformation oder über das Verhalten gebildeter PTP sind jedoch nicht vorhanden. Da QUT vergleichsweise häufig in Psychiatrien und Pflegeheimen eingesetzt wird (Artikel 1), war ein ergänzendes Ziel, für QUT das Verhalten und den Verbleib in der Umwelt sowie das Verhalten bei einer möglichen Wasserbehandlung mit UV-Bestrahlung zu bestimmen. Außerdem war ein Ziel, gegebenenfalls gebildete PTP zu identifizieren und diese mit Hilfe biologischer Abbaubautests und Toxizitätstests zu charakterisieren. Die Anwendbarkeit der Arbeiten im Labormaßstab sollte durch Bestimmung von QUT und gebildeter BTP in Flusswasserproben eines Studiengebietes bestätigt werden. (Artikel 3)

3 METHODEN

3.1 Übersicht

In Abbildung 2 ist die methodische Vorgehensweise zur Erfüllung der Forschungsziele auch in Bezug zu den einzelnen Artikeln schematisch dargestellt. Der nicht veröffentliche methodische Teil, der sich im Wesentlichen auf die Bilanzierung des Arzneimittelverbrauchs in der Stadt Dülmen bezieht, wird in diesem Kapitel und im Abschlussbericht des Projekts "Den Spurenstoffen auf der Spur (DSADS)" (Olsson et al. 2015) ausführlicher beschrieben. Die anderen methodischen Anteile können umfänglich in den im Anhang befindlichen Artikeln nachvollzogen werden.
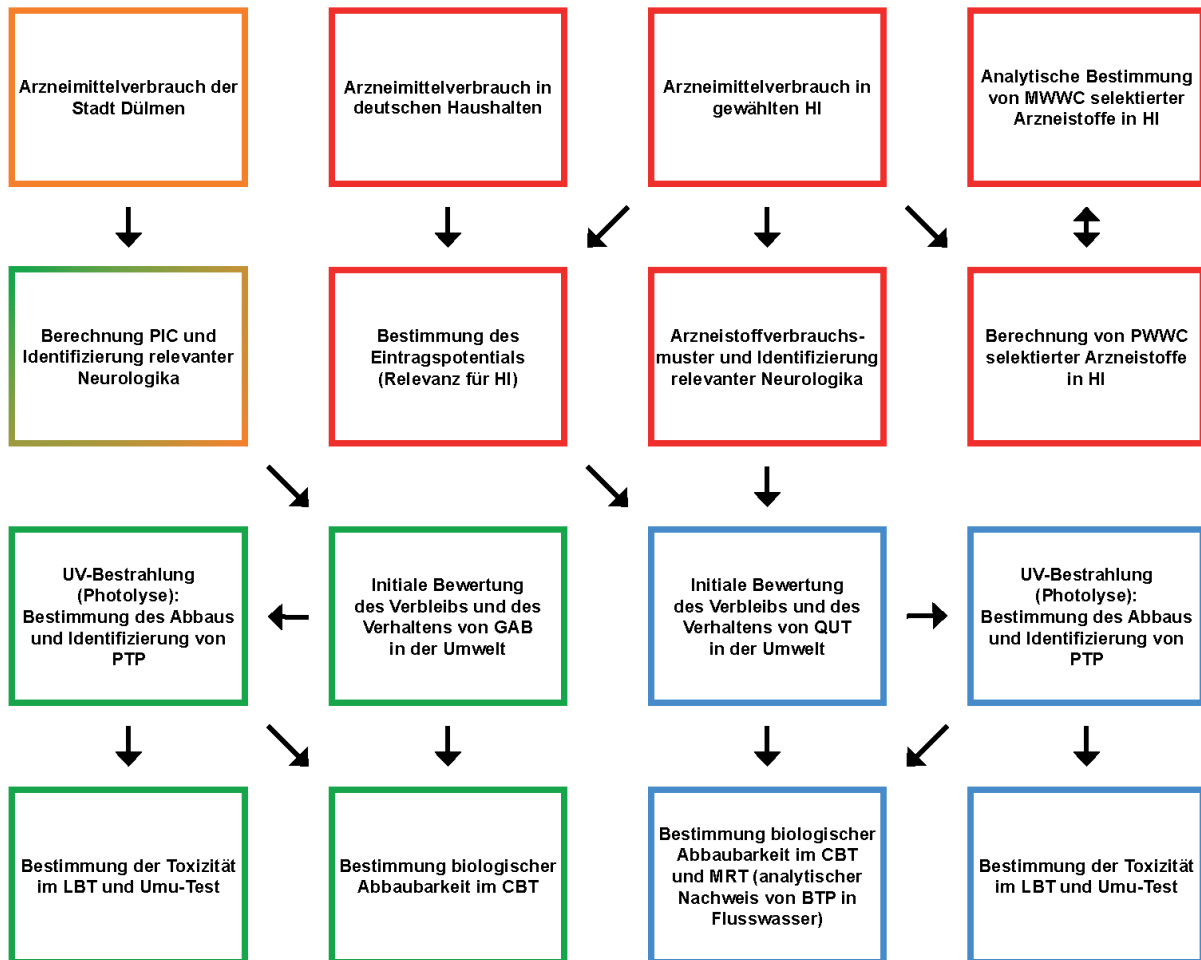

Abbildung 2   Fließschema zum methodischen Vorgehen. Bausteine für Artikel 1 (rot), Artikel 2 (grün), Artikel 3 (blau) und ohne Veröffentlichung (orange) sind farblich gekennzeichnet.

Zur Identifizierung von für den Eintrag in das kommunale Abwasser relevanter Neurologika wurden zwei Studiengebiete betrachtet. Zur Bestimmung diffuser Einträge von Arzneistoffen wurde die Stadt Dülmen in Nordrhein-Westfalen gewählt, wohingegen als Punkteintragsquellen, unabhängig von Dülmen, HI in einem Landkreis in Südwestdeutschland betrachtet wurden. Aus diffuser Quelle leitete sich die weitergehende Betrachtung von GAB ab. QUT wurde im Vergleich zu Haushalten weitestgehend punktuell durch die Anwendung in HI in das kommunale Abwasser eingetragen.

Im weiteren Verlauf wurden GAB und QUT hinsichtlich ihres Verhaltens gegenüber Photolyse betrachtet. Die Muttersubstanzen und Mischungen aus Muttersubstanz und dessen PTP wurden

mit Hilfe des Closed Bottle Test (CBT) und des Manometrischen Respirationstests (MRT) hinsichtlich biologischer Abbaubarkeit überprüft. Mit Hilfe des Leuchtbakterientests (LBT) (Menz et al. 2013) und des Umu-Tests nach ISO/FDIS 13829 (ISO/FDIS 1999) wurde der Verlauf der Toxizität durch UV-Behandlung beurteilt. Außerdem wurden die Konzentrationen von QUT und dessen hauptsächlich gebildetem BTP in ausgewählten Flusswasserproben untersucht.

3.2   Studiengebiete

3.2.1   Diffuser Arzneistoffeintrag in der Stadt Dülmen

Die Stadt Dülmen mit 46 300 Einwohnern ist eine mittelgroße Stadt in Deutschland. Die Altersstruktur entspricht dem bundesweiten Durchschnitt (Olsson et al. 2015). In Dülmen befinden sich fünf Alten- und Pflegeheime, ein allgemeines Krankenhaus mit 173 Betten und eine psychiatrische Fachklinik mit 120 Betten.

Ein lokaler Arzneimittelgroßhandel mit wesentlichem Marktanteil in der Stadt Dülmen stellte die Abgabemengen aller Arzneimittel für das Jahr 2012 zur Verfügung (Datensatz mit 24 085 Einträgen). Die Datensätze beinhalteten die Pharmazentralnummer (PZN), die Artikelbezeichnung, die Darreichungsform, die Packungsgröße sowie die Stückzahl der Arzneimittel. Zudem wurden die Abgabemengen von Arzneimitteln der dort lokalisierten Krankenhäuser durch eine Krankenhausapotheke zur Verfügung gestellt. Da es aufgrund der umfassenden Datenmenge nicht möglich war, alle Arzneistoffe zu bilanzieren, wurde eine Vorauswahl an Arzneistoffen getroffen. Unter Zuhilfenahme einer vorhergehenden Studie über bundesweite Arzneistoffverbrauchsmengen (Ebert et al. 2014) und des Arzneiverordnungs-Reports 2013 (AVR, Schwabe und Paffrath 2013) wurde daher eine Liste von 75 Arzneistoffen erstellt, die hinsichtlich ihres Verbrauchs für das Jahr 2012 bilanziert wurden (siehe Anhang Tabelle 2). Folgende Kriterien wurden der Arzneistoffauswahl zugrunde gelegt:

- Hohe Verbrauchsmenge laut AVR 2013 (Schwabe und Paffrath 2013)
- Hohe Bedeutung des Arzneistoffs innerhalb einer anatomisch-therapeutisch-chemischen Klassifikation[10](ATC Code)-Gruppe
- Ausgeglichenheit der Arzneistoffauswahl über die Gesamtheit der ATC Code-Gruppen

3.2.2   Punktueller Arzneistoffeintrag durch Einrichtungen des Gesundheitswesens (HI)

Es wurden ein allgemeines Krankenhaus (HOSP) mit den drei Standorten MAIN, OPHT und ORTH, eine psychiatrische Fachklinik (PSY) und ein Pflegeheim (NH) als HI hinsichtlich ihres spezifischen Arzneimittelverbrauchs untersucht (Artikel 1). Die HI befinden sich in zwei benachbarten Regionen eines Landkreises in Südwestdeutschland mit etwa 400 000 Einwohnern. Die beiden regionalen Einzugsgebiete der STP sind schematisch in Abbildung 3 dargestellt.

Im größeren Einzugsgebiet 1 mit 85 000 Einwohnern befinden sich der größte Standort MAIN mit 502 Betten, der mittelgroße Standort OPTH mit 131 Betten des allgemeinen Krankenhauses HOSP und PSY mit 146 Betten. Das kleinere Einzugsgebiet 2 hat 13 000 Einwohner. Dort befinden sich die Einrichtungen ORTH mit 108 Betten und NH mit 286 Pflegeheimplätzen.

Die untersuchten Einrichtungen wurden, teilweise mit Einschränkungen, hinsichtlich ihres spezifischen Arzneistoffverbrauchs für die Jahre 2010, 2011 und 2012 bilanziert. Dabei wurden

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[10]   International angewendetes fünfstufiges Klassifikationssystem für Arzneistoffe (WHO 2015b).

bezüglich der Anzahl wenig verordnete Arzneistoffe nicht berücksichtigt (genauere Informationen in Artikel 1). Da jedoch nicht abzusehen war, welche Arzneistoffe in höchsten Mengen verbraucht werden, wurde keine vorherige Arzneistoffeinschränkung, wie zur Bestimmung des diffusen Eintrags (siehe Abschnitt 3.2.1), vorgenommen.
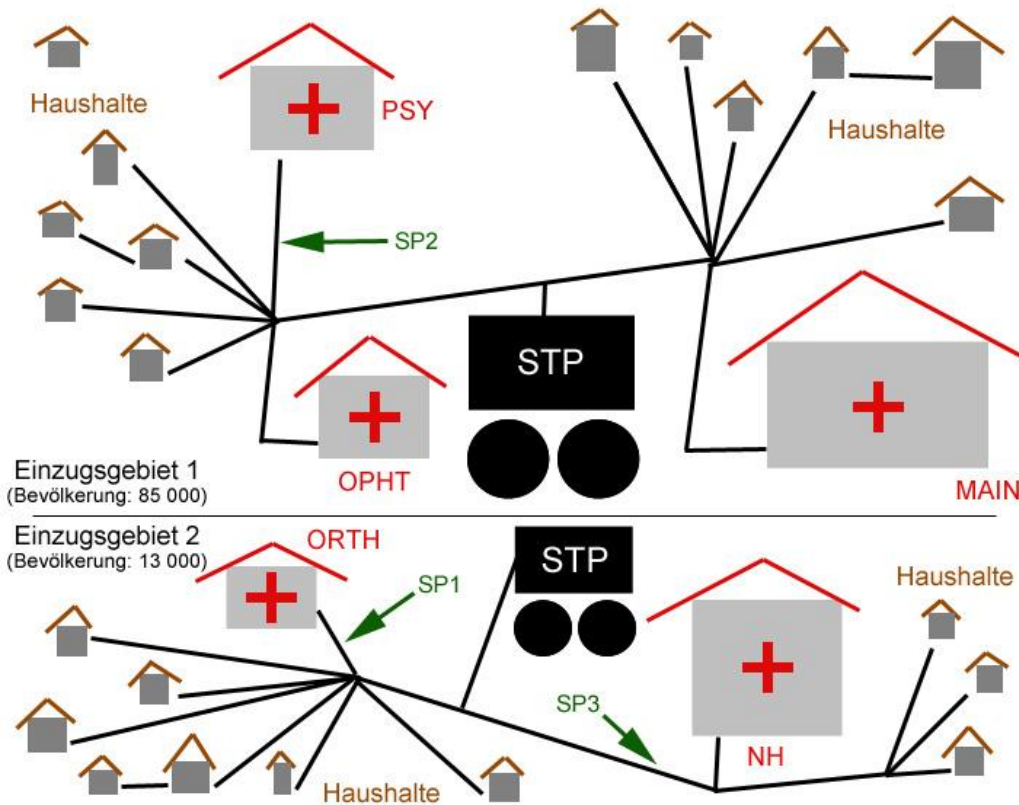

Abbildung 3   Schematische Darstellung der Einzugsgebiete der Kläranlage (STP) mit den Einrichtungen des Gesundheitswesens (HI) allgemeines Krankenhaus mit den Standorten MAIN, OPHT und ORTH, der psychiatrischen Fachklinik (PSY), dem Pflegeheim (NH) sowie den Probeentnahmestellen (SP) (geändert nach Artikel 1).

3.3   Arzneistoffbilanzierung und Verwertung der Daten

3.3.1   Bilanzierung der Verbrauchsmengen

Zur Ermittlung des punktuellen und des diffusen Arzneistoffeintrags wurden die Wirkstärken m_{unit} der Arzneistoffe in den Arzneimitteln mit der Anzahl der Einheiten U_1 und zur Untersuchung des diffusen Arzneistoffeintrags in Dülmen zusätzlich mit den Einheiten in einer Packung u_2 multipliziert. Dabei erhält man die arzneistoffspezifische Verbrauchsmenge für einen Arzneistoff A_{API} in einem Jahr:

$$A_{API} = m_{unit} \cdot U_1 \cdot u_2 \qquad (1)$$

Die einzelnen Arzneistoffverbrauchsmengen der verschiedenen Einrichtungstypen konnten im späteren Verlaufe ihren Arzneistoffgruppen des ATC Codes zugeordnet werden, um die Bedeutung der einzelnen Gruppen hervorzuheben (A_{group}):

$$A_{group} = \sum A_{API} \qquad (2)$$

Um Informationen hinsichtlich des Verbrauchs in den untersuchten Einrichtungen zu bekommen und dabei auch die unterschiedlichen Größen der Einrichtungen zu berücksichtigen, wurden die

Verbrauchsmengen A_{API} von 50 Arzneistoffen auch pro Bett beziehungsweise Bewohner und Jahr angegeben:

$$A_{bed/inh} = \frac{A_{API}}{c_{facility}} \qquad (3)$$

$A_{bed/inh}$ ist der Verbrauch eines Arzneistoffs pro Bett beziehungsweise Pflegeheimbewohner, A_{API} ist der Verbrauch eines Arzneistoffs in einer Einrichtung, $c_{facility}$ ist die Betten- beziehungsweise Bewohneranzahl in einer Einrichtung.

Außerdem wurden für die gleichen 50 Arzneistoffe mit Hilfe des AVR des jeweiligen Jahres Daten für die Verbrauchsmengen in Haushalten berechnet und damit erhoben, um später das Emissionspotential I_{EP} bestimmen zu können (vgl. Abschnitt 3.3.3):

$$A_{HD} = DDD_{SHI} \cdot V_{DDD} \cdot \frac{C_{GER}}{C_{SHI}} \qquad (4)$$

A_{HD} sind die Verbrauchsmengen eines Arzneistoffs in deutschen Haushalten. DDD_{SHI} sind die im AVR angegebenen Tagesdosen, V_{DDD} ist die mittlere Tagesdosis eines Arzneistoffs, C_{GER} ist die Bevölkerung Deutschlands, C_{SHI} sind die Mitglieder in der gesetzlichen Krankenversicherung.

3.3.2 Bestimmung der Kläranlagenzulaufkonzentrationen PIC und der Abwasserkonzentrationen PWWC

Zur Ermittlung relevanter Arzneistoffe aus diffusen Quellen wurden für die gewählten 75 Arzneistoffe in Dülmen die entsprechenden Kläranlagenzulaufkonzentrationen PIC an der dortigen STP berechnet (für GAB in Artikel 2). In diesen Fällen waren die Parameter für Verbrauchsmengen A_{API} und der als Spanne angegebene nicht metabolisierte Anteil f_{nm} variabel.

Außerdem wurden die Abwasserkonzentrationen (PWWC) in den drei Einrichtungen ORTH, PSY und NH für die Arzneistoffe Pregabalin, Gabapentin (GAB), Levetiracetam, Amisulprid, Doxepin und Quetiapin (QUT) berechnet (siehe Artikel 1), um sie im weiteren Verlauf mit den gemessen Abwasserkonzentrationen (MWWC) (siehe Abschnitt 3.4) vergleichen zu können. Mit Hilfe dieses Vorgehens sollte die Methodik der Bilanzierung als Verfahren zur Darstellung der Emissionssituation in HI bewertet werden können.

3.3.3 Emissionspotential I_{EP} von Einrichtungen des Gesundheitswesens (HI)

Zur Beurteilung der Relevanz des Eintrags eines Arzneistoffs für eine HI wurde das Emissionspotential I_{EP} berechnet. I_{EP} ist der Quotient aus Verbrauch in der Einrichtung $A_{facility}$ und dem Verbrauch in den Haushalten $A_{citizens}$:

$$I_{EP} = \frac{A_{facility}}{A_{citizens}} \qquad (5)$$

Ein Wert größer 1 für I_{EP} sagt aus, dass mehr als die Hälfte eines Arzneistoffs in das kommunale Abwasser durch HI eingetragen wird. I_{EP} wurde auf nationaler Ebene sowie zudem in den Kläranlageneinzugsgebieten 1 und 2 auf regionaler Ebene betrachtet. Dazu wurden die Verbrauchsmengen aus Gleichung (4) für die Betrachtung auf regionaler Ebene mit Hilfe der Einwohnerzahl umgerechnet und die Verbrauchsmengen aus Gleichung (1) auf alle HI in Deutschland mit Hilfe der behandelten Fallzahlen beziehungsweise Pflegeheimplätze hochgerechnet (weiterführende Informationen zum Vorgehen in Artikel 1).

3.4 Analytische Bestimmung der Abwasserkonzentrationen (MWWC) in Einrichtungen des Gesundheitswesens (HI)

Die Abwasserkonzentrationen MWWC wurden vergleichend zu PWWC (siehe Abschnitt 3.3.2) von Pregabalin, Gabapentin (GAB), Levetiracetam, Amisulprid, Doxepin und Quetiapin (QUT) bestimmt. Dafür wurden in den Einrichtungen ORTH, PSY und NH je dreimal zwischen Juni 2012 und August 2013 über 24 h Abwassermischproben aus den Abläufen der Einrichtungen entnommen. Die MWWC der Arzneistoffe wurden mit Hilfe der Standardadditionsmethode bestimmt. Eine Aufkonzentrierung der Proben erfolgte durch Festphasenextraktion. Die chromatographische Trennung und die massenspektrometrische Analyse wurden mittels Hochleistungsflüssigkeitschromatographie gekoppelt mit Ion Trap-Massenspektrometrie (LC-ITMS) durchgeführt. Quantifiziert wurde mit Hilfe des höchsten Fragments (Product Ions) im Multiple Reaction Monitoring (MRM)-Modus (genauere Informationen in Artikel 1).

3.5 UV-Bestrahlung (Photolyse): Identifizierung von Phototransformationsprodukten (PTP)

In Artikel 2 und 3 wurden GAB und QUT in Reinstwasser mit polychromatischer UV-Strahlung behandelt, um die Behandlung von Arzneistoff enthaltenden Matrices, wie Abwasser, Kläranlagenablauf oder Trinkwasser, zu simulieren. Die Behandlung wurde bei verschiedenen Startkonzentrationen für 128 min durchgeführt, um das unterschiedliche Eliminations-beziehungsweise Mineralisierungsverhalten und die Bildung von PTP zu beurteilen. GAB wurden bei folgenden Startkonzentrationen photolysiert: 100, 20, 5, 1 und 0,1 mg L^{-1}. Für QUT ergaben sich durch das Vorliegen als Hemifumaratsalz folgende Startkonzentrationen: 86,9; 17,4; 4,3 und 0,9 mg L^{-1}. Mit Hilfe einer Mitteldruckquecksilberdampflampe wurde die Versuchslösung in einem Tauchrohrphotoreaktor aus Quarzglas bestrahlt (weitere Informationen im Anhang).

Während der Behandlung wurden zu definierten Zeitpunkten (0, 2, 4, 8, 16, 32, 64 und 128 min) Proben entnommen, um die Elimination der Arzneistoffe mittels LC-UV-ITMS, die Mineralisation mittels NPOC[11]-Bestimmung und das Auftreten von PTP mittels LC-ITMS zu bestimmen. Außerdem wurden bei GAB die Sauerstoffkonzentration mit einem optischen Sensor und die Wasserstoffperoxidkonzentrationen semiquantitativ mit Teststreifen bestimmt, um mögliche Transformations- und Abbauprozesse von GAB erklären zu können. Die Strukturen der neu auftretenden PTP wurden mittels Hochleistungsflüssigkeitschromatographie gekoppelt mit hochauflösender Massenspektrometrie (LC-HRMS) aufgeklärt. Außerdem wurden zu den gleichen Zeitpunkten bei einer Startkonzentration von 100 mg L^{-1} (GAB) und 86,9 mg L^{-1} (QUT) Proben entnommen, um den Verlauf der Toxizität zu beurteilen (siehe Abschnitt 3.6). GAB als Standard und dessen photolytische Gemische nach 32, 64 und 128 min wurden mit Hilfe des CBT hinsichtlich biologischer Abbaubarkeit beurteilt (siehe Abschnitt 3.7). QUT wurde einem CBT und einem MRT unterzogen. Auch das photolytische Gemisch von QUT nach 128 min Photolyse wurde im CBT und im MRT untersucht (siehe Abschnitt 3.7).

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[11] NPOC: Nicht ausblasbarer organischer Kohlenstoff.

3.6 Toxizitätstests

Zur initialen Beurteilung der Toxizität wurden GAB, QUT und deren photolytische Gemische einem modifizierten LBT nach (Menz et al. 2013) und einem Umu-Test nach ISO/FDIS 13829 (ISO/FDIS 1999) unterzogen.

Beim LBT wird die Hemmung der Lichtemission und die Hemmung des Wachstums des Leuchtbakteriums *Vibrio fischeri* NRRL-B-11177 bestimmt. Die Kurzzeit-Leuchthemmung nach 30 min (LI_{30min}), die Langzeit-Leuchthemmung nach 24 h (LI_{24h}) sowie die Zellvermehrungshemmung (GI_{14h}) werden als Endpunkte prozentual im Vergleich zum unbehandelten Kontrollansatz angegeben.

Beim Umu-Test wird das transgene[12] Bakterium *Salmonella typhimurium* TA1535/pSK1002 verwendet. Als Endpunkt wird das genotoxische Potential bestimmt. Durch die Untersuchungssubstanz hervorgerufene DNA-Schäden haben eine Induzierung des umuC-Gens als Teil des bakteriellen DNA-Reparatursystems zur Folge. Das umuC-Gen ist im Untersuchungsorganismus mit dem lacZ-Gen fusioniert. Daher kann die Induktionsrate des umuC-Gens photometrisch über die Galaktosidase-Aktivität bestimmt werden.

3.7 Biologische Abbaubarkeit

GAB, QUT und deren photolytische Gemische wurden im CBT untersucht. Der CBT wurde in Anlehnung an die Organisation for Economic Co-operation and Development (OECD)-Richtlinie 301 D (OECD 1992) durchgeführt. Er stellt durch den Einsatz von vergleichsweise wenig Mineralmedium und einer geringen Bakteriendichte einen Test zur Bestimmung leichter biologischer Abbaubarkeit in der aquatischen Umwelt dar. Das Ausmaß des Abbaus wird über einen optischen Sauerstoffsensor im Verlauf von 28 Tagen gemessen (Friedrich et al. 2013). Zusätzlich wurde die biologische Abbaubarkeit von QUT und dessen aus der Photolyse resultierenden Gemischen im MRT nach OECD-Richtlinie 301 F (OECD 1992) untersucht. Im Verlaufe von 28 Tagen wird dabei der Verbrauch an Sauerstoff gemessen. Beim MRT wird eine höhere Bakteriendichte eingesetzt. Somit steigt hier die Wahrscheinlichkeit der biologischen Abbaubarkeit. Außerdem werden BTP im Falle einer biologischen Abbaubarkeit im höheren Ausmaß gebildet als beim CBT. Die BTP wurden mittels LC-HRMS aufgeklärt. Zudem wurde im Falle von QUT und dessen PTP der biologische Transformationsweg mittels der Software META (Version 1.8.1, Multicase Inc. Beachwood, USA) und Eawag-Datenbank für Biokatalyse und Bioabbau (Eawag 2016) vorausgesagt, um die Strukturaufklärung zu bestätigen.

Bei beiden Tests gilt eine Substanz als leicht biologisch abbaubar, wenn 60 % der Substanz abgebaut wurde. Der prozentuale Anteil ist der Anteil des Sauerstoffverbrauchs im Vergleich zum theoretischen Sauerstoffbedarf (ThOD). Da diese Berechnung nur bei chemisch definierten Substanzen mit bekannter Summenformel möglich ist, wurde die Auswertung der photolytischen Gemische mit Hilfe des biologischen Sauerstoffbedarfs (BOD) im Vergleich zur Qualitätskontrolle, leicht biologisch abbaubares Natriumacetat, vollzogen.

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[12] gentechnisch verändert

3.8 Analytik der Flusswasserproben

Aus sechs Zuflüssen der Ilmenau, einem Nebenfluss der Elbe, wurden an insgesamt sieben Probeentnahmestellen von Oktober 2014 bis Februar 2015 monatlich Stichproben entnommen (González Alonso et al. 2010; López-Serna et al. 2012). Alle 35 Proben wurden mittels Festphasenextraktion in Kombination mit LC-HRMS auf das Vorkommen von QUT und dessen hauptsächlich gebildetes BTP 398 überprüft. Es wurde angenommen, dass das Verhalten von BTP 398 aufgrund der strukturellen Ähnlichkeit bei der Festphasenextraktion und bei der massenspektrometrischen Analyse ähnlich dem von QUT sei. Ein analytischer Standard für BTP 398 war nicht verfügbar. Daher wurde nur eine qualitative Aussage über den Nachweis von BTP 398 gemacht. Für QUT war die LOD 1,3 ng L^{-1} und die Bestimmungsgrenze (LOQ) 3,8 ng L^{-1}.

4 ERGEBNISSE UND DISKUSSION

4.1 Identifizierung relevanter Arzneistoffe aus diffusem Arzneistoffeintrag in das kommunale Abwasser

Der Gesamtverbrauch der untersuchten 75 Arzneistoffe in Dülmen im untersuchten Jahr 2012 betrug 2 320 kg. Eine Gesamtverbrauchsmengenabschätzung im Vergleich zu den bundesweiten Daten wurde nicht vorgenommen, da nicht klar war, welcher Marktanteil in Dülmen mit dem vorhandenen Datensatz des Arzneimittelgroßhandels abgebildet wurde. Außerdem wurde auch auf eine Darstellung hinsichtlich verschiedener ATC Code-Gruppen an dieser Stelle verzichtet, da aus diesen eine unterschiedlich große Anzahl an Arzneistoffen analysiert wurde und sie daher nicht das tatsächliche Verhältnis der Gruppen zueinander darstellen würden. Die Verbrauchsmengen der einzelnen Arzneistoffe sind in Tabelle 2 (Anhang) zu finden. Die 16 meist verbrauchten Arzneistoffe mit anteiligen Verbrauchsmengen am Gesamtverbrauch sind in Abbildung 4 dargestellt, um einen Vergleich zu den in Abbildung 1 nach Ebert et al. (2014) dargestellten bundesweiten Verbräuchen aus dem gleichen Jahr herstellen zu können. Die fünf meist verbrauchten Arzneistoffe sind in Dülmen mit den bundesweiten Daten identisch. Metformin, Ibuprofen, Metamizol, Acetylsalicylsäure und Paracetamol wurden am meisten verbraucht. Die anderen elf häufig verbrauchten Arzneistoffe unterscheiden sich im Vergleich zu bundesweiten Verbräuchen in ihrem Verhältnis der Verbrauchsmengen zueinander, werden jedoch weitestgehend von den gleichen Arzneistoffen repräsentiert. Der Anteil der übrigen 59 Arzneistoffe liegt in Dülmen bei 16 %, wohingegen der Anteil in Deutschland für diese verbleibenden 59 Arzneistoffe 31 % entspricht. Der größere Anteil kommt dadurch zustande, dass bei Ebert et al. (2014) 1200 Arzneistoffe berücksichtigt wurden, in Dülmen hingegen nur 75 Arzneistoffe bilanziert wurden. Es ist jedoch eindeutig zu erkennen, dass die Verbrauchsmuster im gleichen Zeitraum lokal betrachtet den bundesweiten Verbräuchen ähnlich sind.
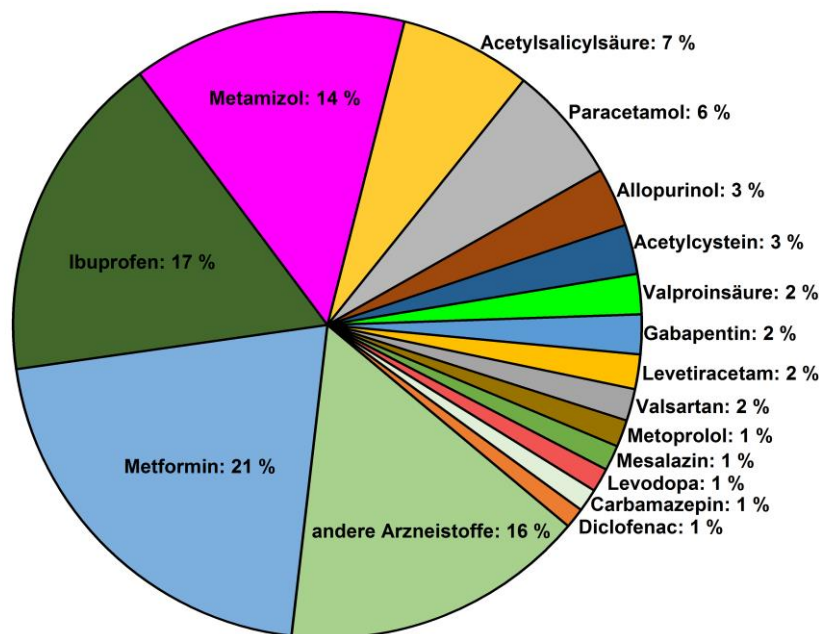

Abbildung 4   Prozentuale Verbrauchsmengenanteile der analysierten Arzneistoffe in Dülmen im Jahr 2012.

In Tabelle 2 (Anhang) sind die Ergebnisse für die PIC unter Einbezug von f_{nm} dargestellt. Da die f_{nm}-Werte je nach Arzneistoff sehr unterschiedlich sind (0,00 bis 1,00 beziehungsweise vollständige bis keine Metabolisierung), bilden die Verbrauchsmengen die berechneten PIC nicht direkt ab. So ergeben sich für Metformin, Ibuprofen, GAB, Valsartan und Levetiracetam die höchsten PIC-Maximalwerte (236,55; 29,82; 23,16; 17,70 und 14,75 µg L^{-1}). Die Arzneistoffe Metamizol, Acetylsalicylsäure und Paracetamol mit den dritt-, viert- und fünfthäufigsten Verbrauchsmengen weisen durch eine extensive Metabolisierung vergleichsweise niedrige maximale PIC auf (1,60; 1,53 und 3,42 µg L^{-1}). Die hohe PIC des Neurologikums GAB konnte durch Messungen und Vorhersagen mittels anderer Studien bestätigt werden (Kasprzyk-Hordern et al. 2009; Ottmar et al. 2010). Deshalb scheint eine Beurteilung des Verhaltens und des Verbleibs von GAB in der Umwelt sowie des Eliminationsverhaltens unter Verwendung technologischer Verfahren und der Bildung relevanter TP sinnvoll. In einer weiteren Studie für zehn andere Arzneistoffe wurde auch gezeigt, dass die PIC und gemessene Konzentrationen an zwei STP gut miteinander korrelierten (Oosterhuis et al. 2013). Unter anderem wurden dabei für Metformin für zwei Einzugsgebiete von Kläranlagen Konzentrationen von 122,01 und 141,38 µg L^{-1} vorhergesagt. Wobei vergleichbare Konzentrationen von 73,73 $\pm$ 9,45 und 84,41 $\pm$ 13,61 µg L^{-1} gemessen wurden. Für Valsartan wurden in der gleichen Studie ähnlich gute Korrelationen erzielt. Die PIC lagen bei 2,63 und 2,71 µg L^{-1}. Die gemessenen Konzentrationen wurden mit 1,93 $\pm$ 0,59 und 2,93 $\pm$ 0,50 µg L^{-1} angegeben. Die Konzentrationen von Valsartan waren in der Studie von Oosterhuis et al. (2013) im Vergleich zu der vorliegenden Arbeit sehr wahrscheinlich geringer, da bei Oosterhuis et al. (2013) die tatsächlichen Abwassermengen der STP für die Berechnung der Konzentrationen als Grundlage verwendet wurden. Im Gegensatz dazu erfolgte die Berechnung der Konzentrationen in dieser Arbeit anhand des pro Kopf-Verbrauchs eines Einwohners von 121 L Wasser (Statistisches Bundesamt DESTATIS 2015). Dieser ist weitaus geringer, da hier industrielle Abwässer nicht mit einbezogen wurden. Dies spielte jedoch bei der Identifizierung von in hohen Konzentrationen eingetragener Arzneistoffe keine Rolle, da für alle Berechnungen die gleiche Abwassermenge zu Grunde gelegt wurde und dies damit keinen Einfluss auf die Konzentrationsverhältnisse zueinander hatte.

4.2 Charakterisierung von Einrichtungen des Gesundheitswesens (HI) hinsichtlich ihres spezifischen Arzneistoffverbrauchs

In Artikel 1 sind die Ergebnisse bezüglich der Charakterisierung der Einrichtungen ausführlich diskutiert. Der Gesamtverbrauch an Arzneistoffen als Mittelwert aus drei Jahren mit entsprechender Standardabweichung in HOSP war für MAIN 1 060,6 $\pm$ 36,0 kg Jahr^{-1}, für OPHT 85,5 $\pm$ 20,0 kg Jahr^{-1} und für ORTH 117,1 $\pm$ 2,8 kg Jahr^{-1}. In MAIN wurden im Wesentlichen Kontrastmittel aus der Gruppe V (Verschiedenes), Antibiotika aus der Gruppe J (Antiinfektiva) und Analgetika aus der Gruppe N (Nervensystem) verabreicht. An den Standorten OPHT und ORTH wurden hauptsächlich Antibiotika und Analgetika verabreicht. In den Einrichtungen PSY (Gesamtverbrauch: 32,3 $\pm$ 1,0 kg Jahr^{-1}) und NH (Gesamverbrauch: 83,0 $\pm$ 13,5 kg Jahr^{-1}) kam es maßgeblich zum Verbrauch von Antiepileptika, Psycholeptika und Psychoanaleptika aus der Gruppe N (Nervensystem). Wesentliche weitere Anteile werden durch die Gruppe A (Alimentäres System und Stoffwechsel) beigetragen.

In HOSP wurde an allen Standorten hauptsächlich Metamizol verwendet, was auch den hohen Verbrauch an Arzneistoffen aus der Gruppe N (Nervensystem) zur Folge hatte (MAIN: 317 ± 10 g Bett^{-1} Jahr^{-1}, OPTH: 132 ± 54 g Bett^{-1} Jahr^{-1}, ORTH: 494 ± 39 g Bett^{-1} Jahr^{-1}). Alle anderen Schmerzmittel, wie Paracetamol, Ibuprofen oder Diclofenac wurden in bedeutend geringeren Mengen verbraucht. Somit ergab sich der höchste Gesamtverbrauch der vier Schmerzmittel für ORTH (617 ± 44 g Bett^{-1} Jahr^{-1}). Im Gegensatz dazu waren die Verbräuche in PSY und NH weitaus geringer (PSY: 52 ± 1 g Bett^{-1} Jahr^{-1}, NH: 54 ± 9 g Bewohner^{-1} Jahr^{-1}). Auch die Verbräuche pro Bett und Jahr der untersuchten Antibiotika Cefuroxim und Sulfamethoxazol waren in den Einrichtungen PSY und NH bis zu tausendfach geringer als an den Standorten von HOSP. Dass in den Einrichtungen PSY und NH im Wesentlichen Antiepileptika, Psycholeptika und Psychoanaleptika verwendet wurden, spiegelte sich auch in den pro Bett/Bewohner-Verbräuchen wider. Valproinsäure (PSY: 33 ± 5 g Bett^{-1} Jahr^{-1}, NH: 23 ± 5 g Bewohner^{-1} Jahr^{-1}) und Quetiapin (PSY: 26 ± 4 g Bett^{-1} Jahr^{-1}, NH: 30 ± 5 g Bewohner^{-1} Jahr^{-1}) wiesen in diesen Arzneistoffgruppen die höchsten Verbräuche auf. Valproinsäure gilt als leicht biologisch abbaubar (Bergheim und Kümmerer 2007) und vollständig in STP eliminierbar (Yu et al. 2006). Quetiapin hingegen wurde schon im Ablauf von STP nachgewiesen und scheint daher in weiterführende aquatische Umweltkompartimente eingetragen zu werden (Oliveira et al. 2015; Yuan et al. 2013).

Die einzeln analytisch bestimmten Abwasserkonzentrationen wurden mit den berechneten Konzentrationsbereichen verglichen. Nur für Levetiracetam und Quetiapin lagen die gemessenen Werte fast immer innerhalb des vorhergesagten Konzentrationsbereichs. Eigentlich sollten berechnete und gemessene Werte in etwa übereinstimmen, da die wesentlichen Parameter wie Verbrauchsmengen und der nicht metabolisierte Anteil mit in die Berechnung von PWWC einbezogen wurden. Allerdings stellte die verwendete Probeentnahmemethode eine wesentliche Limitation dar. Es ist nachvollziehbar, dass durchschnittliche jährliche Verbrauchsmengen nicht die hohen täglichen Fluktuationen an Verbräuchen von Arzneistoffen darstellen können. Auch eine erhöhte Frequenz der Probeentnahmen könnte diese Fluktuationen nicht darstellen.

Die Übereinstimmung von vorausgesagten und gemessenen Konzentrationen wurde auch mit Hilfe des PWWC MWWC^{-1}-Quotienten beurteilt (Coetsier et al. 2009; Ortiz de García, S. et al. 2013). Dazu wurden die mittleren Konzentrationen von PWWC und die Mittelwerte von MWWC verwendet. Auch hier konnte die beste Korrelation für Levetiracetam und Quetiapin festgestellt werden. Alle anderen Arzneistoffe wichen jedoch auch nur im Bereich von nahezu einer Zehnerpotenz voneinander ab. Daher bildeten die vorausgesagten Konzentrationen die tatsächlichen Emissionen zumindest in der Größenordnung gut ab.

4.3 Bestimmung der Bedeutung von Einrichtungen des Gesundheitswesens (HI) am Eintrag relevanter Arzneistoffe in das kommunale Abwasser anhand des Emissionspotentials I$_{EP}$

4.3.1 Nationale und regionale Bedeutung der untersuchten Einrichtungen

Durch Berechnung des Emissionspotentials I$_{EP}$ konnte die Bedeutung der untersuchten HI quantifiziert werden (Artikel 1). Bei der nationalen Betrachtung der 50 untersuchten Arzneistoffe trugen die untersuchten Einrichtungstypen im Allgemeinen wenig zum Arzneimitteleintrag bei (Abbildung 5A). Nur ein I$_{EP}$ bei allgemeinen Krankenhäusern und zwei I$_{EP}$ bei Pflegeheimen nahmen einen höheren Wert als 1 an. Daher wurden nur zwei Arzneistoffe in höherem Ausmaß

durch diese Einrichtungen als durch Haushalte eingetragen. Alle anderen Arzneistoffe nahmen in den Einrichtungen einen geringeren Anteil ein. In allgemeinen Krankenhäusern war die Verwendung von Arzneistoffen, die im alimentären und im kardiovaskulären System wirken, beispielsweise 15 bis 500fach geringer als in Haushalten. Die durchschnittliche Verwendung von Arzneistoffen in psychiatrischen Kliniken war im Vergleich zu Haushalten noch unbedeutender als in allgemeinen Krankenhäusern. Verbräuche von Arzneistoffen, die im alimentären System wirken, waren hier bis zu 2500fach geringer. Innerhalb der Arzneistoffgruppen waren die Verbräuche von Neuroleptika und Beruhigungsmitteln am höchsten, aber immer noch geringer als in Haushalten. In Pflegeheimen waren nur die Verbräuche von Neurologika im Vergleich zu Haushalten hoch. Bei allen anderen bilanzierten Arzneistoffen waren die Verbräuche geringer. Dies liegt an der pflegerischen Ausrichtung, des als Grundlage für die Hochrechnung dienenden Pflegeheims NH. 75 % der Bewohner sind dort älter als 60 Jahre. 2011 waren 95 % der Pflegeheimbewohner bundesweit älter als 60 Jahre. Daran ist die psychiatrisch-pflegerische Ausrichtung von NH zu erkennen. Außerdem scheint der Verbrauch an Neurologika sehr hoch zu sein, da Verbrauchsdaten des AVR die Neurologika der Pflegeheime beinhalten.
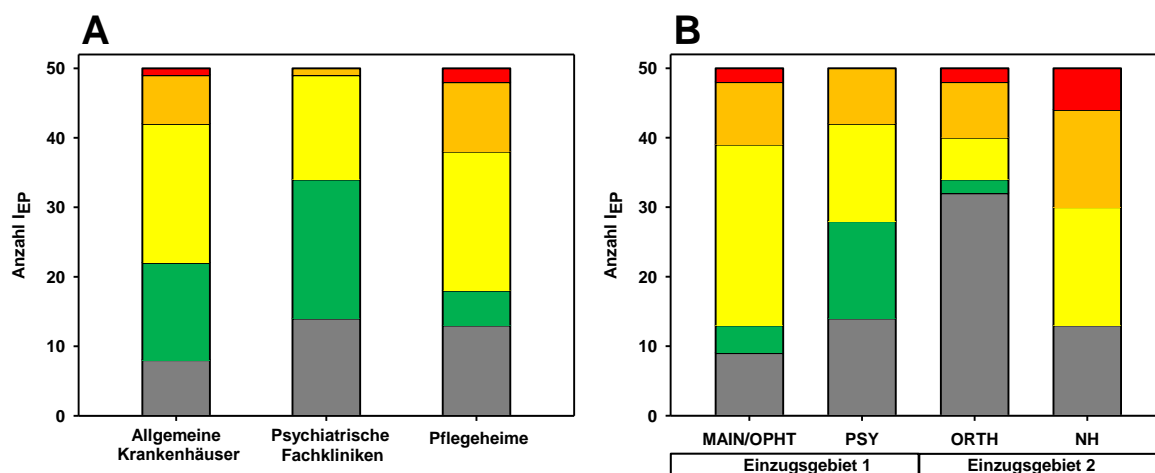

Abbildung 5   Anzahl der Emissionspotentiale für $I_{EP} > 1$ (rot), $I_{EP} > 0,1$ (orange), $I_{EP} > 0,01$ (gelb) und $I_{EP} \leq 0,01$ (grün) für [A] die bundesweite (nationale) und [B] die regionale Bedeutung der Einrichtungen für die ausgewählten 50 Arzneistoffe. Grau zeigt an, dass kein I_{EP} ermittelt werden konnte.

Allgemein wurde bei der nationalen Betrachtung angenommen, dass die Arzneimittelverbräuche nach Art und Menge in den untersuchten Einrichtungen mit allen Einrichtungen in Deutschland weitestgehend übereinstimmen. Eine Bilanzierung zusätzlicher Einrichtungen könnte die Unsicherheit verringern. Übereinstimmungen bei den Arzneimittelverbräuchen mit Literaturangaben (Escher et al. 2011; Schuster et al. 2008) wiesen jedoch auf eine geringe Unsicherheit hin.

Regional wurden zwei Einzugsgebiete von STP betrachtet (Abbildung 3). In Einzugsgebiet 1 mit 85 000 mit den beiden Standorten MAIN und OPHT von HOSP und PSY wurden vergleichsweise höhere I_{EP} festgestellt, als beim nationalen Vergleich. Dies liegt an der höheren Dichte beziehungsweise Größe an HI im Einzugsgebiet und der damit höheren Anzahl an behandelten Fällen im Vergleich zum bundesweiten Durchschnitt. In Einzugsgebiet 2 mit 13 000 Einwohnern machte sich dieser Unterschied noch stärker bemerkbar, da hier die Anzahl behandelter Fälle

beziehungsweise die Anzahl von Pflegeheimbewohnern noch höher als im bundesweiten Durchschnitt waren. Somit kommt es tatsächlich vereinzelt zu hohen Beiträgen von Arzneistoffen in regionale Abwässer durch die einzelnen Einrichtungen. Diese Feststellung trifft jedoch nur auf einzelne Arzneistoffe zu.

Die Darstellung der regionalen Verbräuche in Haushalten mit Hilfe der bundesweiten Verbrauchsdaten des AVR wurde als plausibel angesehen, da die Altersverteilung, die Anzahl der Einwohner mit Migrationshintergrund und die Arbeitslosenrate in den untersuchten Einzugsgebieten etwa dem deutschlandweiten Durchschnitt entsprach (Statistisches Bundesamt DESTATIS 2016).

4.3.2 Identifizierung relevanter Arzneistoffe aus punktuellem Arzneistoffeintrag in das kommunale Abwasser

In Tabelle 1 finden sich alle Arzneistoffe mit einem I_{EP} größer als 1 und den entsprechenden Verbräuchen in den Einrichtungen aus der regionalen Betrachtung zur schnellen Übersicht. Die Emissionspotentiale aller anderen Arzneistoffe finden sich in Artikel 1. Durch die vergleichsweise höhere Anzahl an behandelten Fällen in den beiden Einzugsgebieten sind nahezu alle I_{EP} im regionalen Vergleich höher als im nationalen Vergleich.

Tabelle 1 Emissionspotentiale I_{EP} aus regionaler Betrachtung der Arzneistoffe mit relativer Standardabweichung, die mindestens in einer Einrichtung größer als 1 sind, mit entsprechenden Arzneistoffverbräuchen A_{API} mit relativer Standardabweichung. $I_{EP} > 1$ sind hervorgehoben.

ATC	Arzneistoff	Einzugsgebiet 1				Einzugsgebiet 2			
		MAIN/OPTH		PSY		ORTH		NH	
		I_{EP}	A_{API} (kg)	I_{EP}	A_{API} (kg)	I_{EP}	A_{API} (kg)	I_{EP}	A_{API} (kg)
J01	Cefuroxim	**1,0375** **± 8 %**	26,05 ± 1 %	0,0040 9 %±	0,07 ± 10 %	**3,5621** **± 9 %**	13,67 ± 14 %	0,0234 ± 0 %	0,04 ± 54 %
N03	Oxcarbazepin	0,0134 ± 4 %	0,15 ± 57 %	0,0329 ± 17 %	0,48 ± 20 %	-	-	**1,5893** **± 36 %**	3,67 ± 34 %
N05	Chlorprothixen	-	0,01 ± 62 %	0,0561 ± 35 %	0,15 ± 23 %	-	-	**1,5599** **± 9 %**	0,55 ± 10 %
N05	Clomethiazol	**1,4338** **± 6 %**	1,40 ± 12 %	0,4495 ± 17 %	0,45 ± 11 %	**2,5021** **± 20 %**	0,36 ± 20 %	-	-
N05	Melperon	0,1378 ± 13 %	0,58 ± 20 %	0,1146 ± 32 %	0,50 ± 38 %	0,1508 ± 0 %	0,08 ± 13 %	**1,1013** **± 6 %**	0,72 ± 6 %
N05	Quetiapin	0,0157 ± 10 %	0,33 ± 9 %	0,1803 ± 21 %	3,79 ± 14 %	-	-	**2,6462** **± 11 %**	8,60 ± 15 %
N06	Moclobemid	0,3462 ± 39 %	0,15 ± 55 %	0,1608 ± 0 %	0,08 ± 64 %	-	-	**6,9241** **± 74 %**	0,70 ± 29 %
R06	Promethazin	0,0000 ± 0 %	0,02 ± 15 %	0,2005 ± 1 %	0,19 ± 11 %	-	-	**2,1899** **± 38 %**	0,32 ± 38 %

Acht Arzneistoffe mit einem $I_{EP} > 1$ konnten identifiziert werden. Davon kommen sechs Arzneistoffe aus dem Bereich der Neurologika. Wobei Promethazin (R06) auch in der Regel bei psychiatrischen Erkrankungen verwendet und nur auf Grund seines Wirkmechanismus zu den Antihistaminika gezählt wird. Cefuroxim als Antibiotikum spielt im Besonderen bei Krankenhäusern eine Rolle. Der insbesondere in MAIN/OPHT und ORTH hohe Eintrag an Cefuroxim lässt sich durch die infektionsprophylaktische Gabe bei Operationen erklären. Die

hohen I_{EP} von Clomethiazol lassen sich durch die Zulassungsvorgaben erklären. Dieser Arzneistoff sollte bei akuter Entzugssymptomatik nur unter stationärer Kontrolle verwendet werden (Cheplapharm Arzneimittel GmbH 2011). Deshalb hat Clomethiazol auch keine Bedeutung in NH. Auch Chlorprothixen, Melperon und Promethazin werden bevorzugt zur Beruhigung und zur Behandlung von Schlafstörungen eingesetzt. Der Einsatz lässt sich sehr wahrscheinlich durch die ungewohnte Umgebung bei stationären Aufenthalten erklären. Das Antiepileptikum Oxcarbazepin, Derivat[13] des Carbamazepin, und das Antidepressivum Moclobemid wurden vermutlich vornehmlich durch die Ärztinnen und Ärzte für die Bewohner von NH verschrieben. Quetiapin mit hohen Emissionspotentialen und Verbrauchsmengen in PSY und NH sollte hohe Aufmerksamkeit geschenkt werden. Insbesondere aufgrund der hohen Bedeutung und aufgrund der Verbrauchsmengen scheint eine Beurteilung hinsichtlich des Umweltverhaltens sowie des Eliminationsverhaltens unter Verwendung technologischer Verfahren und der Bildung relevanter TP sinnvoll.

4.4 Photolyse und initiale Bewertung des Verbleibs und des Verhaltens von Gabapentin (GAB) in der Umwelt

In Artikel 2 werden die Ergebnisse von Photolyse und initialer Bewertung des Verbleibs und des Verhaltens von GAB in der Umwelt ausführlich diskutiert. Bei einer Startkonzentration von 100 mg L^{-1} war GAB nach 128 min zu 80 % eliminiert, wohingegen nur 9 % von GAB mineralisiert wurden. Dieses Ergebnis lässt auf die Bildung von PTP schließen. Die Elimination von GAB folgte einer Kinetik 0. Ordnung mit einer Geschwindigkeitskonstanten k von 0,640 min^{-1}. Die Elimination von GAB für abnehmende Startkonzentrationen folgte einer Kinetik 1. Ordnung mit abnehmenden Halbwertszeiten und entsprechend zunehmenden Geschwindigkeitskonstanten (20 mg L^{-1}: $t_{1/2}$ = 18 min, k = 0,039 min^{-1}; 5 mg L^{-1}: $t_{1/2}$ = 8 min, k = 0,086 min^{-1}; 1 mg L^{-1}: $t_{1/2}$ = 3 min, k = 0,217 min^{-1}). Auch bei Startkonzentrationen von 20 und 5 mg L^{-1} konnte eine Residualkonzentration an NPOC am Ende der Photolyse festgestellt werden, was auch in diesem Fall auf eine Bildung von PTP schließen lässt. Für die Startkonzentration von 0,1 mg L^{-1} konnte kein kinetisches Modell aufgestellt werden. Nach 4 min Photolyse war die GAB-Konzentration bereits unter der LOD.

Da GAB im Vergleich zu anderen Arzneistoffen im niedermolekularen Bereich nur wenige chromophore Gruppen[14] aufweist (Abbildung 11, Anhang), und es damit auch nur zu einer vergleichsweise geringen Überlappung mit dem Emissionsspektrum der UV-Lampe kommt (siehe Abbildung 10, Anhang), liegt die Vermutung nahe, dass es zu indirekter Photolyse von GAB kam. Es wird angenommen, dass GAB hauptsächlich über reaktive Sauerstoffspezies (ROS) abgebaut wurde. Bei einer Startkonzentration von 100 mg L^{-1} nahm die Konzentration des gelösten Sauerstoffs kontinuierlich ab. Nach etwa 2 h konnte kein Sauerstoff mehr in der Lösung nachgewiesen werden. So könnten sich aus gelöstem Sauerstoff Superoxidanionen und Hydroperoxylradikale gebildet haben (Du et al. 2014). Diese haben die Elimination von GAB und die Bildung von PTP verursacht. Nach 32 min bildete sich erstmalig Wasserstoffperoxid (< 0,5 mg L^{-1}). Bis zum Testende stieg dieser Wert auf 0,5 – 2 mg L^{-1} an. Wasserstoffperoxid wurde

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[13] Abkömmling, chemisch verwandte Substanz.
[14] Gruppen, die die selektive Lichtabsorption entscheidend beeinflussen (Beyer und Walter 2004).

dabei wahrscheinlich durch die Rekombination von Superoxidanionen und Hydroperoxylradikalen gebildet (Bielski et al. 1985).

Bei einer Startkonzentration von 100 mg L^{-1} konnten 27 PTP identifiziert werden. Es wurden mehrere Strukturisomere gebildet. Aus Gründen der Übersichtlichkeit wurden nur die Strukturen von allen PTP mit einem Peakflächenverhältnis A/A_0, das größer als 1 % war, aufgeklärt (A ist die Peakfläche eines PTP zu einem bestimmten Zeitpunkt, A_0 ist die Peakfläche von GAB zum Zeitpunkt 0 min). Im Wesentlichen wurden PTP durch Mono- oder Dihydroxylierungen gebildet (Abbildung 6). Das durch Dehydrierung gebildete Cyanid PTP 168 wird im US-amerikanischen Arzneibuch (USP 2013) als Verunreinigung von GAB angegeben. Für PTP 154 wird ein zyklisches Laktam als Struktur angeboten, das durch Abspaltung von Wasser entstanden sein könnte. Diese Struktur stellt ein durch Hitze gebildetes Abbauprodukt von GAB dar und wurde schon in anderen Studien beschrieben (Ciavarella et al. 2007; Lin et al. 2010). Als weiteres nicht hydroxyliertes Produkt wird das hier gefundene PTP 128 als decarboxyliertes Produkt von GAB beschrieben. Decarboxylierung wurde schon zuvor als photolytischer Transformationprozess beschrieben (Wang und Lin 2012).
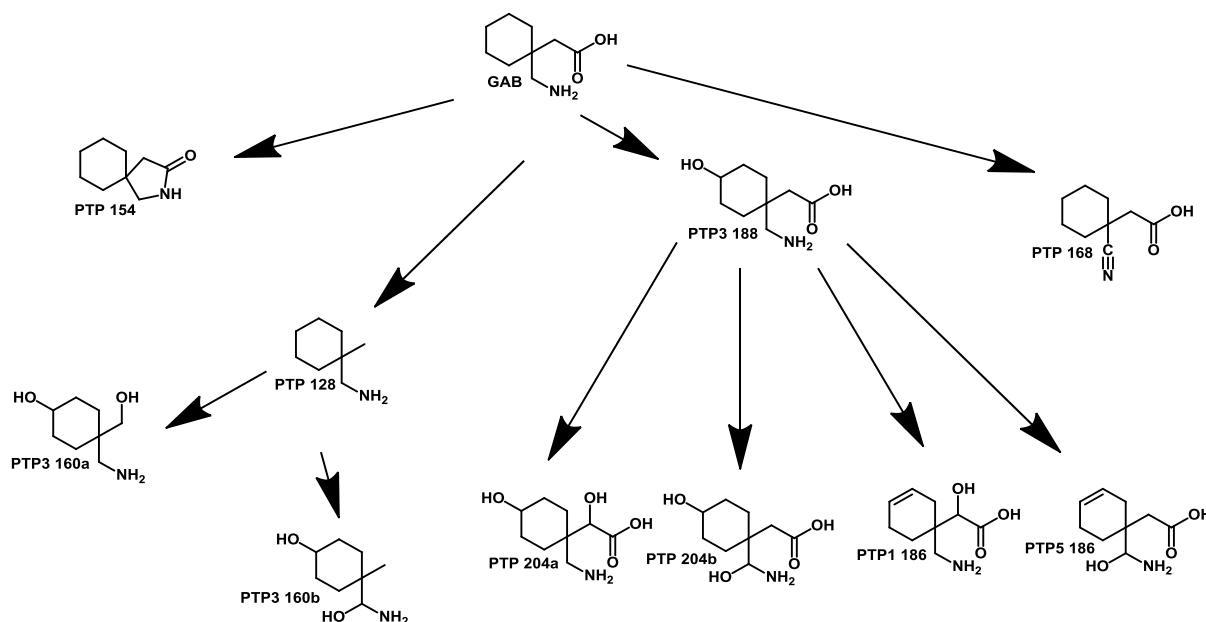
Abbildung 6   Vorgeschlagener Phototransformationsweg von Gabapentin (GAB) unter Bildung von Phototransformationprodukten (PTP) (nach Artikel 2).

Nach OECD-Richtlinien (OECD 1992) konnte für GAB im CBT keine leichte biologische Abbaubarkeit nachgewiesen werden (7,9 ± 3,6 %). 99,6 ± 0,1 % von GAB wurden im CBT bezogen auf das Peakflächenverhältnisses S/S_0 nach 28 Tagen wiedergefunden. Die Ergebnisse zeigen, dass GAB, sobald es in die Umwelt gelangt, möglicherweise nicht durch den Einfluss von Bakterien abgebaut werden kann und auch nicht transformiert wird. In anderen Studien konnte GAB in STP nicht eliminiert werden (De la Cruz, N. et al. 2012; Reungoat et al. 2010) und kann deshalb auch in Oberflächengewässer gelangen (Writer et al. 2013). Auch die photolytischen Gemische waren nicht biologisch abbaubar. Der BOD stieg über die Testdauer von 28 Tagen nicht signifikant an. Auch die Peakflächen der strukturell aufgeklärten PTP wiesen keine signifikante Änderung nach 28 Tagen auf.

Unbehandeltes GAB bei einer 1:2-Verdünnung hatte keinen signifikant toxischen Effekt auf Leuchtbakterien (C_0 = 100 mg L^{-1}). Die photolytischen Gemische hingegen sorgten nach einer Behandlungszeit von 64 und 128 min für eine signifikante Langzeit-Leuchthemmung (LI_{24h}) beziehungsweise Zellvermehrungshemmung (GI_{14h}). Die effektivste Hemmung trat nach 128 min ein. GAB war zu diesem Zeitpunkt marginal mineralisiert (10 % NPOC-Elimination) und fast vollständig eliminiert (etwa 90 % GAB-Elimination). Dies lässt vermuten, dass PTP einen toxischen Effekt auf Leuchtbakterien haben. Nach dem Verlauf des Peakflächenverhältnisses wären PTP 168, PTP5 186, PTP 204a und PTP 204b mögliche Kandidaten für die toxischen Effekte. Allerdings könnten Mischtoxizität und Reaktionsnebenprodukte wie ROS, auch einen Einfluss auf die ansteigende Toxizität gehabt haben. Beim Umu-Test konnte keine signifikante Induktion des umuC-Gens für GAB und die photolytischen Gemische erkannt werden. Falsch negative Ergebnisse aufgrund von Zytotoxizität können jedoch nicht ausgeschlossen werden.

4.5 Photolyse und initiale Bewertung des Verbleibs und Verhaltens von Quetiapin (QUT) in der Umwelt

In Artikel 3 werden die Ergebnisse zur Photolyse und initialer Bewertung des Verbleibs und des Verhaltens von QUT in der Umwelt ausführlich diskutiert. Bei einer hohen Startkonzentration von 86,9 mg L^{-1} folgte auch QUT bei einer Elimination von 61 % einer Kinetik 0. Ordnung (k = 0,497 min^{-1}). Der NPOC wurde zu 3 % eliminiert. Die niedrigeren Startkonzentrationen (17,4 und 4,3 mg L^{-1}) folgten einer Kinetik 1. Ordnung (k = 0,016 und 0,069 min^{-1}) bei abnehmender Halbwertszeit $t_{1/2}$ von 45 und 10 min. In beiden Fällen wurde QUT nicht vollständig mineralisiert (NPOC-Elimination: 5 und 70 %). Bei einer Startkonzentration von 0,9 mg L^{-1} war die Konzentration von QUT nach 16 min unter der LOD. Die Bestimmung der Abbaukinetik und des NPOC waren daher in diesem Falle nicht möglich.

Die relative Eliminationsgeschwindigkeit von QUT nahm mit abnehmender Startkonzentration, wie auch schon bei GAB, zu. Bei jeweils vergleichbaren Konzentrationen war die Geschwindigkeit jedoch langsamer als bei GAB. Die molare Extinktion von QUT im Bereich von 200 bis 350 nm ist weitaus höher als bei GAB (Abbildung 10, Anhang). Daher ist davon auszugehen, dass die direkte Photolyse eine sehr große Rolle für die Elimination von QUT spielt. Die höhere Absorption lässt sich durch die höhere Anzahl an chromophoren Gruppen erklären (Abbildung 12, Anhang).

Während der Photolyse wurden PTP gebildet. Sieben davon wurden strukturell aufgeklärt, die allesamt polarer als QUT waren (Abbildung 7). Alle hatten über den gesamten Verlauf der Behandlung ein Peakflächenverhältnis A/A_0 < 7 %. PTP1 und PTP3 400 und PTP 414 entstanden sehr wahrscheinlich durch Mono- beziehungsweise Dihydroxylierung. Die Position für die Hydroxylierung am Piperazinring von PTP 414 ließ sich mit Hilfe der Fragmentierungsmuster nicht eindeutig bestimmen. PTP2 400 ist das Sulfoxid, das auch beim humanen Metabolismus gebildet wird (Fisher et al. 2012). PTP 251 entstand nach Elimination der Seitenkette und Aufbruch des Piperazinrings, PTP 358 nach Elimination zweier Kohlenstoffatome im Piperazinring.
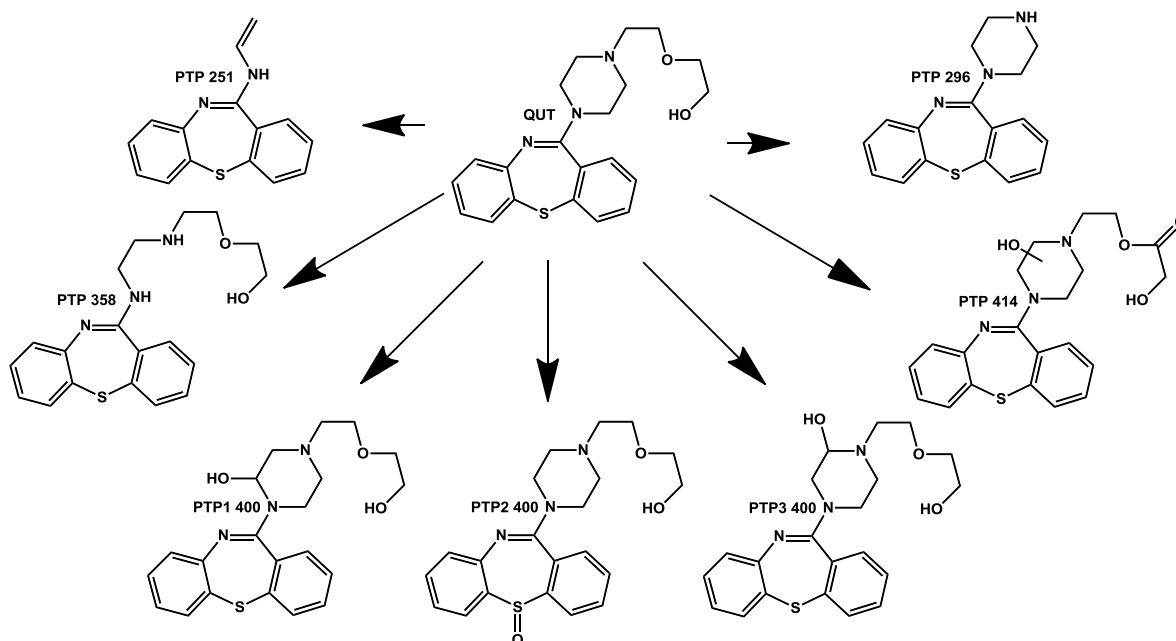
Abbildung 7 Vorschlag für Phototransformationsprodukte (PTP), die unter Photolyse von Quetiapin (QUT) gebildet werden (nach Artikel 3).

Nach OECD-Richtlinien (OECD 1992) war QUT im CBT und im MRT nicht leicht biologisch abbaubar (16,6 ± 1,5 % und -1,9 ± 3,2 %). Der geringfügige Abbau von QUT im CBT ist sehr wahrscheinlich durch Fumarsäure zu erklären. Fumarsäure ist leicht biologisch abbaubar (86,1 ± 1,0 %) und hat somit zum teilweisen Abbau von QUT als Hemifumaratsalz beigetragen. Beim CBT wurden bezogen auf das mittlere Peakflächenverhältnis S/S_0 80 ± 4 % an QUT wiedergefunden. Beim MRT wurden sogar nur 11 ± 11 % wiedergefunden. Dies ließ auf eine biologische Transformation von QUT schließen, die sich im MRT in einem höheren Ausmaß aufgrund der höheren Bakteriendichte zeigte. Das photolytische Gemisch von QUT nach 128 min Photolyse war im CBT und im MRT nicht besser biologisch abbaubar, wenn man den BOD zur Beurteilung heranzieht. Die Peakflächenflächenverhältnisse S/S_0 änderten sich allerdings für nahezu alle PTP. Dies lässt vermuten, dass die meisten PTP biologisch transformiert wurden.

In Abbildung 8 sind Strukturvorschläge für BTP zu sehen, die bei beiden biologischen Abbaubarkeitstests (CBT und MRT) gebildet wurden. BTP 398 wurde als Haupt-BTP identifiziert. Es wurde ausgehend von QUT gebildet, da es in den Bioabbaubarkeitstests von QUT und im photolytischen Gemisch zu finden war. Durch die Software META wurde BTP 398 auch als mögliches TP vorausgesagt. Sehr wahrscheinlich wurde die endständige Alkoholgruppe in der Seitenkette zur Carbonsäure oxidiert. BTP 398 entsteht ebenfalls beim humanen Metabolismus (DeVane und Nemeroff 2001). Bei der Untersuchung von Flusswasserproben konnte QUT in keiner Proben nachgewiesen werden. Die BTP 398-Konzentrationen lagen dagegen in 12 der untersuchten 35 Flussproben über der LOD. Dies hängt wohl maßgeblich damit zusammen, dass BTP 398 beim humanen Metabolismus und auch durch bakterielle Transformation in Flusswasser gebildet werden kann. Da durch das Fehlen eines analytischen Standards letztendlich keine quantitative Aussage über das Vorkommen von BTP getroffen werden kann, sollten weiterführende Untersuchungen diesbezüglich unternommen werden.

Entsprechend simultan zu BTP 398 entstand BTP 414 als Oxidationsprodukt von PTP2 400. Auch BTP 414 konnte mit der Software META bestätigt werden. Laut Fragmentationsmuster hat BTP 356 eine verkürzte Seitenkette mit dihydroxyliertem Piperazinringsystem. Aus welchem PTP die Strukturen BTP 356 und 400 entstanden sind, ließ sich nicht bestimmen.
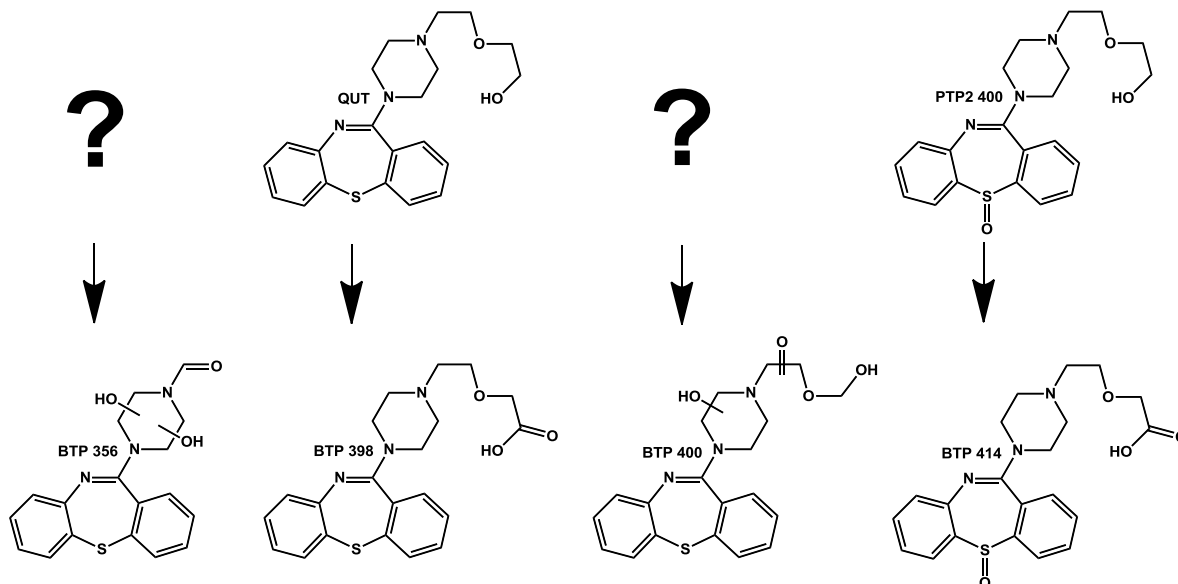

Abbildung 8   Vorschlag für Biotransformationsprodukte (BTP), die im Closed Bottle Test (CBT) und im Manometrischen Respirationstest (MRT) ausgehend von Quetiapin (QUT) und Phototransformationsprodukten (PTP) gebildet wurden. Ein Fragezeichen ist angegeben, wenn nicht bestimmbar war, aus welchem PTP das jeweilige BTP entstanden ist. (nach Artikel 3)

QUT selbst hatte abhängig vom betrachteten Endpunkt einen unterschiedlich ausgeprägten toxischen Effekt auf Leuchtbakterien. Am deutlichsten war die Langzeit-Leuchthemmung (LI_{24h}) zu erkennen. Auch die photolytischen Gemische zeigten einen deutlich toxischen Effekt bei der Langzeit-Leuchthemmung. Beispielhaft konnte damit anhand der Langzeit-Leuchthemmung gezeigt werden, dass die Mischung aus PTP einen ähnlichen zytotoxischen Effekt wie QUT auf Leuchtbakterien ausübt. Beim Umu-Test konnte keine signifikante Induktion des umuC-Gens für QUT und die photolytischen Gemische erkannt werden. Falsch negative Ergebnisse aufgrund von Zytotoxizität können jedoch nicht ausgeschlossen werden.

5 SCHLUSSFOLGERUNGEN UND AUSBLICK

Diese Arbeit belegt die grundlegende Notwendigkeit, den Einsatz von Arzneistoffen in Humanarzneimitteln auch unter Einbezug eines späteren Umweltvorkommens zu bewerten. Dies beinhaltet auch in der Umwelt weit verbreitete und damit bedeutende Substanzen tiefergehend zu charakterisieren.

So konnte gezeigt werden, dass bei einem diffusen Eintrag von Arzneistoffen eine regelmäßige Überprüfung der Verbrauchsmengen von Arzneimitteln unter Einbeziehung des humanen Metabolismus erfolgen sollte, um die Dynamik des Arzneimittelmarktes auch auf die Umweltforschung übertragen zu können. Es wurde erstmalig gezeigt, dass Arzneistoffe mit moderaten Verbrauchsmengen wie GAB, in sehr hohen Konzentrationen am Zulauf von STP zu finden sind und deshalb genauer auf ihr Umweltverhalten hin untersucht werden sollten. Durch die Identifikation für den Abwassereintrag hoch relevanter Substanzen wird eine selektive und gezielte Untersuchung hinsichtlich ihres Abbauverhaltens und Verbleibs in aquatischen Systemen ermöglicht. Außerdem ist es möglich, durch Identifikation relevanter Arzneistoffe Ärztinnen und Ärzte, Apothekerinnen und Apotheker, Zuständige für die Abwasserentsorgung und involvierte Vertreterinnen und Vertreter aus der Wissenschaft für einen entsprechenden nachhaltigeren Umgang mit Arzneimitteln zu sensibilisieren.

Alle untersuchten HI waren nicht die Hauptverursacher für den Eintrag von Arzneistoffen in das kommunale Abwasser. Dies konnte erstmalig für psychiatrische Kliniken und Pflegeheime mit Hilfe einer neu entwickelten Methode zur Abschätzung des Emissionspotentials gezeigt werden. Bei detaillierter Betrachtung auf der Ebene lokaler Einzugsgebiete von STP konnte in Einzelfällen für bestimmte Arzneistoffe, wie QUT, eine hervorzuhebende Bedeutung für HI gezeigt werden. Da diese Bedeutung jedoch nur in Einzelfällen in Betracht kommt, sollten unter Berücksichtigung der lokalen Begebenheiten die zu untersuchenden Einrichtungen im Einzelfall betrachtet und mit Hilfe des in dieser Arbeit präsentierten Vorgehens analysiert werden. So können bei Bedarf pro HI oder Haushalte im Einzugsgebiet bedeutende Arzneistoffe identifiziert werden. Die entsprechenden Ergebnisse können dann als Information zur Entwicklung von Reduktionsmaßnahmen dienen. Die Wirkung der Maßnahmen kann später nach einem definierten Zeitraum nochmals überprüft werden.

Durch die erfolgreiche Anwendung einer neu entwickelten Methode zur Modellierung der Arzneistoffkonzentrationen im Abwasser basierend auf einer Verbrauchsmengenbilanzierung und in Kombination mit einer analytischen Bestimmung von ausgewählten Arzneistoffen, liefert diese Arbeit einen wertvollen Beitrag zur Erhebung und Auswertung von Untersuchungsdaten zur Bestimmung von potenziellen Emissionsquellen und ihrer Beiträge zum Arzneistoffeintrag ins kommunale Abwasser. Somit ist dieses entwickelte Konzept auch ein Beitrag zur wissenschaftlichen Diskussion im Bereich möglicher Alternativen zu aufwändigen Monitoringprogrammen.

Diese Arbeit zeigt zudem, wie wichtig es ist, Arzneistoffe hinsichtlich ihres Verbleibs und Verhaltens in der Umwelt zu charakterisieren, da für vor 2006 zugelassene Arzneistoffe selten diesbezüglich Daten verfügbar sind. Seit 2006 ist zwar eine einheitliche Umweltrisikoprüfung bei nationalen und europäischen Zulassungen erforderlich, die Ergebnisse sind jedoch nicht zwingend

öffentlich zugänglich und zudem für Humanarzneimittel nicht zulassungsentscheidend (European Medicines Agency 2006). Umweltrisikoprüfungen beinhalten außerdem hauptsächlich Informationen bezüglich der Muttersubstanzen. Tiefergehende Untersuchungen von Metaboliten und TP von Arzneistoffen werden in der Regel derzeit nicht durchgeführt. Die in dieser Arbeit durchgeführten Untersuchungen zu GAB und QUT zeigen, dass dies insbesondere auch für die TP wichtig wäre.

Die Ergebnisse zeigen außerdem, dass eine Photolyse kritisch als mögliche Behandlungsmethode von entsprechenden Wassermatrices betrachtet werden sollte. Die strukturelle Aufklärung von PTP mittels hochauflösender Massenspektrometrie bei verschiedenen Startkonzentrationen ist bei dieser Betrachtung von wesentlicher Bedeutung. Darauf aufbauend sollten im weiteren Verlauf diese PTP hinsichtlich biologischer Abbaubarkeit unter Einbeziehen der möglichen Bildung von BTP und toxischer Eigenschaften beurteilt werden. Zukünftig sollten generell die Eigenschaften strukturell bekannter TP mittels computerbasierter Methoden ((quantitative) structure-activity relationships, (Q)SAR) tiefergehend charakterisiert werden, um erste Informationen bezüglich des Verhaltens und der Wirkung zu bekommen.

Das in dieser Arbeit genutzte Set von Methoden ermöglicht letztendlich die Entwicklung von Strategien zur Identifizierung häufig verwendeter Arzneistoffe und zur Beurteilung der ökologischen Relevanz gebildeter TP und Metaboliten. Mit diesen Ergebnissen können an den richtigen Stellen die entsprechenden Emissionsminderungsmaßnahmen entwickelt und so ein Beitrag zum nachhaltigeren Einsatz von Arzneimitteln geleistet werden.

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8 ANHANG

Arzneistoffbilanzierung in Dülmen

Tabelle 2 Gewählte bilanzierte Arzneistoffe für Dülmen mit Verbrauchsmengen (A), nicht metabolisierter Anteil (f_{nm}) und vorhergesagte Kläranlagenzulaufkonzentrationen PIC im Jahr 2012. Der niedrigste und höchste Wert für f_{nm} aus der jeweiligen Fachinformation und der Micromedex Datenbank (Truven Health Analytics Inc. 2013) sind angegeben. Im Ergebnis wird die PIC als Konzentrationsbereich angegeben.

Arzneistoff	Therapeutische Untergruppe des ATC Code (Stufe 2)	A (kg)	f_{nm}	PIC (µg L^{-1})
Esomeprazol	A02 Mittel bei säurebedingten Erkrankungen	2,0	0,00 – 0,01	0,00 – 0,07[15]
Omeprazol	A02 Mittel bei säurebedingten Erkrankunge	12,8	0,00 – 0,01	
Pantoprazol	A02 Mittel bei säurebedingten Erkrankunge	22,1	0,00 – 0,01	0,00 – 0,11
Metoclopramid	A03 Mittel bei funktionellen GI-Störungen	1,2	0,20	0,12
Mesalazin	A07 Antidiarrhoika und intestinale Antiphlogistika/Antiinfektiva	30,3	0,50 – 0,70	7,38 – 10,33
Metformin	A10 Antidiabetika	485,0	1,00	236,55
Sitagliptin	A10 Antidiabetika	7,0	0,79 – 0,87	2,70 – 2,97
Acetylsalicylsäure	A01 Antithrombotische Mittel	157,3	0,00 – 0,02	0,00 – 1,53
Clopidogrel	A01 Antithrombotische Mittel	6,1	0,50	1,49
Amiodaron	C01 Herztherapie	4,7	0,50 – 0,51	1,14 – 1,15
Furosemid	C03 Diuretika	13,3	0,93 – 0,94	6,04 – 6,11
Hydrochlorothiazid	C03 Diuretika	18,8	0,61 – 0,96	5,60 – 8,81
Spironolacton	C03 Diuretika	3,8	0,27 – 0,31	0,50 – 0,56
Triamteren	C03 Diuretika	4,3	0,20 – 0,24	0,42 – 0,51
Torasemid	C03 Diuretika	5,4	0,24	0,63
Bisoprolol	C07 Beta-Adrenorezeptoren-Antagonisten	5,7	0,50	1,38
Metoprolol	C07 Beta-Adrenorezeptoren-Antagonisten	32,3	0,05 – 0,15	0,79 – 2,36
Sotalol	C07 Beta-Adrenorezeptoren-Antagonisten	3,3	0,66 – 0,88	1,08 – 1,44
Amlodipin	C08 Calciumkanalblocker	4,8	0,10	0,24
Verapamil	C08 Calciumkanalblocker	9,2	0,03 – 0,23	0,13 – 1,03
Candesartan	C09 Mittel mit Wirkung auf das Renin-Angiotensin-System	2,0	0,56 – 0,89	0,56 – 0,89
Enalapril	C09 Mittel mit Wirkung auf das Renin-Angiotensin-System	7,7	0,52	1,95
Losartan	C09 Mittel mit Wirkung auf das Renin-Angiotensin-System	5,1	0,04	0,10
Ramipril	C09 Mittel mit Wirkung auf das Renin-Angiotensin-System	2,2	0,45 – 0,57	0,48 – 0,61
Valsartan	C09 Mittel mit Wirkung auf das Renin-Angiotensin-System	38,2	0,92 – 0,95	17,14 – 17,70

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[15] Für das Eutomer Esomeprazol und das Racemat Omeprazol ist eine Gesamtkonzentration PIC angegeben.

Fortsetzung Tabelle 2

Arzneistoff	Therapeutische Untergruppe des ATC Code (Stufe 2)	A (kg)	f_{nm}	PIC ($\mu g\, L^{-1}$)
Bezafibrat	C10 Mittel, die den Lipidstoffwechsel beeinflussen	7,7	0,40 – 0,50	1,49 – 1,87
Simvastatin	C10 Mittel, die den Lipidstoffwechsel beeinflussen	18,1	0,05	0,44
Ethinylestradiol	G03 Sexualhormone und andere Modulatoren des Genitalsystems	0,0	0,05	0,00
Prednisolon	H02 Corticosteroide zur systemischen Anwendung	2,4	0,05	0,06
Azithromycin	J01 Antibiotika zur systemischen Anwendung	2,8	0,06 – 0,14	0,08 – 0,19
Cefuroxim	J01 Antibiotika zur systemischen Anwendung	13,8	1,00	6,75
Ciprofloxacin	J01 Antibiotika zur systemischen Anwendung	18,1	0,77	6,80
Clarithromycin	J01 Antibiotika zur systemischen Anwendung	10,3	0,20 – 0,40	1,00 – 2,01
Doxycyclin	J01 Antibiotika zur systemischen Anwendung	3,2	0,81 – 1,00	1,26 – 1,55
Levofloxacin	J01 Antibiotika zur systemischen Anwendung	2,1	0,95 – 1,00	1,07 – 1,14[16]
Ofloxacin	J01 Antibiotika zur systemischen Anwendung	0,2	0,80 – 0,90	
Sulfamethoxazol	J01 Antibiotika zur systemischen Anwendung	16,2	0,15 – 0,20	1,18 – 1,58
Diclofenac	M01 Antiphlogistika und Antirheumatika	24,3	0,00 – 0,01[17]	2,18 – 2,27
Etoricoxib	M01 Antiphlogistika und Antirheumatika	2,2	0,00 – 0,02	0,00 – 0,02
Ibuprofen	M01 Antiphlogistika und Antirheumatika	396,2	0,01 – 0,15[18]	2,92 – 29,82
Naproxen	M01 Antiphlogistika und Antirheumatika	5,9	0,00 – 0,10	0,00 – 0,29
Allopurinol	M04 Gichtmittel	70,9	0,00 – 0,10	0,00 – 3,46
Metamizol	N02 Analgetika	327,9	0,00 – 0,01	0,00 – 1,60
Oxycodon	N02 Analgetika	1,4	0,00 – 0,19	0,00 – 0,13
Paracetamol	N02 Analgetika	140,2	0,00 – 0,05	0,00 – 3,42
Tramadol	N02 Analgetika	11,2	0,10 – 0,40	0,55 – 2,18
Carbamazepin	N03 Antiepileptika	27,6	0,03	0,40
Gabapentin	N03 Antiepileptika	47,5	1,00	23,16
Lamotrigin	N03 Antiepileptika	7,0	0,00 – 0,10	0,00 – 0,34
Levetiracetam	N03 Antiepileptika	41,5	0,66 – 0,73	13,35 – 14,75

[16] Für das Eutomer Levofloxacin und das Racemat Ofloxacin ist eine Gesamtkonzentration PIC angegeben.

[17] f_{nm} für systemische Darreichungsform von Diclofenac. f_{nm} für topische Darreichungsform ist 0,95.

[18] f_{nm} für systemische Darreichungsform von Ibuprofen. f_{nm} für topische Darreichungsform ist 0,95.

Fortsetzung Tabelle 2

Arzneistoff	Therapeutische Untergruppe des ATC Code (Stufe 2)	A (kg)	f_{nm}	PIC (μg L^{-1})
Oxcarbazepin	N03 Antiepileptika	11,5	0,01 – 0,06	0,06 – 0,33
Pregabalin	N03 Antiepileptika	14,8	0,90 – 0,98	6,51 – 7,10
Valproinsäure	N03 Antiepileptika	48,1	0,00 – 0,05	0,00 – 1,17
Levodopa	N04 Antiparkinsonmittel	28,6	0,01 – 0,06	0,14 – 0,84
Amisulprid	N05 Psycholeptika	2,7	0,70 – 0,98	0,91 – 1,27
Chlorprothixen	N05 Psycholeptika	0,5	0,00 – 0,05	0,00 – 0,01
Clozapin	N05 Psycholeptika	3,0	0,06 – 0,11	0,09 – 0,16
Melperon	N05 Psycholeptika	1,7	0,05 – 0,10	0,04 – 0,09
Quetiapin	N05 Psycholeptika	15,2	0,00 – 0,05	0,00 – 0,37
Amitriptylin	N06 Psychoanaleptika	4,7	0,01 – 0,05	0,02 - 0,11
Doxepin	N06 Psychoanaleptika	2,1	0,00 – 0,03	0,00 – 0,03
Citalopram	N06 Psychoanaleptika	4,1	0,10 – 0,23	0,20 – 0,47[19]
Escitalopram	N06 Psychoanaleptika	0,1	0,08 – 0,23	
Methylphenidat	N06 Psychoanaleptika	1,2	0,00 – 0,01	0,00 – 0,01
Mirtazapin	N06 Psychoanaleptika	4,5	0,00 – 0,01	0,00 – 0,02
Moclobemid	N06 Psychoanaleptika	1,8	0,00 – 0,01	0,00 – 0,01
Opipramol	N06 Psychoanaleptika	6,1	0,10	0,30
Sertralin	N06 Psychoanaleptika	3,1	0,00 – 0,14	0,00 – 0,21
Trimipramin	N06 Psychoanaleptika	4,5	0,01 – 0,10	0,02 – 0,22
Venlafaxin	N06 Psychoanaleptika	14,6	0,05 – 0,13	0,36 – 0,93
Acetylcystein	R05 Husten- und Erkältungspräparate	59,4	0,00 – 0,05	0,00 – 1,45
Cetirizin	R06 Antihistaminika zur systemischen Anwendung	0,8	0,50 – 0,67	0,22 – 0,28[20]
Levocetirizin	R06 Antihistaminika zur systemischen Anwendung	0,1	0,80 – 0,86	
Promethazin	R06 Antihistaminika zur systemischen Anwendung	1,9	0,00 – 0,01	0,00 – 0,01
Acetazolamid	S01 Ophthalmika	1,3	1,00	0,65

[19] Für das Racemat Citalopram und das Eutomer Escitalopram ist eine Gesamtkonzentration PIC angegeben.
[20] Für das Racemat Cetirizin und das Eutomer Levocetirizin ist eine Gesamtkonzentration PIC angegeben.

Mitteldruckquecksilberdampflampe

Die Mitteldruckquecksilberdampflampe tauchte in die behandelte Lösung unter Abtrennung durch ein Quarzglas ein. Das Quarzglas ermöglichte uneingeschränkte Bestrahlung emittierter Wellenlängen. Die Lösung wurde durch eine Kühlung bei 19 – 21 °C gehalten. Mit Hilfe eines Magnetrührers wurde die Durchmischung gewährleistet. Die Probe wurde durch einen Hahn abgenommen.
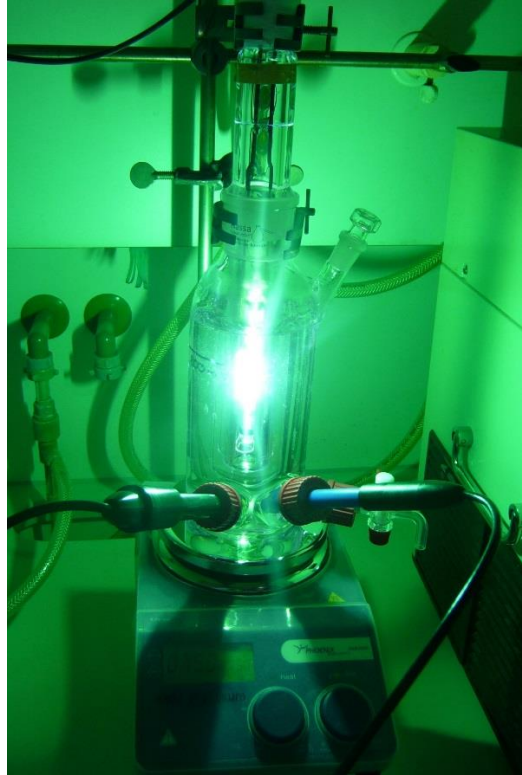

Abbildung 9   Versuchsaufbau Photolyse mit UV-Mitteldruckquecksilberdampflampe (abgeändert von graphical abstract aus Artikel 2).
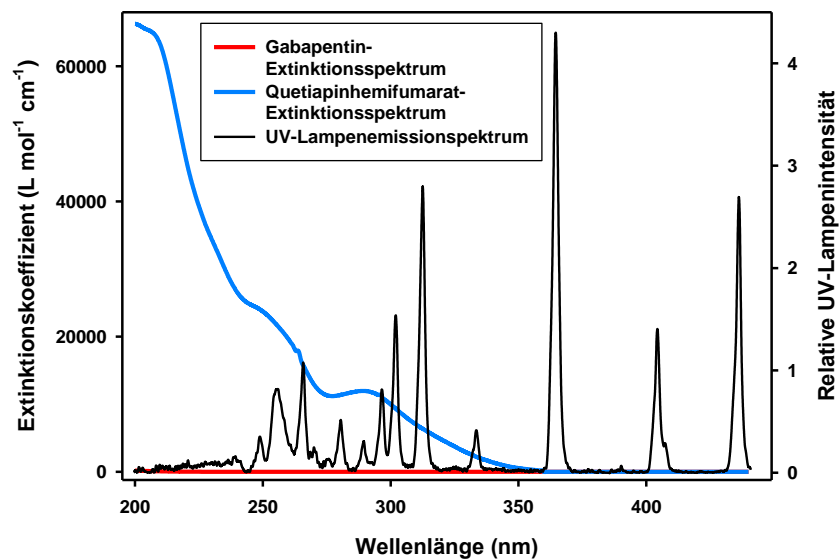
Abbildung 10   Emissionsspektrum der UV-Mitteldruckquecksilberdampflampe, Gabapentin-Extinktionsspektrum und Quetiapinhemifumarat-Extinktionsspektrum.

Strukturformeln von Gabapentin (GAB) und Quetiapin (QUT)

Im Folgenden sind die Strukturformeln der beiden als relevant identifizierten Neurologika dargestellt:
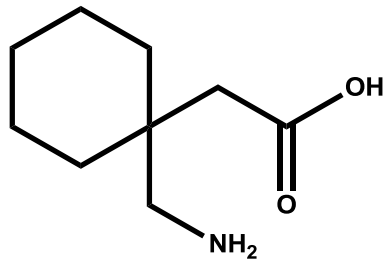
Abbildung 11   Strukturformel von Gabapentin.
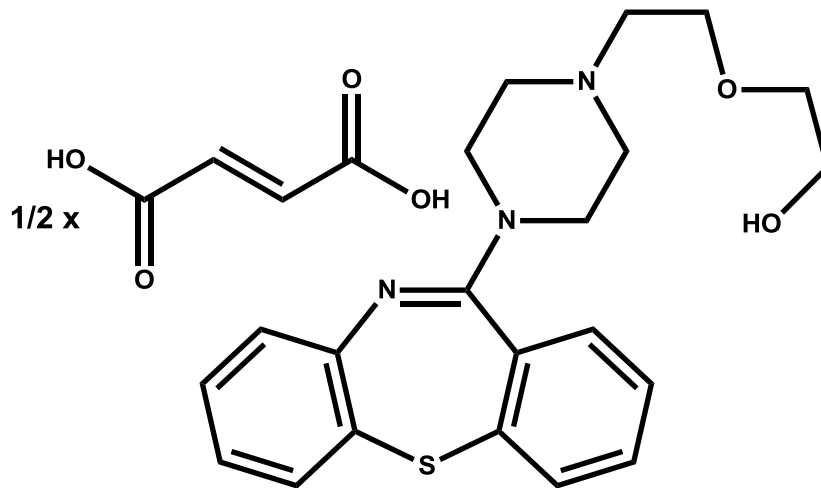
Abbildung 12   Strukturformel von Quetiapinhemifumarat.

Artikel zur kumulativen Dissertation

Artikel 1

Manuel Herrmann, Oliver Olsson, Rainer Fiehn, Markus Herrel, Klaus Kümmerer (2015).

The Significance of Different Health Institutions and Their Respective Contributions of Active Pharmaceutical Ingredients to Wastewater.

Environment International 85:61-76. DOI: 10.1016/j.envint.2015.07.020.

Artikel 2

Manuel Herrmann, Jakob Menz, Oliver Olsson, Klaus Kümmerer (2015).

Identification of Phototransformation Products of the Antiepileptic Drug Gabapentin: Biodegradability and Initial Assessment of Toxicity.

Water Research 85:11-21. DOI: 10.1016/j.watres.2015.08.004.

Artikel 3

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Experimental and *in silico* Assessment of Fate and Effects of the Antipsychotic Drug Quetiapine and Its Bio- and Phototransformation Products in Aquatic Environments.

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Artikel 1

The Significance of Different Health Institutions
and Their Respective Contributions
of Active Pharmaceutical Ingredients to Wastewater

Manuel Herrmann, Oliver Olsson, Rainer Fiehn, Markus Herrel, Klaus Kümmerer

Full length article

The significance of different health institutions and their respective contributions of active pharmaceutical ingredients to wastewater

Manuel Herrmann [a,b], Oliver Olsson [a], Rainer Fiehn [b], Markus Herrel [c], Klaus Kümmerer [a,*]

[a] Sustainable Chemistry and Material Resources, Institute of Sustainable and Environmental Chemistry, Leuphana University of Lüneburg, C13, Scharnhorstrasse 1, DE-21335 Lüneburg, Germany
[b] Hospital Pharmacy, Ortenau Klinikum Offenburg-Gengenbach, Ebertplatz 12, DE-77654 Offenburg, Germany
[c] Department of Occupational Safety and Environmental Protection, Ortenau Klinikum Offenburg-Gengenbach, Ebertplatz 12, DE-77654 Offenburg, Germany

ARTICLE INFO

ABSTRACT

Active pharmaceutical ingredients (APIs) have been frequently found in the environment. It is, however, still not quite clear who is mainly responsible for API emissions. Hospitals have been considered to be the main contributing point sources for wastewater (WW) discharge of APIs. However, recent studies have shown that the contribution of hospitals to the input of APIs into the aquatic environment is quite low. Due to demographic change and the increase of psychiatric diseases, health institutions (HIs) such as psychiatric hospitals and nursing homes are likely to be important sources as well, but no data is available in this respect. This study aims to assess the impact of HIs and to provide a methodology to measure their respective contributions. Drawing on pharmaceutical consumption data for the years 2010, 2011, and 2012, this study identified API usage patterns for a psychiatric hospital (146 beds), a nursing home (286 inhabitants), and a general hospital (741 beds), the latter of which comprises three separate locations. All the HIs are located in two sub-regions of a county district with about 400,000 citizens in southwestern Germany. A selection of neurological drugs was quantified in the sewer of these facilities to evaluate the correlation between consumption and emission. The API contribution of HIs was assessed by comparing the specific consumption in the facilities with the consumption in households, expressed as the emission potential (I_{EP}). The study shows that the usage patterns of APIs in the psychiatric hospital and the nursing home were different from the general hospital. Neurological drugs such as anticonvulsants, psycholeptics, and psychoanaleptics were mainly consumed in the psychiatric hospital and the nursing home (74% and 65%, respectively). Predicted and average measured concentrations in the effluent of the investigated HIs differed mostly by less than one order of magnitude. Therefore, the consumption-based approach is a useful method to assess usage patterns of APIs in HIs and to predict their respective contributions to WW. The national contribution of HIs on total WW discharge of APIs compared to households was very low. Only the results for the sedative clomethiazole in general hospitals as well as the antidepressant moclobemide and the antipsychotic quetiapine for the nursing homes were found to deserve some attention. The regional comparison showed that in sub-regions with a comparably higher density of HIs, the allocated facilities could be seen as point sources emitting particular APIs. However, in general, the bulk of the consumed pharmaceuticals to WW discharge has to be attributed to households.

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1. Introduction

In many studies, active pharmaceutical ingredients (APIs), contained in human pharmaceuticals, have been found in the environment in every possible country, with wastewater (WW) being the primary entry route (Halling-Sørensen et al., 1998; Klosterhaus et al., 2013; Langford and Thomas, 2009; Nikolaou et al., 2007; Verlicchi et al., 2012a). Studies have identified health institutions (HIs) such as hospitals as main polluters and tried to measure their environmental input of APIs (Escher et al., 2011; Gupta et al., 2009; Verlicchi et al., 2012b). Several studies reported that the contribution is depending on the bed density of HIs in the investigated area (Ort et al., 2010; Verlicchi et al., 2012b). Other studies showed that hospitals cannot be seen as a main source for APIs in the environment (Heberer and Feldmann, 2005; Kümmerer and Henninger, 2003; Schuster et al., 2008). By means of a

mass balance, Kümmerer and Henninger (2003) identified households as the main contributors in terms of total environmental input of antibiotics. Le Corre et al. (2012) came to the same conclusion by evaluating the consumption-based contribution of all consumed APIs emitted from selected hospitals in comparison to the entire national consumption in Australia.

Data on the emission of APIs from HIs other than hospitals are, however, still not available, and psychiatric hospitals and nursing homes in particular have rarely been investigated as point sources for API emission. It is, however, necessary to understand their impact more clearly, as the importance of psychiatric hospitals will increase in the future, because the prevalence of mental diseases is associated with the increasing mental pressure in a meritocratic society (Janssens et al., 2014). Moreover, the increasing importance of mental diseases is also associated with demographic change, which is likely to make nursing homes even more important (Laidlaw and Pachana, 2009). In Germany, for example, the number of cases treated in psychiatric hospitals and people living in nursing homes has increased consecutively by about 13% between 2005 to 2011 (Federal Health Report Germany, 2014a, 2014b). Despite these significant developments, there is a considerable lack of information about the usage patterns of APIs in these facilities. Escher et al. (2011) described the usage patterns of a psychiatric center in Switzerland, but the authors could not provide a general conclusion concerning the contribution of APIs.

Many studies have already discussed how to determine environmental concentrations and, accordingly, the emission sources of APIs in the environment. In general, it is possible to model concentrations in the environment (predicted environmental concentration (PEC)) or to measure concentrations (measured environmental concentration (MEC)) (Johnson et al., 2008; Liebig et al., 2006). In some studies, analytical monitoring of API concentrations in receiving waters is considered to depict the actual situation in the environment (Al Aukidy et al., 2012). However, Le Corre et al. (2012) claimed that analytical determination is not always a suitable approach to determine the importance of APIs in terms of environmental impact, because analytical determination is often limited to the availability of suitable sampling sites. Likewise, samples are often not representative for the study area. As a result, this approach can lead to results that either overestimate or underestimate the contribution of different emission sources. In contrast to monitoring, the prediction of concentrations makes it possible to consider longer time periods, but this method is still not able to depict the actual situation. Therefore, recent studies have focused on the comparison of PEC and MEC illustrating limitations for both approaches. Mullot et al. (2010) compared PEC and MEC in hospital effluent and the receiving sewage treatment plant (STP), and they noted that there is a strong correlation between PEC and MEC when it comes to hospital WW. In contrast, Coetsier et al. (2009) focused on concentrations in STPs and concluded that PEC gives a first approximation, but MEC should be preferred. Verlicchi et al. (2014) revealed high variations between PEC and MEC in surface water. The authors noted that predicted concentrations are just theoretical values. Nevertheless, monitoring campaigns over a long period would become too complex to be feasible.

In this article (i) pharmaceutical usage patterns of APIs in a psychiatric hospital, a nursing home, and a general hospital are presented to determine emitted APIs of concern. Additionally, (ii) the method applicability of a consumption-based approach to identify emitted APIs from HIs was assessed by comparing PEC and MEC. Moreover, (iii) the respective contributions of APIs emitted to WW from such HIs in comparison to households were determined by means of the emission potential I_{EP} (national and regional scales). To meet these objectives, pharmaceutical consumption data of the HIs under consideration for the years 2010, 2011, and 2012 are presented and analyzed.

2. Material and methods

2.1. Calculations

2.1.1. Data sets and study site

In this study, a hospital (HOSP) with three locations (MAIN, OPHT, ORTH) was used to analyze the usage patterns of APIs. HOSP is located in southwestern Germany. With its three locations, it is classified, according to the Federal Statistical Office, as a general hospital (Federal Health Report Germany, 2014c). It is the largest part of a clinic association located in two sub-regions of a county with 400,000 citizens (Fig. 1). 2300 people are employed to treat approximately 30,000 cases each year. MAIN is the biggest location of HOSP and hosts patients with a focus on gastroenterology, general pediatrics, general surgery, nephrology, neurology, obstetrics, radiology, and urology. Data for every consumed pharmaceutical was collected to allow for a comparison to the psychiatric hospital and the nursing home. For the other two HOSP locations, data was collected on the most important 75% of pharmaceuticals in terms of number of prescriptions. The medium-sized location OPHT is located in the same region like MAIN. Its main focus is on cardiology, oncology, ophthalmology, pulmonology, and radio oncology. The smallest location ORTH is geared to orthopedic surgery and orthopedic rheumatology, and contains a small ward for general internal medicine.

The study also investigated the biggest psychiatric hospital (PSY) in the study region with 146 beds and approximately 1500 medical cases per year. PSY provides treatment for adults and juveniles, and it has three departments: psychiatric, psychotherapeutic, and psychosomatic. PSY is classified under the category 'other hospitals with exclusive psychiatric, psychotherapeutic or psychiatric, psychotherapeutic and neurologic beds' (Federal Health Report Germany, 2014d). In addition to a complete list of neurological drugs, data on the most important 75% of pharmaceuticals in terms of number of prescriptions consumed at this institution was collected.

With its 286 inhabitants, the nursing home (NH) under consideration is embedded in the clinic association of HOSP. NH provides care for the elderly, people with psychiatric disorders, and people suffering from alcoholism. In NH, data on every pharmaceutical was collected due to the availability of different raw data.

The data sets for each facility included the units consumed per pharmaceutical (HOSP, PSY) and the number and size of the delivered pharmaceutical boxes (NH) for the years 2010, 2011, and 2012. Data sets were obtained from the hospital pharmacy of HOSP and, in the case of NH, from the delivering public pharmacy. The entire data table comprised 45,343 entries in total.

In Table 1, the corresponding variety of entries per facility is presented together with the information about the capacity, treated cases/inhabitants, and water consumption of the facilities.

Data on the consumption of APIs in households in Germany, as defined daily doses (DDDs), was taken from the annually published report about prescriptions of pharmaceuticals in Germany, the so-called Arzneiverordnungs-Report (AVR) (Schwabe and Paffrath, 2011, 2012, 2013). AVR contains information on all pharmaceuticals prescribed at public expense in doctors' offices in Germany. Hence, it represents the overall consumption of pharmaceuticals for people insured by the statutory health insurance (SHI) in households and in nursing homes.

Two regions were chosen to measure the contribution of APIs. These two regions were classified according to the STP that processes the WW. The schematic constitution of the public sewer system is shown in Fig. 1. In Region 1, 85,000 citizens are connected to the STP. Around six million cubic meters of WW is treated every year at this site. Two facilities of the general hospital HOSP (MAIN and OPHT) and PSY are located in the same region. Region 2, which is adjacent to Region 1, with 13,000 citizens and WW treatment of one million cubic meters per year, is smaller. ORTH and NH are located in this region.
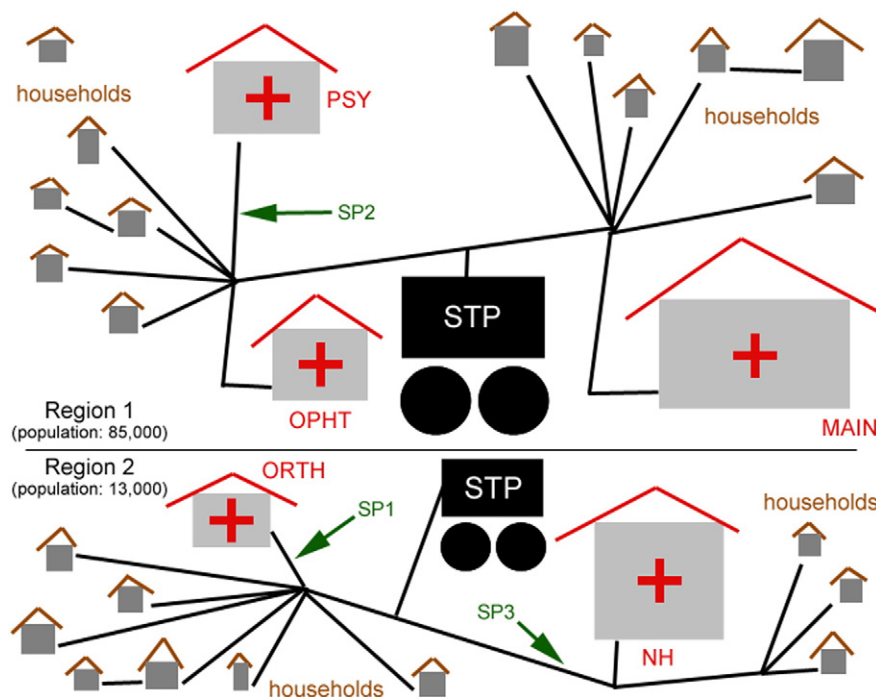
Fig. 1. Schematic diagram of the study regions showing allocated health institutions (HIs) (the general hospital with its three locations (MAIN, OPHT, ORTH), the psychiatric hospital (PSY), and the nursing home (NH)), households, sewage treatment plants (STPs), and sampling points (SPs).

2.1.2. Classification of active pharmaceutical ingredients (APIs), anatomical therapeutical chemical code (ATC Code), and defined daily dose (DDD)

To study the usage patterns of each HI, the APIs were classified according to anatomical therapeutical chemical code (ATC Code) (WHO, 2012a). Other studies have already successfully applied this code (Schuster et al., 2008). Van der Aa et al. (2011), for example, classified the APIs to Level 5 of the ATC Code. Fig. 2 shows the example of metformin, which is classified to Level 5. In this study, APIs were classified to the first Level of the ATC Code to show consumption by facility. A classification to the second Level of ATC Code was used to compare 50 APIs.

V_{DDD}, which can be defined as "the assumed average maintenance dose per day for a drug used for its main indication in adults" (WHO, 2012b), is used to compare given units of APIs. V_{DDD} is defined for each API listed in the ATC Code.

2.1.3. List of selected active pharmaceutical ingredients (APIs)

All pharmaceuticals consumed in the facilities in 2010, 2011, and 2012 were included in the mass balance post processing. The focus of this study was on small molecules (<900 g mol^{-1}). Biopharmaceuticals were excluded because proteins, with their amino acid structure, are completely metabolized in human body and altered by bacteria in the environment. In addition, they are not considered to have a significant negative impact on the environment. Accordingly, herbal medicinal products, vitamins, and natural minerals were also excluded (EMEA, 2006).

For the assessment of usage patterns in the facilities studied here and for the comparison with German households, 50 APIs were selected (Table 2). Selection criteria were mainly determined by existing consumption data. They were not influenced by any effect data existing in literature. Therefore, this approach constitutes to identify newly occurring APIs of concern which have not yet been investigated. At least one of the following selection criteria had to be met:

- Consumption had to exceed one kg per year in at least one HI.
- The API is a highly consumed "representative" of a group of the ATC Code.
- Usage is common in psychiatric hospitals or nursing homes.

2.1.4. Pharmaceutical usage patterns and assessment of active pharmaceutical ingredient (API) emission

The study combined different approaches using different consumption-based criteria to predict the potential emissions of psychiatric hospitals and nursing homes. It also compared these emissions in municipal WW to those of general hospitals and the general population (Fig. 3).

Each pharmaceutical was assigned to its API, and then all units were added to calculate the total amount consumed each year at each HI (Eq. (1)). The APIs were assigned to their anatomical main group

Table 1
Information about the general hospital with its three locations (MAIN, OPHT, ORTH), the psychiatric hospital (PSY), and the nursing home (NH) for 2010, 2011 and 2012: For MAIN, OPHT, ORTH, and PSY, the number of beds and treated cases are shown. For NH, the availability of places for inhabitants and the annual average number of inhabitants are shown. Additionally, entries in raw data table received from the delivering pharmacy and the water consumption are provided.

| Health Institution	Year	Beds/places for inhabitants	Treated cases/average inhabitants	Entries in raw data table	Water consumption (m³)
MAIN	2010	502	23,850	1407	67,863
	2011	502	23,523	1407	61,895
	2012	502	23,202	1443	51,299
OPHT	2010	131	3866	1529	22,164
	2011	131	4488	1572	27,783
	2012	131	4543	1562	16,934
ORTH	2010	108	3203	972	8179
	2011	108	3390	1044	7109
	2012	108	3434	1029	5918
PSY	2010	146	1465	621	7286
	2011	146	n.a.	649	7129
	2012	146	1527	629	7632
NH	2010	286	273	10,784	16,156
	2011	286	270	8642	15,978
	2012	286	271	12,053	14,854
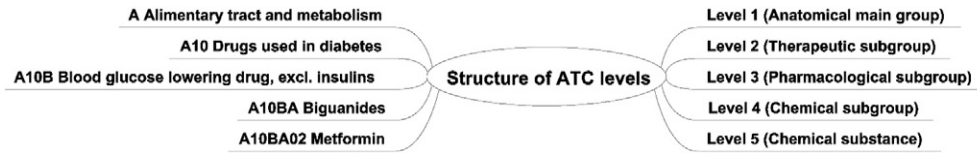
Fig. 2. Levels of the anatomical therapeutical classification (ATC) Code (right side) and an example for the active pharmaceutical ingredient (API) metformin (left side).

(Level 1) and added in the same manner (Eq. (2)), which would then show the API usage patterns of HIs (Fig. 3).

$$A_{local} = m_{unit} \times U \qquad (1)$$

$$A_{group} = \sum A_{local} \qquad (2)$$

A_{local} is the consumption of APIs in the facilities given in kg year^{-1}, m_{unit} is API per pharmaceutical unit given in mg, U is the number of consumed pharmaceutical units, and A_{group} is the API in Level 1 of the ATC Code given in kg year^{-1}.

To study the usage patterns of the APIs (Fig. 3), the consumed amount per bed and inhabitant $A_{bed/inh}$ was calculated as follows:

$$A_{bed/inh} = \frac{A_{local}}{c_{facility}} \qquad (3)$$

A_{local} is the consumption in the facilities given in kg year^{-1}, $c_{facility}$ is the number of beds in hospitals and inhabitants in NH, respectively.

To compare facilities and households in terms of I_{EP} (see Eq. (9)), it is necessary to turn DDD year^{-1} into kg year^{-1} (Eq. (4)).

$$A_{SHI} = DDD_{SHI} \times V_{DDD} \qquad (4)$$

A_{SHI} is the consumed amount in kg year^{-1} of APIs given in AVR, DDD_{SHI} are the doses of a certain API, and V_{DDD} is the average daily dose applied per day in its main indication.

AVR only collects the pharmaceuticals for the 69.7 million members in the German SHI (Federal Ministry of Health, 2013). In 2012, 80.5 million people lived in Germany (Federal Statistical Office DESTATIS, 2014a). The study assumed that the prescription patterns for people with private health insurance is very similar to, if not identical with, the one for people in SHI. Therefore, the consumption of each API investigated in households A_{HD} was calculated as follows (Eq. (5)):

$$A_{HD} = A_{SHI} \times \frac{C_{GER}}{C_{SHI}} \qquad (5)$$

A_{HD} is the consumed amount of APIs in households in Germany given in kg year^{-1}, C_{GER} and C_{SHI} are the citizens of Germany and the people in SHI, respectively.

The extrapolation of pharmaceutical consumption of the HIs investigated here to general hospitals in Germany was based on the treatment of medical cases, because these are indicators for activities at a given hospital (Magee, 2003). In the three locations of HOSP (MAIN, OPHT, ORTH), around 30,000 cases are treated each year (Table 1). According to the Federal Statistical Office, around 18 million cases are treated in general hospitals in Germany (Federal Health Report Germany, 2014a). In PSY, about 1500 cases are treated each year (Table 1). Consumption was calculated based on information provided for hospitals with only psychiatric, psychotherapeutic, or psychiatric, psychotherapeutic, and neurological facilities in Germany (Federal Health Report Germany, 2014a). The following Eq. (6) shows the consumption in either general hospitals or psychiatric hospitals A_{hos} in Germany.

$$A_{hos} = A_{local} \times \frac{Z_{GER}}{Z_{local}} \qquad (6)$$

A_{local} is the consumption in the facilities given in kg year^{-1}, Z_{GER} are the medical cases treated in Germany, and Z_{local} are the medical cases treated in HIs.

To calculate the consumption of nursing homes, the average number of inhabitants living in the NH under consideration and the number of places for the elderly in Germany were used. This data was obtained from the Federal Statistical Office DESTATIS (2013); for example, 743,120 places for long-term care were provided in Germany in 2011.

Based on these numbers, $A_{nurs.home}$ was calculated as follows (Eq. (7)):

$$A_{nurs.home} = A_{local} \times \frac{I_{GER}}{I_{local}} \qquad (7)$$

A_{local} is the consumption in the facilities given in kg year^{-1}, I_{GER} are the places for long-term care provided in Germany, I_{local} is the number of inhabitants living in NH.

The consumption in households of the Study Regions 1 (A_{R1}) and 2 (A_{R2}) (Fig. 1) was calculated as shown below (Eq. (8)):

$$A_{R1,2} = A_{HD} \times \frac{C_{R1,2}}{C_{GER}} \qquad (8)$$

C_R is the population relying on the STP of Study Regions 1 or 2, and C_{GER} is the population of Germany.

To assess the emission potential for the medical facilities, the quotient I_{EP} was calculated for each API, illustrating the relevance of WW discharge (national and regional). I_{EP} assesses the relevance of pharmaceuticals investigated in this study for each facility in terms of consumption. To calculate I_{EP}, the consumption of a facility $A_{facility}$ was divided by the consumption in households $A_{citizens}$ (Eq. (9)). The I_{EP} was calculated for every type of facility (general hospitals, psychiatric hospitals, and nursing homes) separately and compared to German households (national comparison). Additionally, I_{EP} was calculated for the investigated facilities in the study regions and compared to the households in the study regions (regional comparison: comparison Region 1 and comparison Region 2).

$$I_{EP} = \frac{A_{facility}}{A_{citizens}} \qquad (9)$$

If I_{EP} exceeds 1, then more APIs are released by medical facilities than by households, and it is possible to state that these facilities are relevant in terms of WW discharge.

2.1.5. Comparison of predicted wastewater concentration (PWWC) and measured wastewater concentration (MWWC)

A selection of APIs was quantified in the effluents of three facilities (ORTH, PSY, NH), a process that was used to determine MWWC (Section 2.2). PWWCs were calculated for the same six neurological APIs for a period of three years, and the results were given both as absolute values and as ranges (compare Sections 2.1.6 and 2.1.7). Neurological drugs were chosen, because information about their environmental occurrence and effects is scarce. Additionally, they were assumed to be predominantly consumed in PSY and NH. Likewise, the APIs were also chosen due to a high consumption and/or a high excretion rate (f_{nm}).

Table 2
Active pharmaceutical ingredients (APIs) for evaluation of usage patterns and contribution comparison to households.

API	Therapeutic subgroup (Level 2)	Anatomical main group (Level 1)
Pantoprazole	A02 Drugs for acid related disorders	A Alimentary system and metabolism
Mesalazine	A07 Antidiarrheals, intestinal antiinflammatory/antiinfective agents	A Alimentary system and metabolism
Metformin	A10 Drugs used in diabetes	A Alimentary system and metabolism
Acetylsalicylic acid	B01 Antithrombotic agents	B Blood and blood forming agents
Amiodarone	C01 Cardiac therapy	C Cardiovascular system
Furosemide	C03 Diuretics	C Cardiovascular system
Metoprolol	C07 Beta blocking agents	C Cardiovascular system
Valsartan	C09 Agents acting on the renin-angiotensin system	C Cardiovascular system
Simvastatin	C10 Lipid modifying agents	C Cardiovascular system
Cefuroxime	J01 Antibacterials for systemic use	J Antiinfectives for systemic use
Sulfamethoxazole	J01 Antibacterials for systemic use	J Antiinfectives for systemic use
Fluorouracil	L01 Antineoplastic agents	L Antioneoplastic and immunomod. agents
Diclofenac	M01 Antiinflammatory & antirheumatic products	M Musculoskeletal system
Ibuprofen	M01 Antiinflammatory & antirheumatic products	M Musculoskeletal system
Allopurinol	M04 Antigout preparations	M Musculoskeletal system
Lidocaine	N01 Anesthetics	N Nervous system
Mepivacaine	N01 Anesthetics	N Nervous system
Propofol	N01 Anesthetics	N Nervous system
Metamizole	N02 Analgesics	N Nervous system
Paracetamol	N02 Analgesics	N Nervous system
Tilidine	N02 Analgesics	N Nervous system
Tramadol	N02 Analgesics	N Nervous system
Carbamazepine	N03 Antiepileptics	N Nervous system
Eslicarbazepine acetate	N03 Antiepileptics	N Nervous system
Gabapentin	N03 Antiepileptics	N Nervous system
Lamotrigine	N03 Antiepileptics	N Nervous system
Levetiracetam	N03 Antiepileptics	N Nervous system
Oxcarbazepine	N03 Antiepileptics	N Nervous system
Pregabalin	N03 Antiepileptics	N Nervous system
Valproic acid	N03 Antiepileptics	N Nervous system
Entacapone	N04 Anti-parkinson drugs	N Nervous system
Levodopa	N04 Anti-parkinson drugs	N Nervous system
Amisulpride	N05 Psycholeptics	N Nervous system
Chlorprothixene	N05 Psycholeptics	N Nervous system
Clomethiazole	N05 Psycholeptics	N Nervous system
Clozapine	N05 Psycholeptics	N Nervous system
Melperone	N05 Psycholeptics	N Nervous system
Quetiapine	N05 Psycholeptics	N Nervous system
Amitriptyline	N06 Psychoanaleptics	N Nervous system
Bupropion	N06 Psychoanaleptics	N Nervous system
Doxepin	N06 Psychoanaleptics	N Nervous system
Duloxetine	N06 Psychoanaleptics	N Nervous system
Mirtazapine	N06 Psychoanaleptics	N Nervous system
Moclobemide	N06 Psychoanaleptics	N Nervous system
Sertraline	N06 Psychoanaleptics	N Nervous system
Trimipramine	N06 Psychoanaleptics	N Nervous system
Venlafaxine	N06 Psychoanaleptics	N Nervous system
Promethazine	R06 Antihistamines for systemic use	R Respiratory system
Acetazolamide	S01 Ophthalmologicals	S Sensory organs
Iomeprol	V08 Contrast media	V Various

2.1.6. Calculation of predicted wastewater concentration (PWWC)

PWWC for three antiepileptic drugs (gabapentin, levetiracetam, pregabalin), two antipsychotics (amisulpride, quetiapine), and one antidepressant (doxepin) were calculated based on annual consumption data, water consumption, and the excretion rate f_{nm}. For ORTH and PSY, PWWC was calculated with the help of Eq. (10). The annual consumption of the selected APIs in the HIs A_{local} was calculated using Eq. (1). Detailed information concerning f_{nm} was taken from the corresponding summaries of product characteristics and the Micromedex Database (Truven Health Analytics Inc., 2013). The annual water consumption V_{local} is equal to the annual WW amount. PWWC will be calculated as follows:

$$PWWC = \frac{A_{local} \times f_{nm}}{V_{local}} \qquad (10)$$

For NH, PWWC was calculated in a different manner (Eq. (11)). NH is located near the bottom of the western slope of a mid-sized mountain range. The sewer system starts in a suburban village of 800 inhabitants, and NH is located slightly below this village (Fig. 1). Therefore, the total WW amount at the sampling point consists of the effluents produced by 800 inhabitants (c_{SV}), which consume, on average, 121 L of water per day (V_{capita}) (Federal Statistical Office DESTATIS, 2015), and by the inhabitants of NH. PWWC for NH will be calculated as follows:

$$PWWC = \frac{\left(A_{local} + A_{SHI} \times \dfrac{c_{SV}}{c_{SHI}}\right) \times f_{nm}}{V_{local} + V_{capita} \times c_{SV} \times 365} \qquad (11)$$

2.1.7. Sensitivity analysis of predicted wastewater concentration (PWWC)

For the calculation of PWWC one constant value for each variable was defined. These values were calculated mean values from annual data or obtained from literature, respectively (Tables 3 and 4). The calculation results of PWWC with constant parameters can be found in Table 5. In addition, a range for PWWC was calculated to compare it to MWWC (Fig. 8), since parameters from Eqs. (10) and (11) were actually varying. To quantify the influence of the different varying parameters (Tables 3 and 4) on the results of PWWC, a sensitivity analysis similar to Verlicchi et al. (2014) was conducted. Following ranges for variables according to literature and availability of data were set:

- Excretion rate f_{nm}: Due to different findings in literature, a specific range for the excretion rate of each API was given.
- API consumption in HIs A_{local} and API consumption of members in SHI A_{SHI}: As the annual API consumption was varying during the three years, the interval was determined from the lowest to the highest annual consumption.
- Water consumption in HIs V_{local}: The annual water consumption was varying during the three years of consideration. The WW amount was set equal to water consumption given as the highest annual volume and the lowest annual volume consumed in three years.
- Water consumption per capita and day V_{capita}: The individual water consumption is varying from 100 to 350 L (Verlicchi et al., 2014).
- Population in the suburban village c_{sv}: The suburban village does not show high variability concerning number of citizens. Therefore, the population is assumed to vary between -5% and $+5\%$.
- Members of the SHI c_{SHI}: Annual average members of the SHI were varying during the three years of consideration. The interval was determined from the highest to the lowest number of members.

Varying parameters and the respective constant parameters for each variable are shown in Tables 3 and 4. In Section 3.3.3, PWWC ranges are compared to MWWC. Influencing variables on the result of PWWC are reported in Section 3.3.2.
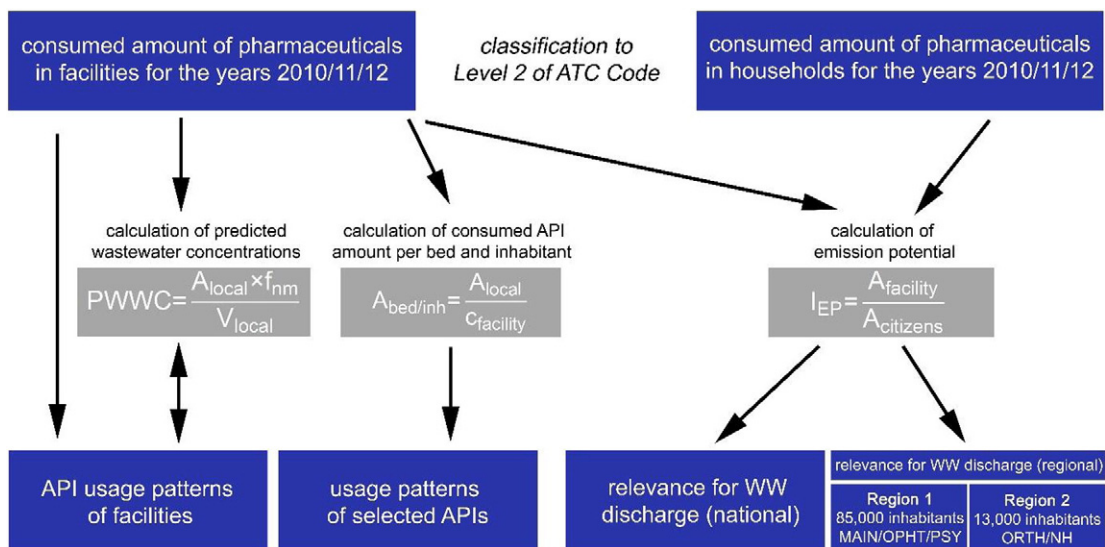
Fig. 3. Evaluation scheme for the significance of active pharmaceutical ingredient (API) emissions to wastewater (WW) discharge.

2.2. Chemical analysis

2.2.1. Chemicals, reagents, and standard mix solution

Amisulpride (purity ≥98%), doxepin hydrochloride (purity ≥98%), gabapentin (certified purity 99.9%), and levetiracetam (purity ≥98%) were purchased from Sigma-Aldrich GmbH (Steinheim, Germany). Pregabalin (certified purity 98.7%) and quetiapine hemifumarate (certified purity 98%) were purchased from LGC Standards GmbH (Wesel, Germany). A standard mix solution was freshly prepared with water/methanol (50/50, v/v) containing 100 mg L^{-1} doxepin and levetiracetam, 34 mg L^{-1} pregabalin and quetiapine, and 10 mg L^{-1} amisulpride and gabapentin.

2.2.2. Sampling, sample preparation, and analytical conditions

Effluent samples of ORTH, PSY, and NH were investigated for three different seasons of the year. At every facility, time-proportional 24 h-mixed samples were taken similar to the studies of Gómez et al. (2006) and Oliveira et al. (2015). In ORTH, one sample was collected manually at the main sewer (Fig. 1) in June 2012, November 2012, and April 2013. In the same way, samples were taken for PSY in June 2012, December 2012, and July 2013. In the case of NH, samples were collected at a mixed sewer system (Fig. 1, Section 2.1.5) in November 2012, March 2013, and August 2013, respectively. A main sewer is not available at NH, as NH consists of seven buildings each directly discharging to the public sewer system. Sampling points are shown in Fig. 1. A sample of 300 mL of effluent was taken every 2 h resulting in a total amount of 3600 mL. During sampling, the samples were kept at 2 to 8 °C and stored at −80 °C until sample preparation. No significant rain event occurred during the sampling day and the day before the sampling day. Dry days for sampling were chosen to avoid dilution in the sewer system during rainfall.

To prepare the samples, the standard addition method with a four-point calibration was used to compensate varying matrix effects. 40 mL of a given sample were subsequently spiked with 30 μL, 60 μL, and 90 μL of standard mix solution, respectively. Solid phase extraction, after filtration through a fluted filter, was conducted using a MN Chromabond® HR-XC 6 mL/200 mg (Macherey-Nagel, Düren,

Table 3
For active pharmaceutical ingredients (APIs), values (ranges shown in parentheses) used in the sensitivity analysis of predicted wastewater concentration (PWWC) for the following variables: Excretion rate f_{nm}, consumption in health institutions (HIs) A_{local}, and consumption for members in the statutory health insurance (SHI) A_{SHI}.

API	f_{nm}	A_{local} (kg year^{-1})			A_{SHI} (t year^{-1})
		ORTH	PSY	NH	
Pregabalin	0.94[a,b]	0.16	0.82	1.08	17.98[h]
	(0.90–0.98)[a,b]	(0.16–0.17)	(0.37–1.21)	(0.82–1.28)	(16.29–19.65)[h]
Gabapentin	1.00[a,c]	0.28	0.11	1.41	68.16[h]
		(0.21–0.35)	(0.05–0.14)	(0.97–2.08)	(62.82–73.26)[h]
Levetiracetam	0.70[a,d]	0.49	0.49	2.85	64.15[h]
	(0.66–0.73)[a,d]	(0.25–0.71)	(0.28–0.83)	(1.87–4.06)	(55.80–72.00)[h]
Amisulpride	0.84[a,e]	0.00	0.70	0.00	3.08[h]
	(0.70–0.98)[a,e]	(0.00–0.01)	(0.54–1.01)	(0.00–0.00)	(2.68–3.56)[h]
Doxepin	0.02[a,f]	0.01	0.05	0.18	5.16[h]
	(0.00–0.03)[a,f]	(0.00–0.02)	(0.02–0.08)	(0.14–0.21)	(4.98–5.34)[h]
Quetiapine	0.03[a,g]	0.17	3.79	8.70	17.36[h]
	(0.00–0.05)[a,g]	(0.11–0.20)	(3.14–4.40)	(6.93–10.40)	(15.84–18.48)[h]

[a] Truven Health Analytics Inc. (2013).
[b] Pfizer, Inc. (2013).
[c] Pfizer, Inc. (2015).
[d] UCB, Inc. (2013).
[e] Ratiopharm GmbH (2010).
[f] Cheplapharm Arzneimittel GmbH (2011).
[g] AstraZeneca Pharmaceuticals (2013).
[h] Schwabe and Paffrath (2011, 2012, 2013).

Table 4

Values (ranges shown in parentheses) used in the sensitivity analysis of predicted wastewater concentration (PWWC) for the following variables: Water consumption in HIs V_{local}, per capita and day water consumption in the suburban village V_{capita}, members of the SHI c_{SHI}, and the population of the suburban village c_{SV}.

V_{local} (m^3)			V_{capita} (L)	c_{SHI}	c_{SV}
ORTH	PSY	NH			
7,069	7,349	15,663	121[a]	69,714,945[c,e]	800
(5918–8179)	(7129–7632)	(14,854–16,156)	(100–350)[b]	(69,637,277–69,803,236)[d,e]	(760–840)

[a] Federal Statistical Office DESTATIS (2015).
[b] Verlicchi et al. (2014).
[c] Federal Ministry of Health (2011).
[d] Federal Ministry of Health (2012).
[e] Federal Ministry of Health (2013).

Germany) strong mixed-mode cation exchanger. After extraction, the leachate was dried under a gentle nitrogen stream and reconstituted in 400 μL of aqueous ammonium acetate 10 mM/methanol (50/50, v/v).

Samples were analyzed with an Agilent 1100 series HPLC system (Agilent Technologies, Waldbronn, Germany) coupled to a Bruker Esquire 6000^{plus} ion trap mass spectrometer (Bruker Daltonics, Bremen, Germany). Elution was performed on an MN Nucleodur® RP-phenyl-hexyl column (EC 125/3 mm, 100–3 μm) protected by a MN Nucleodur® RP-phenyl-hexyl guard column (EC 4/3 mm, 100–3 μm) (Macherey-Nagel, Düren, Germany). Binary mobile phase consisted of 10 mM ammonium acetate in ultrapure water (A) and methanol (B). The flow rate was 0.4 mL min^{-1}, and the oven temperature 30 °C. Gradient was as follows: 0 min: 10% B, 4 min 10% B, 15 min 90% B, 21 min 90% B, 25 min 10% B, and 28 min 10% B. The injected sample volume was 5 μL. Target compounds were measured in positive MRM mode using the highest abundant amplitude for fragmentation. The highest product ion was used for quantification. The method detection limits (MDL) and method quantification limits (MQL) were determined in ultrapure water with a signal-to-noise ratio of three and ten, respectively (see Fig. 8).

2.2.3. Uncertainty analysis of measured wastewater concentration (MWWC)

An uncertainty analysis regarding the chemical analysis procedure of the APIs was carried out. The total uncertainty was calculated from the recovery of the APIs in WW ($U_{recovery}$) and the precision of the analytical instrument ($U_{precision}$) (Eq. (12)). The relative recovery was determined from the recoveries of one spiked sample in each WW matrix (n = 3). To determine the precision, the inter-day instrumental precision of the analytical instrument was used (three injections of standard at 1 mg L^{-1}).

$$U_{total} = \sqrt{U_{recovery}^2 + U_{precision}^2} \qquad (12)$$

Table 5

Predicted wastewater concentrations (PWWCs) calculated with constant values for one location of the general hospital (ORTH), the psychiatric hospital (PSY), and the nursing home (NH).

API	PWWC (ORTH) (μg L^{-1})	PWWC (PSY) (μg L^{-1})	PWWC (NH) (μg L^{-1})
Pregabalin	21.6	104.4	23.8
Gabapentin	40.1	14.7	43.0
Levetiracetam	48.2	46.1	48.8
Amisulpride	0.4	80.4	0.6
Doxepin	0.0	0.1	0.1
Quetiapine	0.6	12.9	4.4

3. Results and discussion

3.1. Mass balance of consumed active pharmaceutical ingredients (APIs) by anatomical chemical therapeutical code (ATC Code) classes

The basic mass balance study of the average pharmaceutical consumption for the years 2010, 2011, and 2012, including standard deviation in the locations of HOSP (MAIN, OPHT, ORTH), PSY, and NH, is summarized in Fig. 4. The average annual consumption is presented according to ATC Code Level 1 (Eq. (2)).

The consumption of all APIs in the locations of HOSP was 1060.6 ± 36.0 kg year^{-1} (MAIN), 85.5 ± 20.0 kg year^{-1} (OPHT), and 117.1 ± 2.8 kg year^{-1} (ORTH). In MAIN, the consumption increased by 53.5 kg during the three years under consideration. In OPHT, the consumption decreased by approximately the same amount of 45.2 kg in the same period. These developments corresponded to the fact that some medical departments were moved from one location to another. The mass balance in HOSP showed values between almost no consumption (e.g. ATC Level P, MAIN) and 540.7 ± 21.2 kg year^{-1} (ATC Level V, MAIN). 14 out of 14 ATC Levels are covered by MAIN, 10 ATC Levels by OPHT, and 9 ATC Levels by ORTH.

MAIN showed the highest consumption of 540.7 ± 21.2 kg year^{-1} (approximately 50% of the total in MAIN) in group various (V), which includes contrast agents (V08), which most likely have been used in the radiological department. Escher et al. (2011) also identified X-ray contrast media with 58% as the most important APIs emitted from a general hospital. Even though contrast agents are primarily administered at hospitals, they are not necessarily emitted by these institutions. Weissbrodt et al. (2009) determined that 51% of the applied APIs were excreted off-site. The group nervous system (N) with 223.1 ± 9.0 kg year^{-1} is followed by antiinfectives (J) with 206.8 ± 10.3 kg year^{-1}. The contributions for the second level of ATC (groups J, M and N) are shown in Fig. 5a. In group N, analgesics (N02), which are typically used for pain relief after surgery, are the most important. Antibacterials (J01) is the main contributing group for antiinfectives (J), which are used to prevent or to treat bacterial infections. Le Corre et al. (2012) also identified antibiotics as a pharmaceutical class that is consumed to a great extent in hospitals. APIs from other ATC groups show values below 30 kg year^{-1}. Thus, they were consumed to a significant lower amount.

The highest consumption of APIs in OPHT was found for the group of antiinfectives (J) with 50.4 ± 10.5 kg year^{-1}, nervous system (N) with 20.3 ± 6.9 kg year^{-1}, and musculoskeletal system (M) with 9.2 ± 3.9 kg year^{-1}. APIs from other ATC groups were lower than 2 kg year^{-1}. On the second ATC Level for group J, mainly antibacterials (J01) were represented. In group N, analgesics (N02), and for group M, anti-inflammatory and antirheumatic products (M01) (Fig. 5a) were predominantly consumed. Therefore, OPHT showed, with the possible exception of contrast agents, a similar distribution of APIs like MAIN.

ORTH showed the highest consumption for the group nervous system (N) with 64.2 ± 5.2 kg year^{-1}, antiinfectives (J) with 38.0 ± 2.0 kg year^{-1}, and musculoskeletal system (M) with 8.1 ± 1.1 kg year^{-1}. APIs from other ATC groups contributed 4 kg year^{-1} and lower. The
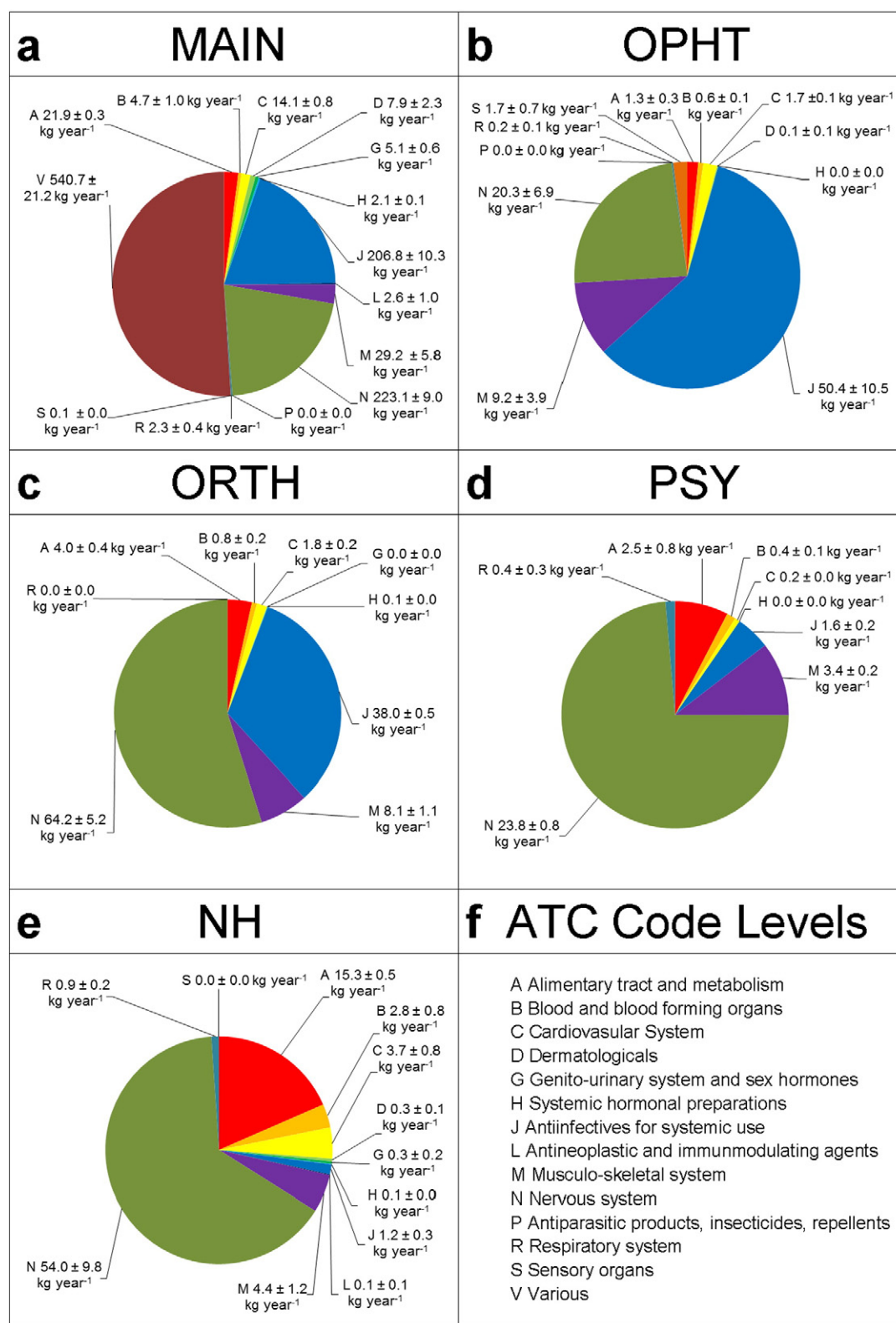
Fig. 4. Average annual consumption of groups of active pharmaceutical ingredients (APIs) according to anatomical therapeutical chemical (ATC) Code Level 1 within 2010 and 2012 for the general hospital with its three locations (MAIN, OPHT, ORTH), the psychiatric hospital (PSY), and the nursing home (NH) in kg year^{-1} with corresponding standard deviation.

comparably high consumption of analgesics (N02) (shown in Fig. 5a) was due to the focus on orthopedics in this location.

Considering results in all three locations of HOSP, it becomes obvious that group antiinfectives (J), nervous system (N), and musculoskeletal (M) had the highest consumption in terms of on-site consumed APIs. A closer look at the second ATC Level shows that antibacterials (J01),

anti-inflammatory drugs (M01), and analgesics (N02) were the most relevant groups of pharmaceuticals in general hospitals. Other studies have identified similar usage patterns for general hospitals (Le Corre et al., 2012; Schuster et al., 2008).

On average, 32.3 ± 1.0 kg year^{-1} of APIs were consumed in PSY. These findings are similar to those of a study of a psychiatric center
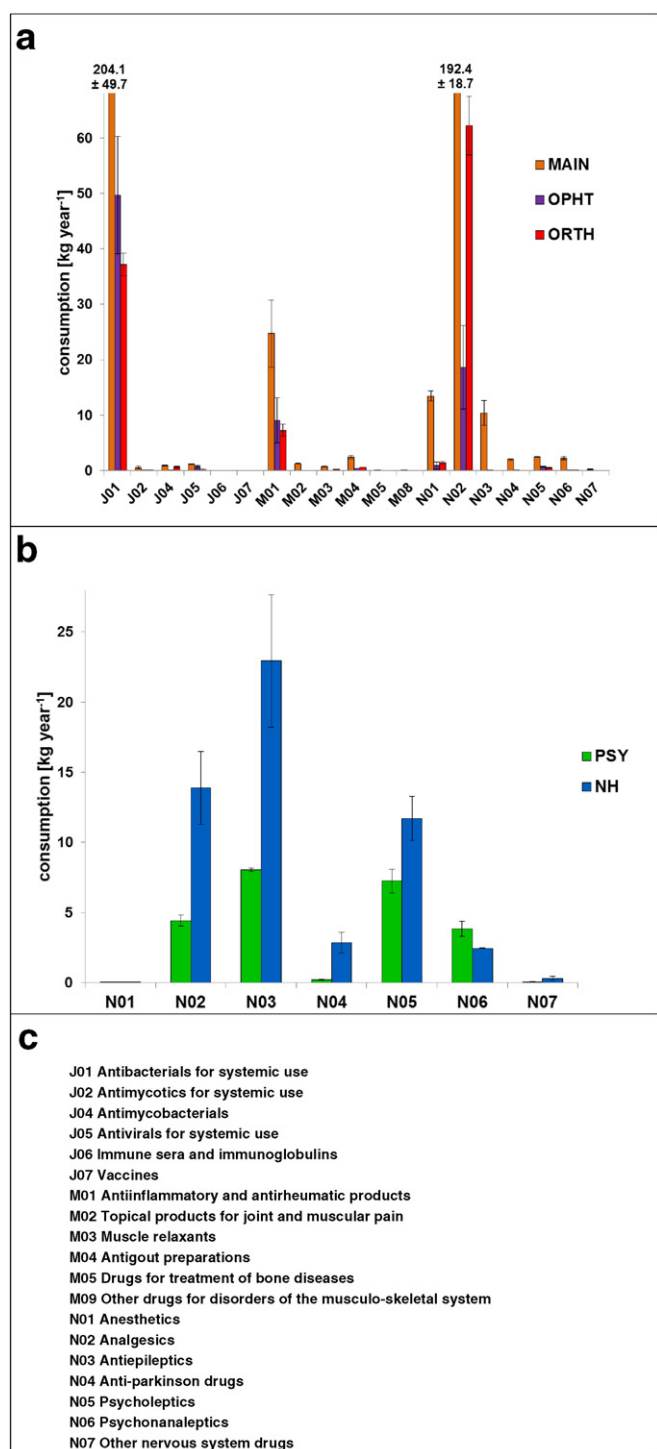
Fig. 5. Average annual consumption of highest consumed groups classified in anatomical therapeutical chemical (ATC) Code Level 2 in the three locations of the general hospital (MAIN, OPHT, ORTH), the psychiatric hospital (PSY), and the nursing home (NH) within 2010 and 2012.

with 211 beds, which showed that 52 kg of APIs were consumed in Switzerland in 2007 (Escher et al., 2011). The distribution of groups consumed at PSY is shown in Fig. 4d.

APIs acting on the nervous system (N) showed the highest consumption (23.8 ± 0.8 kg year^{-1}). These findings differ from those of Escher et al. (2011), who identified laxatives, analgesics, and antidiabetics as the primary APIs emitted by a psychiatric center. This can be due to a different methodological approach. For the mass balance, only 75% of the most important APIs in terms of number of prescriptions

were included in the present study. Additionally, only chemically defined small molecules were included for post processing of data (see Section 2.1.3). Therefore, polymeric laxatives like macrogol, usually consumed in high amounts, would be excluded in our conducted mass balance. Moreover, non-steroidal anti-inflammatory drugs and analgesics (M01 and N02, 7.8 ± 0.2 kg year^{-1}), antiinfectives (J, 1.6 ± 0.2 kg year^{-1}), and APIs affecting the cardiovascular system (C, 0.2 ± 0.0 kg year^{-1}) were rarely applied.

In the group nervous system, antiepileptics (N03, 8.1 ± 0.1 kg year^{-1}) were, in terms of consumption, most significant (Fig. 5b). In general, they are used to treat epilepsy, but some substances such as gabapentin, pregabalin, or valproic acid are also used to treat bipolar disorders or neuropathic pain. For this reason, they have also been used in psychiatric hospitals. Psycholeptics (N05) were consumed more frequently than psychoanaleptics (N06). This demonstrates that antipsychotics, tranquilizers, and hypnotics (N05) are more important than antidepressants (N06), because people suffering from depression are, in general, treated more often as outpatients.

The overall average API consumption in NH was 83.0 ± 13.5 kg year^{-1}. The APIs with the highest consumption rate have an effect on the nervous system (N, 54.0 ± 9.8 kg year^{-1}) (Fig. 4e). In addition, there was a high consumption of drugs affecting the alimentary system and metabolism (A, 15.3 ± 0.5 kg year^{-1}). PSY has a similar consumption pattern. Less important include painkillers (M01 and N02, 16.2 ± 2.6 kg year^{-1}), substances affecting the cardiovascular system (C, 3.7 ± 0.8 kg year^{-1}), and antibiotics (J, 1.2 ± 0.3 kg year^{-1}).

In terms of consumption, antiepileptics (A03, 22.9 ± 4.7 kg/year) were the most significant (Fig. 5b) in the group nervous system. Moreover, psycholeptics (A05) were the APIs with the third highest consumption and, therefore, highly significant pollutants from NH. It is important to note here that only approximately 75% of the inhabitants in the facility are older than 60 years. In 2011, around 95% of people living in German nursing homes were older than 60 years (Federal Statistical Office DESTATIS, 2013). The lower average age of the inhabitants could be explained by the psychiatric focus of NH. This could also explain the fact that the consumption of neurologicals for the treatment of psychiatric diseases is high compared to other nursing homes.

The comparison by ATC classes allows for the identification of the most important groups in terms of consumption at each facility. One major problem in this respect is the missing distinction between analgesics (N02) and the other neurologicals for the treatment of epilepsy (N03 antiepileptics) and psychiatric disorders (N05 psycholeptics and N06 psychoanaleptics). Therefore, the investigation of the second Level of ATC was necessary. Pie charts show the distribution of pharmaceutical groups, which were characteristic for facilities investigated here. The results showed a similar distribution of APIs in every year for every facility.

3.2. Usage patterns of selected active pharmaceutical ingredients (APIs) in the facilities

Based on the selection criteria introduced in Section 2.1.3, the consumption of 50 APIs in each facility were compared to identify the different usage patterns of the different types of HIs. To account for the differences in terms of size, the average consumed amount of APIs per year as well as bed or inhabitant $A_{bed/inh}$ was calculated according to Eq. (3).

Fig. 6 shows the results for all facilities studied here. It indicates that at every location of the general hospital, metamizole (N02) was, in terms of amount consumed per bed, the most significant (MAIN: 317 ± 10 g bed^{-1} year^{-1}, OPHT: 132 ± 54 g bed^{-1} year^{-1}, ORTH: 494 ± 39 g bed^{-1} year^{-1}). The use of metamizole is frequently discussed in international medical science (Hamerschlak and Cavalcanti, 2005; Hedenmalm and Spigset, 2002; Huber et al., 2014), because of its most problematic side effect, agranulocytosis. Metamizole is even banned from the US market, and therefore, a generally high
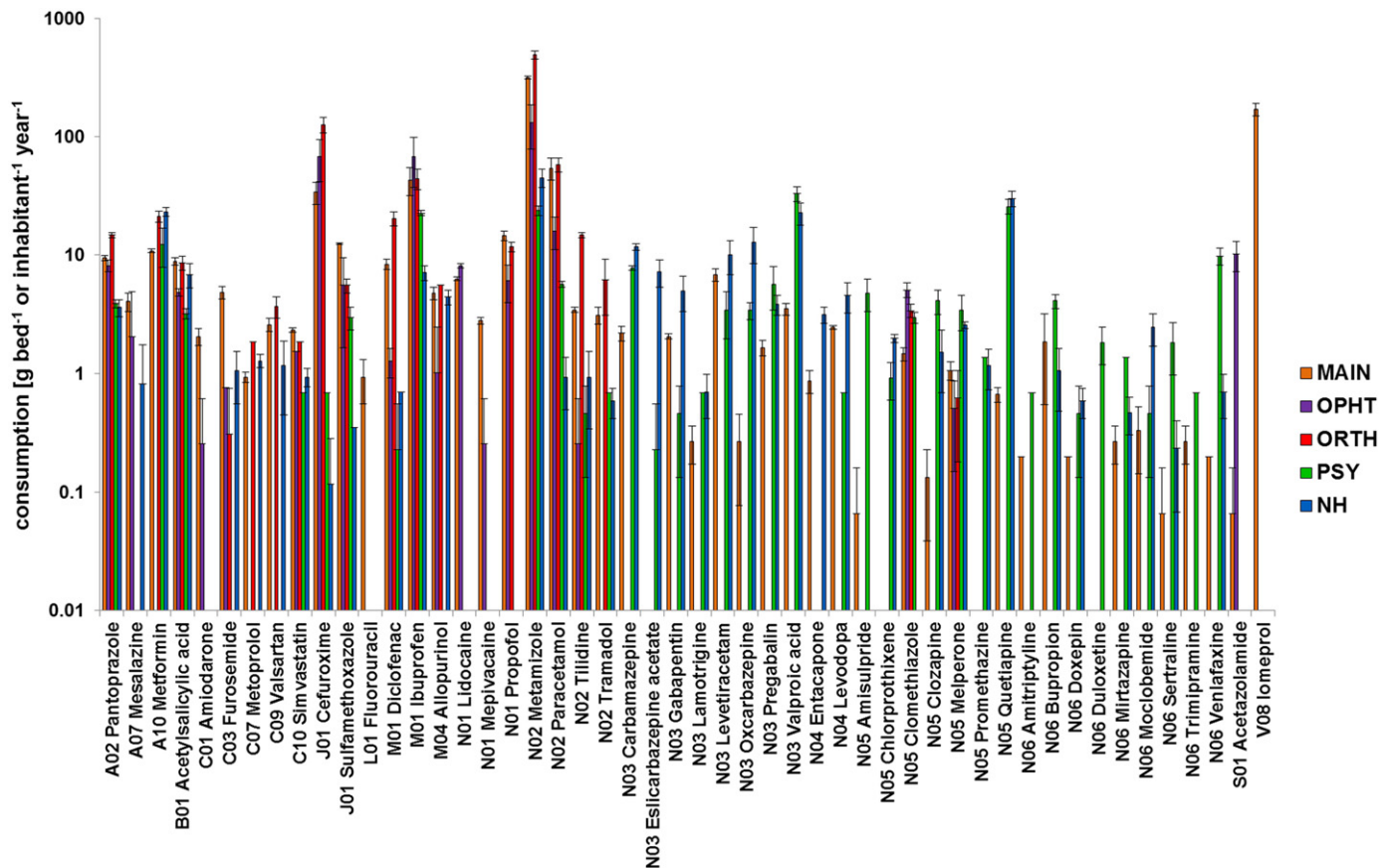
Fig. 6. Annual average consumption of APIs in the three locations of the general hospital (MAIN, OPHT, ORTH), the psychiatric hospital (PSY), and the nursing home (NH).

consumption in hospitals in all countries is very unlikely. The results for the general hospital show that other painkillers such as paracetamol, ibuprofen, or diclofenac as single APIs were consumed in lower amounts. The highest consumption of painkillers, including the four APIs mentioned above, per bed is found in ORTH (MAIN: 422 ± 32 g bed^{-1} year^{-1}, OPHT: 218 ± 90 g bed^{-1} year^{-1}, ORTH: 617 ± 44 g bed^{-1} year^{-1}), which includes the orthopedic department. The four painkillers were consumed to a lesser extent at NH and PSY (PSY: 52 ± 1 g bed^{-1} year^{-1}; NH: 54 ± 9 g inhabitant^{-1} year^{-1}).

The antibiotics cefuroxime and sulfamethoxazole are insignificant due to very low consumption: 0.7 g bed^{-1} year^{-1} and 0.1 g bed^{-1} year^{-1}, as well as 3.0 g inhabitant^{-1} year^{-1} and 0.3 g inhabitant^{-1} year^{-1} in PSY and NH, respectively. These numbers are very low compared to the high amounts typically applied in HOSP (cefuroxime: MAIN: 34.0 ± 0.7 g bed^{-1} year^{-1}, OPHT: 68.5 ± 26.6 g bed^{-1} year^{-1}, ORTH: 126.5 ± 18.1 g bed^{-1} year^{-1}). Patients in psychiatric clinics and inhabitants in nursing homes do not undergo any surgeries and are not hospitalized because of infections. Thus, antibiotics are rarely applied there.

In PSY and NH, most of the drugs that are applied are neurologicals like antiepileptics (N03), psycholeptics (N05), and psychoanaleptics (N06) (Fig. 5b). Similar patterns was found for the consumption of APIs (Fig. 6).

In PSY, the anticonvulsant valproic acid was identified and with 33.1 ± 4.8 g bed^{-1} year^{-1}, it was the most significant API in terms of amount consumed. In MAIN, with 3.5 ± 0.4 g bed^{-1} year^{-1}, the amount consumed was ten times lower. The antipsychotic quetiapine, with 25.8 ± 3.6 g bed^{-1} year^{-1}, and the painkiller metamizole, with 24.7 ± 2.4 g bed^{-1} year^{-1}, are ranked second and third. The painkiller ibuprofen, with 22.6 ± 1.1 g bed^{-1} year^{-1}, and the antidiabetic

metformin, with 12.3 ± 4.5 g bed^{-1} year^{-1}, are ranked fourth and fifth. Other APIs applied in lower amounts were mostly psycholeptics (N05), antiepileptics (N03), and psycholeptics (N05) (Fig. 6).

In terms of distribution, NH and PSY were similar. Although metamizole was, in terms of amount consumed, the most significant, quetiapine (30.1 ± 4.6 g inhabitant^{-1} year^{-1}), valproic acid (22.7 ± 4.9 g inhabitant^{-1} year^{-1}), and other neurologicals such as antiepileptics and psycholeptics were more important.

The results showed that in NH and PSY, neurologicals (group N) were, in terms of amount consumed, the most significant. In particular, quetiapine was consumed in high amounts. To our knowledge, there is no study that assesses the environmental behavior of quetiapine. Yuan et al. (2013) showed that there is a high primary elimination in a STP. The anticonvulsant valproic acid is also consumed in high amounts, but many studies indicate that it is readily biodegradable (Bergheim and Kümmerer, 2007; Yu et al., 2006) and almost completely biologically removed from WW (Onesios and Bouwer, 2012; Yu et al., 2012). Carbamazepine, oxcarbazepine, and eslicarbazepine acetate, as structural relatives, can also be traced in the environment. Carbamazepine showed persistence after irradiation with sunlight (Matamoros et al., 2009) and has frequently been detected in the environment (Capdeville and Budzinski, 2011; Singh et al., 2014). For this reason, it has been used as an environmental tracer (Gasser et al., 2011). With regard to the highly consumed anticonvulsants pregabalin, levetiracetam, and gabapentin, there is a lack of knowledge concerning their respective environmental behaviors. For gabapentin, studies about UV treatment, ozonation, activated carbon treatment, and elimination in STPs are available, but results about the elimination are not consistent (Reungoat et al., 2010a; Yuan et al., 2013), and further investigations are needed to determine the environmental impact of gabapentin.

Table 6
Mean measured wastewater concentrations (MWWC) and the corresponding number of detections (n) higher than method quantification limit (MQL) for the selected active pharmaceutical ingredients (APIs) in the effluent of one location of the general hospital (ORTH), the psychiatric hospital (PSY), and the nursing home (NH). Additionally, the uncertainty of the chemical analysis for APIs is given (68% confidence interval).

API	ORTH		PSY		NH		$U_{recovery}$	$U_{precision}$	U_{total}
	MWWC ($\mu g\ L^{-1}$)	n	MWWC ($\mu g\ L^{-1}$)	n	MWWC ($\mu g\ L^{-1}$)	n			
Pregabalin	217.0 ± 277.0	3	345.9 ± 309.4	3	146.0 ± 137.3	3	5	3	6
Gabapentin	9.4	1	<MDL	0	4.3 ± 1.4	3	9	3	9
Levetiracetam	13.3 ± 11.6	2	107.6 ± 3.3	2	72.5 ± 32.7	3	22	2	22
Amisulpride	<MDL	0	26.1 ± 11.2	3	2.4 ± 0.4	2	5	1	5
Doxepin	<MDL	0	<MDL	0	9.1	1	24	1	24
Quetiapine	<MDL	0	15.9 ± 13.5	3	5.3 ± 1.2	3	7	4	8
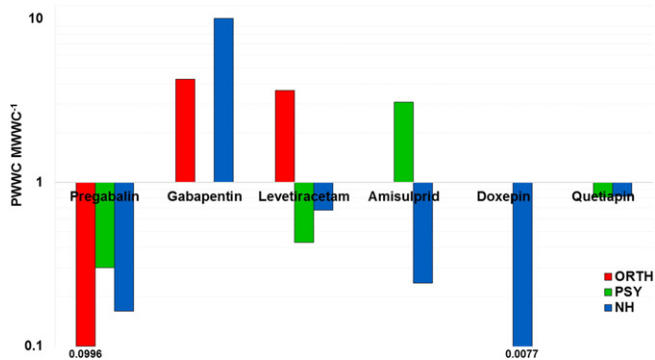
Fig. 7. Accompaniment of predicted wastewater concentration (PWWC) and measured wastewater concentration (MWWC) by means of PWWC MWWC^{-1} for the six investigated active pharmaceutical ingredients (APIs) pregabalin, gabapentin, levetiracetam, amisulpride, doxepin, and quetiapine in the location of the general hospital with orthopedic focus (ORTH), the psychiatric hospital (PSY), and the nursing home (NH). PWWC was calculated by means of Eqs. (10) and (11), respectively, with constant values for variables defined in Table 3. For MWWC, the average of three samples was used.

3.3. Comparison of predicted wastewater concentration (PWWC) and measured wastewater concentration (MWWC)

3.3.1. PWWC MWWC^{-1} ratios

Average MWWCs, with corresponding uncertainty of the analysis, are shown in Table 6. Results were evaluated based on PWWC MWWC^{-1} ratios (Fig. 7). Similar approaches were followed by other studies (Coetsier et al., 2009; Ortiz de García et al., 2013; Verlicchi et al., 2014). The authors calculated PEC MEC^{-1} ratios in effluent of STPs or surface water.

In this study, the ratios ranged mainly from 0.1 to 10, except for doxepin and pregabalin. Morasch et al. (2010) also showed MEC PEC^{-1} correlation within one order of magnitude for 21 APIs in the influent and effluent of a STP. On the other hand, Verlicchi et al. (2014) demonstrated strong underestimation of more than one order of magnitude for the measured concentrations of three antibiotics in the influent and effluent of a STP.

In general, pregabalin was underestimated in every facility (Fig. 7). In contrast to pregabalin, gabapentin was overestimated in every facility. In comparison, Morasch et al. (2010) revealed good MEC PEC^{-1} for gabapentin at the influent of a STP. Because of the high stability of gabapentin (Reungoat et al., 2010b), a transformation of gabapentin in WW is unlikely. For levetiracetam and amisulpride, slight overestimation and underestimation, respectively, were demonstrated. Doxepin was predicted in very low concentrations, which were not covered by the determined MDL and corresponding MQL. Quetiapine showed very good correlation for every HI.

3.3.2. Influencing variables on the result of predicted wastewater concentrations (PWWCs)

A sensitivity analysis was carried out to assess the influence of each varying parameter from Eqs. (10) and (11) on the result of PWWC. The influence was calculated by changing one parameter in its defined range. At the same time, the other variables were to set the defined constant value (Tables 3 and 4). The deviation for each variable from PWWC, calculated with constant values (Table 5), is shown in Table 7. Variations for excretion rate f_{nm}, consumption of APIs in the facility A_{local}, and the water consumption in the facilities V_{local} had an influence on PWWC in all the three facilities. API consumption for members in SHI A_{SHI}, the water consumption per capita and day V_{capita}, the number of members in SHI c_{SHI}, and the population in the suburban village c_{SV} only influence PWWC in NH.

As the excretion factor f_{nm} is specific for each respective API, the influence on PWWC in each HI is the same. f_{nm} for doxepin and quetiapine is very low down to zero. As a result, individual metabolic variations have a high influence on the result of PWWC. For antiepileptic drugs pregabalin, gabapentin, and levetiracetam f_{nm} is comparably high, and therefore, subject to low variations. Likewise, the influence on PWWC is comparably low.

In ORTH and PSY, PWWC is mainly affected by the consumption of APIs in the facilities. Additionally, in ORTH, PWWC is influenced by the local water consumption V_{local}. This was due to water savings during years of consideration. In PSY and NH, the impact of local water consumption V_{local} was comparably low, because it remained constant during years (Table 1). In contrast, for NH, the water consumption in the suburban village V_{capita} had a very high influence on PWWC.

It can be said that the excretion rate and the local consumption of APIs can have a high influence on PWWC. Additionally, annual API consumption in households has a high influence on the results of PWWC.

Table 7
Minimum and maximum percentage deviation from predicted wastewater concentration (PWWC) (Table 5) due to changing one variable in its defined range (Tables 3 and 4).

| API	f_{nm}	A_{local}			A_{SHI}	V_{local}			V_{capita}	c_{SHI}	c_{SV}
		ORTH	PSY	NH		ORTH	PSY	NH			
Pregabalin	−4/+4	−3/+4	−54/+48	−21/+15	−2/+1	−19/+14	−3/+4	−2/+1	−14/+57	0/0	−3/+3
Gabapentin	0/0	−26/+24	−56/+31	−20/+30	−3/0	−19/+14	−3/+4	−2/+1	−14/+57	0/0	−2/+2
Levetiracetam	−5/+5	−49/+45	−43/+70	−27/+34	−3/0	−19/+14	−3/+4	−2/+1	−14/+57	0/0	−3/+2
Amisulpride	−17/+17	−100/+200	−23/+44	−/−	−13/+16	−19/+14	−3/+4	−2/+1	−14/+57	0/0	−2/+1
Doxepin	−100/+100	−86/+78	−54/+49	−15/+12	−1/+1	−19/+14	−3/+4	−2/+1	−14/+57	0/0	−2/+2
Quetiapine	−100/+100	−32/+13	−17/+16	−20/+19	0/0	−19/+14	−3/+4	−2/+1	−14/+57	0/0	−3/+3
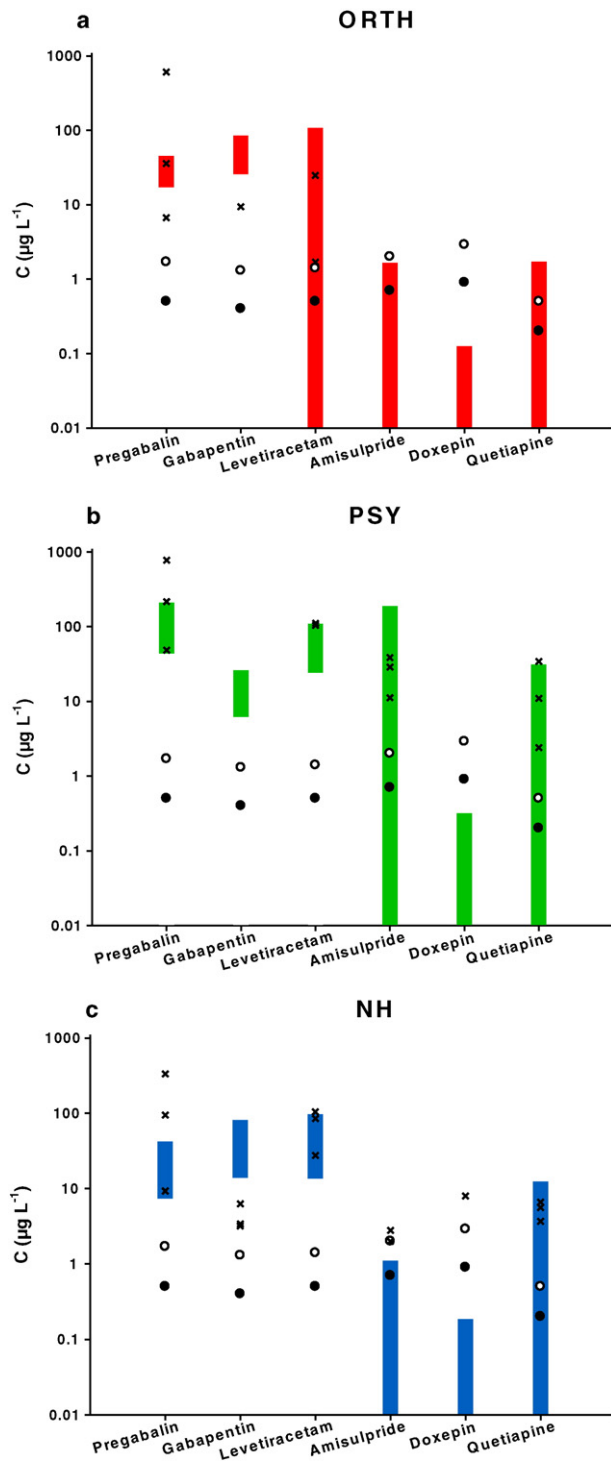
Fig. 8. Accompaniment of predicted wastewater concentration (PWWC) and measured wastewater concentration (MWWC) in the location of the general hospital with the orthopedic focus (ORTH), the psychiatric hospital (PSY), and the nursing home (NH). PWWC range, given from different varying parameters during years under consideration, is shown as bars. MWWC of samples are shown as (×). Method detection limit (MDL) is shown as (●). Method quantification limit (MQL) is shown as (○).

3.3.3. Comparison of predicted range with measured concentrations

With Eqs. (10) and (11) and corresponding varying parameters, obtained from Tables 3 and 4, a range for PWWC was calculated. The comparison to MWWC is shown in Fig. 8.

In ORTH, only the anticonvulsants pregabalin, gabapentin, and levetiracetam were detected. Pregabalin was detected three times. Once it was detected within the range of PWWC with 35.9 µg L^{-1}, once it was overestimated by 158% (MWWC = 6.7 µg L^{-1}), and once it was underestimated by 2065% (MWWC = 608.4 µg L^{-1}) by the PWWC range. Although for gabapentin, a range from 25.8 to 59.1 µg L^{-1} was predicted, it was detected only once. In addition, the MWWC of gabapentin with 9.4 µg L^{-1} was lower than the range of PWWC (174% overestimated). Levetiracetam was determined at the lower edge of PWWC range (MWWC = 24.8 µg L^{-1}). In addition, it was detected in very low amounts (MWWC = 1.7 µg L^{-1}). Moreover, the two antipsychotics amisulpride and quetiapine, and the antidepressant doxepin were consumed in very low amounts and as a result not detected in any sample.

In PSY, pregabalin, levetiracetam, and quetiapine were measured in amounts similar to those predicted. Only pregabalin was two times underestimated by 31% (MWWC = 216.8 µg L^{-1}) and 365% (MWWC = 772.5 µg L^{-1}). Pregabalin was once detected within the range of PWWC. In contrast, the three measured concentrations of amisulpride were all overestimated (29%, 73%, and 341%). Considering that the concentration predicted for doxepin was comparably low (up to 0.3 µg L^{-1}), it is not surprising that this API could not be measured in a single sample in PSY. On the other hand, there is no explanation why gabapentin was not detected in a single sample (PWWC up to 19.8 µg L^{-1}).

The samples taken at NH showed a good correlation for PWWC and MWWC for levetiracetam, and quetiapine. The three measured concentrations for pregabalin were, however, too high twice (MWWC = 95.0 µg L^{-1} and 333.8 µg L^{-1}), and once within the predicted range (MWWC = 9.3 µg L^{-1}). In contrast, gabapentin was measured in lower concentrations than predicted (MWWC = 3.2 µg L^{-1}, 3.4 µg L^{-1}, and 6.3 µg L^{-1}), and was, therefore, overestimated by 337%, 311%, and 122%, respectively. All measured concentrations for amisulpride and doxepin were underestimated.

3.3.4. Discrepancies, bias, and uncertainties

Ideally, predicted concentrations in WW, calculated based on accurate information concerning drug and water consumption as well as excretion rates, should be identical with measured results. In the present study, PWWC and MWWC showed similar patterns. Nevertheless, differences between the two approaches regarding resulting API concentrations were observed. As the calculated uncertainty was low (Table 6), these differences cannot be justified by the uncertainty of the chemical analysis. On the other hand, WW measurements were limited by the sampling method used. 300 mL-grab samples taken every 2 h correspond to twelve single samples and cannot cover the high fluctuations of APIs at the sewer of a HI. More sophisticated sampling campaigns (e.g. flow-proportional sampling) such as those conducted in the studies of Mullot et al. (2010) or Ort et al. (2010) could reduce the bias. Even so, the average consumption during a given day is very different from the average consumption during a given year. Sensitivity analysis showed that the consumption has a high influence on the result PWWC. Therefore, simply by increasing the amount of samples, it would not be possible to arrive at a better understanding of the API emission situation.

Since real data about daily consumption are virtually impossible to obtain, daily drug consumption in HIs must just be assumed or calculated from monthly or annual consumption. It is, therefore, pointless to include the high variability of daily drug consumption in the calculation of WW concentration. Furthermore, only the range of concentrations is of interest. An extensive monitoring campaign yields values of high variable environmental concentrations over the day or over 1 week. Further, it is almost impossible to determine the emission of APIs to the environment over 1 year or a longer period. Although PWWC can lead to underestimation or overestimation of results, several studies (e.g. Kümmerer and Henninger, 2003; Schuster et al., 2008) have confirmed

Table 8

Mean values of emission potential (I_{EP}), as absolute values, with corresponding relative standard deviation. I_{EP} was calculated from annual consumption data (years 2010, 2011 and 2012) for active pharmaceutical ingredients (APIs) used in households, the general hospital with its three locations (MAIN, OPHT, ORTH), the psychiatric hospital (PSY), and the nursing home (NH) for the scenarios national comparison, and regional comparison in Region 1 and in Region 2, respectively. $I_{EP} > 1$ is highlighted in bold. Blank cell indicates no consumption in either households or in the investigated facility.

ATC	API	Comparison national			Comparison Region 1		Comparison Region 2	
		General hospitals	Psychiatric hospitals	Nursing homes	MAIN/OPHT	PSY	ORTH	NH
A02	Pantoprazole	0.0642 ± 18%	0.0032 ± 9%	0.0385 ± 15%	0.0848 ± 21%	0.0081 ± 11%	0.1499 ± 14%	0.0948 ± 4%
A07	Mesalazine	0.0127 ± 10%	–	0.0089 ± 70%	0.0212 ± 11%	–	–	0.0202 ± 70%
A10	Metformin	0.0027 ± 4%	0.0004 ± 36%	0.0108 ± 8%	0.0032 ± 2%	0.0010 ± 35%	0.0087 ± 10%	0.0251 ± 4%
B01	Acetylsalicylic acid	0.0438 ± 5%	0.0023 ± 9%	0.0680 ± 24%	0.0620 ± 7%	0.0057 ± 11%	0.0740 ± 12%	0.1557 ± 21%
C01	Amiodarone	0.0668 ± 9%	–	–	0.1119 ± 11%	–	–	–
C03	Furosemide	0.0619 ± 4%	–	0.0354 ± 47%	0.1022 ± 7%	–	0.0280 ± 0%	0.0794 ± 47%
C07	Metoprolol	0.0022 ± 8%	–	0.0057 ± 12%	0.0026 ± 10%	–	0.0072 ± 0%	0.0132 ± 13%
C09	Valsartan	0.0208 ± 8%	–	0.0187 ± 53%	0.0267 ± 8%	–	0.0533 ± 13%	0.0422 ± 52%
C10	Simvastatin	0.0182 ± 4%	0.0008 ± 1%	0.0146 ± 19%	0.0265 ± 6%	0.0019 ± 3%	0.0254 ± 3%	0.0337 ± 15%
J01	Cefuroxime	0.9427 ± 3%	0.0016 ± 6%	0.0104 ± 0%	**1.0375 ± 8%**	0.0040 ± 9%	**3.5261 ± 9%**	0.0234 ± 0%
J01	Sulfamethoxazole	0.1335 ± 7%	0.0051 ± 25%	0.0088 ± 6%	0.2056 ± 7%	0.0128 ± 24%	0.1142 ± 4%	0.0193 ± 9%
L01	Fluorouracil	–	–	–	–	–	–	–
M01	Diclofenac	0.0750 ± 17%	0.0008 ± 0%	0.0111 ± 5%	0.0830 ± 14%	0.0020 ± 0%	0.2743 ± 19%	0.0248 ± 5%
M01	Ibuprofen	0.0341 ± 4%	0.0021 ± 4%	0.0092 ± 13%	0.0493 ± 0%	0.0053 ± 6%	0.0503 ± 14%	0.0214 ± 8%
M04	Allopurinol	0.0112 ± 2%	–	0.0219 ± 17%	0.0152 ± 3%	–	0.0235 ± 1%	0.0496 ± 15%
N01	Lidocaine	–	–	–	–	–	–	–
N01	Mepivacaine	–	–	–	–	–	–	–
N01	Propofol	–	–	–	–	–	–	–
N02	Metamizole	0.2829 ± 7%	0.0029 ± 10%	0.0736 ± 18%	0.3635 ± 7%	0.0071 ± 12%	0.7192 ± 11%	0.1717 ± 12%
N02	Paracetamol	0.2560 ± 20%	0.0040 ± 5%	0.0094 ± 47%	0.3521 ± 23%	0.0099 ± 3%	0.4872 ± 10%	0.0211 ± 48%
N02	Tilidine	0.0609 ± 3%	0.0012 ± 1%	0.0225 ± 62%	0.0533 ± 3%	0.0029 ± 2%	0.3165 ± 5%	0.0514 ± 60%
N02	Tramadol	0.0414 ± 20%	0.0013 ± 6%	0.0153 ± 31%	0.0489 ± 12%	0.0031 ± 4%	0.1346 ± 48%	0.0345 ± 31%
N03	Carbamazepine	0.0115 ± 12%	0.0080 ± 11%	0.1716 ± 11%	0.0192 ± 13%	0.0199 ± 8%	–	0.3874 ± 10%
N03	Eslicarbazepine acetate	–	–	–	–	–	–	–
N03	Gabapentin	0.0075 ± 6%	0.0005 ± 2%	0.0485 ± 36%	0.0125 ± 7%	0.0013 ± 4%	–	0.1126 ± 33%
N03	Lamotrigine	0.0091 ± 35%	0.0046 ± 10%	0.0619 ± 44%	0.0152 ± 35%	0.0114 ± 13%	–	0.1448 ± 38%
N03	Levetiracetam	0.0268 ± 21%	0.0025 ± 36%	0.0995 ± 28%	0.0450 ± 23%	0.0062 ± 34%	–	0.2345 ± 22%
N03	Oxcarbazepine	–	0.0133 ± 18%	0.7023 ± 37%	0.0134 ± 4%	0.0329 ± 17%	–	**1.5893 ± 36%**
N03	Pregabalin	0.0229 ± 17%	0.0150 ± 36	0.1393 ± 19%	0.0384 ± 18%	0.0371 ± 34%	–	0.3252 ± 13%
N03	Valproic acid	0.0105 ± 13%	0.0192 ± 12%	0.1856 ± 23%	0.0175 ± 12%	0.0479 ± 14%	–	0.4217 ± 22%
N04	Entacapone	0.0184 ± 21%	–	0.1833 ± 17%	0.0309 ± 23%	–	–	0.4179 ± 15%
N04	Levodopa	0.0026 ± 4%	0.0001 ± 0%	0.0133 ± 30%	0.0044 ± 4%	0.0004 ± 2%	–	0.0304 ± 28%
N05	Amisulpride	–	0.0778 ± 39%	–	0.0230 ± 0%	0.1952 ± 42%	–	–
N05	Chlorprothixene	0.0000 ± 0%	0.0226 ± 36%	0.6876 ± 11%	–	0.0561 ± 35%	–	**1.5599 ± 9%**
N05	Clomethiazole	**1.0876 ± 7%**	0.1813 ± 18%	–	**1.4338 ± 6%**	0.4495 ± 17%	**2.5021 ± 20%**	–
N05	Clozapine	0.0075 ± 71%	0.0473 ± 22%	0.2334 ± 55%	0.0185 ± 3%	0.1177 ± 22%	–	0.5330 ± 52%
N05	Melperone	0.0916 ± 14%	0.0457 ± 30%	0.4832 ± 4%	0.1378 ± 12%	0.1146 ± 32%	0.1508 ± 0%	**1.1013 ± 6%**
N05	Quetiapine	0.0094 ± 11%	0.0722 ± 18%	**1.1256 ± 17%**	0.0157 ± 10%	0.1803 ± 21%	–	**2.6462 ± 11%**
N06	Amitriptyline	0.0069 ± 2%	0.0047 ± 3%	–	0.0116 ± 1%	0.0116 ± 1%	–	–
N06	Bupropion	0.1425 ± 72%	0.0541 ± 5%	0.1706 ± 47%	0.3615 ± 14%	0.1346 ± 7%	–	0.4015 ± 40%
N06	Doxepin	0.0095 ± 4%	0.0066 ± 3%	0.0762 ± 27%	0.0159 ± 3%	0.0162 ± 2%	–	0.1721 ± 27%
N06	Duloxetine	0.0000 ± 0%	0.0321 ± 25%	–	–	0.0795 ± 23%	–	–
N06	Mirtazapine	0.0147 ± 34%	0.0149 ± 3%	0.0690 ± 38%	0.0246 ± 34%	0.0370 ± 6%	–	0.1606 ± 34%
N06	Moclobemide	0.2101 ± 40%	0.0666 ± 0%	**3.8607 ± 34%**	0.3462 ± 39%	0.1608 ± 0%	–	**6.9241 ± 74%**
N06	Sertraline	0.0040 ± 141%	0.0246 ± 42%	0.0642 ± 10%	0.0199 ± 0%	0.0606 ± 41%	–	0.1496 ± 13%
N06	Trimipramine	0.0139 ± 38%	0.0069 ± 3%	–	0.0231 ± 36%	0.0172 ± 1%	–	–
N06	Venlafaxine	0.0040 ± 13%	0.0380 ± 9%	0.0351 ± 36%	0.0067 ± 15%	0.0945 ± 8%	–	0.0828 ± 28%
R06	Promethazine	0.0000 ± 0%	0.0807 ± 3%	0.9617 ± 40%	0.0000 ± 0%	0.2005 ± 1%	–	**2.1899 ± 38%**
S01	Acetazolamide	0.5987 ± 31%	–	–	0.9961 ± 29%	–	–	–
V08	Iomeprol	–	–	–	–	–	–	–

that such a calculation, providing consumption data and including metabolism for each investigated API, is a suitable approach to determine discharge of APIs to the environment over a longer time period. In fact, it is the approximate concentration range that is of relevance and not the exact concentration at a certain time point or within a very short time period.

3.4. Contribution of health institutions (HIs) compared to households

3.4.1. National comparison of consumed pharmaceuticals

In order to evaluate the relevance of the selected facilities and their respective contributions of APIs to STP inflow, the average consumption of the selected pharmaceuticals in the selected HIs in Germany was compared to the average consumption in German households. Table 8 shows the results for the emission potential I_{EP} of the 50 selected APIs

and demonstrates, with an I_{EP} higher than 1, a higher relevance for the API emitting from the facility type than from households.

The results illustrate that in general hospitals, the consumption of pharmaceuticals affecting the alimentary tract (A) and the cardiovascular system (C) was between 15 and 500 times lower than in households (e.g. I_{EP} (metoprolol) = 0.0022). In terms of amount consumed, the most significant painkiller in general hospitals, metamizole, (see Section 3.2) represents only approximately 22% of the total consumption in Germany. Only the amounts for the antibiotic cefuroxime (I_{EP} = 0.9427), the antipsychotic clomethiazole (I_{EP} = 1.0876), and the carbonic anhydrase inhibitor acetazolamide (I_{EP} = 0.5987) were similar to the one for households. The overall contribution of cefuroxime and sulfamethoxazole from general hospitals is almost 49% and 12%, respectively, which is consistent with the findings of other studies (Kümmerer and Henninger, 2003) (cefuroxime: 65%, sulfamethoxazole: 8%). The

high contribution of clomethiazole is due to its regulatory registration and use as a pharmaceutical for alcohol withdrawal (Morgan, 1995). Acetazolamide is commonly used only to treat glaucoma or edema.

Compared to households, the consumption of pharmaceuticals was almost negligible in psychiatric hospitals. In German households, the consumption of substances affecting the alimentary tract (A) or the cardiovascular system (C) was up to 2500 times higher than in psychiatric hospitals (e.g. I_{EP} (metformin) = 0.0004). Typical APIs used in general hospitals, such as cefuroxime (I_{EP} = 0.0016), were also used in lower amounts. Only antipsychotics and tranquilizers are noticeably high, with the highest I_{EP} quotients of 0.1813 for clomethiazole, 0.0807 for promethazine, and 0.0722 for quetiapine.

In nursing homes consumption of substances used to treat alimentary system disorders or cardiovascular disorders was quite low. However, the application of anticonvulsants (e.g. I_{EP} (oxcarbazepine) = 0.70), antipsychotics (e.g. I_{EP} (quetiapine) = 1.1256), and antidepressants (e.g. I_{EP} (moclobemide) = 3.86) in nursing homes led to emission potentials up to 1 or even higher. Moclobemide is used to treat depression and social anxiety. It did not seem to be a highly consumed API in the mass balance, but apparently it is very important in nursing homes. The NH examined in this study was described as a nursing home that takes care of elderly people with psychiatric disorders and of people suffering from alcoholism. The median age is lower than the median age in German nursing homes in general (see Section 3.1) (Federal Statistical Office DESTATIS, 2013). It can, therefore, be assumed that compared to other nursing homes, the consumption of drugs for cardiovascular diseases is lower, and the consumption of neurologicals higher.

3.4.2. Data quality regarding national comparison

It was assumed that the consumption of pharmaceuticals in the hospitals and nursing home is very similar to comparable facilities in Germany. The general hospital provides treatment for all possible diseases with 31,179 treated cases, in 2012. This scaled up to 18,020,968 treated cases throughout Germany. Increasing the number of hospitals could reduce the bias, therefore, upscaling was a rough estimation. However, it is quite reasonable to assume that the usage patterns of pharmaceutical classes closely resemble that of the rest of Germany. Accordingly, PSY with 1527 treated cases, was scaled up to 599,474 cases in Germany.

For nursing homes, an extrapolation of the average of 271 inhabitants to 743,120 provided places for care in Germany was conducted. Pharmaceuticals consumed in nursing homes are collected in AVR, which leads to the conclusion that the consumption of pharmaceuticals in NH is very high. This could be due to the psychiatric focus of the facility mentioned above. Inhabitants in need of long-term care include not only the elderly, but also people with mental health problems.

3.4.3. Regional comparison of consumed pharmaceuticals

The results of the comparison of consumed APIs at the facilities with two sub-regions in the study area, expressed as I_{EP}, are shown in Table 8 (regional comparisons 1 and 2).

The results for Region 1, with 85,000 citizens and two out of three locations of the general hospitals MAIN and OPHT as well as PSY, illustrate that due to a high density of medical facilities, I_{EP} is higher than the national average. The number of treated medical cases was used to assess the density of the HIs in the investigated areas, because treated cases can be used as an indicator of the activity of a hospital (Magee, 2003). In 2012, 0.22 medical cases per citizen were treated at German hospitals, whereas in Region 1, 0.33 medical cases were treated. In terms of medical cases treated, the ratio between the medical facilities and households was higher in Region 1 than the national average. Likewise, more medical cases in psychiatric hospitals were treated in this region as well (Germany: 0.007 cases citizen^{-1}, Region 1: 0.018 cases citizen^{-1}).

For this reason, cefuroxime (I_{EP} = 1.0375) and clomethiazole (I_{EP} = 1.4338) were consumed in higher amounts in MAIN and OPHT than in

households (I_{EP} > 1). Still, the I_{EP} of APIs affecting the alimentary tract (A) and the cardiovascular system (C) were comparably low. Compared to households, antinflammatory drugs such as diclofenac (I_{EP} = 0.0830) and ibuprofen (I_{EP} = 0.0493), contribute 8% and 5%, respectively, to WW discharge. Other studies revealed similar results: between 1 and 10% for diclofenac and ibuprofen (Beier et al., 2011; Heberer and Feldmann, 2005; Langford and Thomas, 2009; Verlicchi et al., 2012b).

The overall contribution from PSY was very low. The amount of APIs listed in antiepileptics (N03) and psycholeptics (N05) used in PSY was still less than the amount used in households. The highest comparable contribution was due to clomethiazole with approximately 31% (I_{EP} = 0.4495). Other APIs were used up to 2500 times less than in households (e.g. I_{EP} (metformin) = 0.001 or I_{EP} (levodopa) = 0.0004).

Table 8 also shows the results of I_{EP} for the smaller Region 2 with 13,000 citizens and one location of the general hospital (ORTH) as well as NH. Eight APIs were identified with an I_{EP} higher than 1.

In ORTH, clomethiazole (I_{EP} = 2.5021) and cefuroxime (I_{EP} = 3.5261) have a higher I_{EP} than 1. The higher consumption of cefuroxime is due to the high amount of prophylactic antibiotic used in orthopedic operations, which are often done to treat gonarthrosis. Before and after this operation cefuroxime is given to prevent infections.

In NH, there are six I_{EP} higher than 1. Anticonvulsants (N03) were consumed up to 10 times less in NH than by 13,000 comparable citizens in households. Nevertheless, the antiepileptic drug oxcarbazepine (I_{EP} = 1.5893), the antihistamine promethazine (I_{EP} = 2.1899), the psycholeptics chlorprothixene (I_{EP} = 1.5599), melperone (I_{EP} = 1.1013), and quetiapine (I_{EP} = 2.6462), as well as the antidepressant moclobemide (I_{EP} = 2.6462) were even more frequently used than in households.

3.4.4. Data quality regarding regional comparison

Reliable data on the local consumption at the facilities was provided by the pharmacies that supplied drugs to these HIs. The consumption in households was scaled down from the national consumption in Germany. The demographic situation in the investigated regions was compared to Germany. This comparison showed almost the same distribution in terms of age, migration background, and employment status (Federal Statistical Office DESTATIS, 2014b), which is important since age as well as social and economic environment are factors affecting individual health (WHO, 2013). A calculation of consumption in households based on public pharmacies would not offer a more nuanced perspective on the actual consumption in the regions, because travelers or patients receiving their pharmaceuticals from facilities located outside the study site could also cause a bias.

4. Conclusions

In this study annual pharmaceutical consumption of different HIs data was presented, processed, and assessed. Psychiatric hospitals and nursing homes can be characterized based on their pharmaceutical usage patterns, which can be discerned by means of a mass balance. The patterns of these two HIs turned out to be different from those of general hospitals and households. Most of the pharmaceuticals consumed in psychiatric hospitals and nursing homes are APIs that have an effect on the nervous system. Due to their respective properties, these APIs are unlikely to harm the environment. There is, however, no conclusive empirical evidence that neurological drugs are benign. Further investigations on the ecotoxicological risk of neurological drugs are, therefore, needed.

The methods herein presented were able to support the assessment of usage patterns of APIs in HIs and to predict their respective contributions to WW. The information in this article brings new insights to provide practical support for the decision making process for managing API WW loads from HIs and decentralized water quality control. This study moved the focus from the development of comprehensive and expensive monitoring campaigns to a more simplified approach. Hence, the

presented methods can at least partially replace extensive monitoring campaigns. Likewise, it is an efficient tool for the forecast of pollution problems, an important prerequisite for sustainable management of resources.

By applying this tool, this study demonstrated that psychiatric hospitals and nursing homes are, in general, insignificant emitters of APIs into sewage. The findings indicate that this is also true for general hospitals. However, in some instances, specific APIs were used to a great extent at specific HIs. In regions with a high density of medical facilities, these HIs can, therefore, be regarded as point sources for singular API emission, which could lead to higher concentrations in WW and may pose an environmental threat. Nevertheless, this has to be proven for every particular situation, and can be confirmed by the methods presented in this article. With the results obtained, a solid basis is created for an informed decision concerning emission reduction strategies.

Acknowledgments

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Artikel 2

Identification of Phototransformation Products
of the Antiepileptic Drug Gabapentin:
Biodegradability and Initial Assessment of Toxicity

Manuel Herrmann, Jakob Menz, Oliver Olsson, Klaus Kümmerer

Identification of phototransformation products of the antiepileptic drug gabapentin: Biodegradability and initial assessment of toxicity

Manuel Herrmann [a, b], Jakob Menz [a], Oliver Olsson [a], Klaus Kümmerer [a, *]

[a] Sustainable Chemistry and Material Resources, Institute of Sustainable and Environmental Chemistry, Leuphana University of Lüneburg, C13, Scharnhorstrasse 1, DE-21335 Lüneburg, Germany
[b] Hospital Pharmacy, Ortenau Klinikum Offenburg-Gengenbach, Ebertplatz 12, DE-77654 Offenburg, Germany

ARTICLE INFO

ABSTRACT

The anticonvulsant drug Gabapentin (GAB) is used for the treatment of various diseases (e.g. epilepsy, bipolar disorder, neuropathic pain) and is being consumed in high amounts. As GAB is not metabolized and shows a weak elimination in sewage treatment plants (STPs), it has been detected in surface water and even in raw potable water. Moreover, the confirmed teratogenic effects of GAB indicate the need for further investigations regarding options for the elimination of GAB in the water cycle. Little is known about the behavior of GAB during treatment with UV light, which is normally used for the disinfection of potable water and discussed for advanced wastewater treatment. In this study, GAB was exposed to polychromatic UV irradiation at different initial concentrations in aqueous solution. Afterwards the structures of the resulting phototransformation products (PTPs) were identified and elucidated by means of high-resolution mass spectrometry. GAB and photolytic mixtures were submitted to the Closed Bottle Test (CBT; OECD 301 D) to assess biodegradability. Furthermore, the toxicity of GAB and its photolytic mixtures was initially addressed on screening level using a modified luminescent bacteria test (LBT) and the umu-test (ISO/FDIS 13829). Environmentally realistic concentrations of GAB were disclosed by predicting STP influent concentrations (24.3 and 23.2 μg L^{-1}). GAB with initial concentration of 100 mg L^{-1} was eliminated by 80% after 128 min of direct UV irradiation, but just 9% of non-purgeable organic carbon (NPOC) was removed indicating the formation of dead-end transformation products (TPs). Structures of different PTPs were elucidated and several identical PTPs could also be identified at lower initial treatment concentrations (20 mg L^{-1}, 5 mg L^{-1}, 1 mg L^{-1} and 0.1 mg L^{-1}). GAB was classified as not readily biodegradable. Moreover, photo treatment did not result in better biodegradable PTPs. With increasing UV treatment duration, photolytic mixtures of GAB showed an increased inhibition of both, the bacterial luminescence emission as well as the growth in the modified LBT. In the umu-test no significant induction of the umuC gene as an indicator of genotoxicity was observed. Our results show that UV irradiation of GAB containing water would lead to the formation of recalcitrant PTPs. Considering that GAB was found in raw drinking water, the formation of toxic PTPs during drinking water treatment with UV light might be possible. Therefore, further studies should be conducted regarding the fate and effects on human health and the environment of GAB and the PTPs identified within this study.

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1. Introduction

The occurrence and assessment of pharmaceuticals in the environment plays an important role in environmental research, since the variety and the consumption of pharmaceuticals is still rising (OECD, 2011). Many different compounds from several classes, like analgesics, antihypertensive agents or antibiotics have been detected in different environmental compartments (Capdeville and Budzinski, 2011; Nödler et al., 2010). The environmental risk emanating from pharmaceuticals has been frequently assessed by several studies (Escher et al., 2011; Sanderson et al., 2004; Verlicchi et al., 2012). For some pharmaceuticals the potential environmental risk is obvious. Antibiotics can affect the

population dynamics of microbial communities, hormones can cause changes in the endocrine system of water organisms and cytostatics are apparently highly toxic to actively dividing eukaryotic cells. In contrast, the effect being caused by pharmaceuticals such as psychotropic drugs in the environment is not easily assessable. Still, they should receive more attention, because they have been found in different environmental aqueous compartments (Writer et al., 2013), as well as in drinking water (Huerta-Fontela et al., 2011).

For medical use the antiepileptic drug gabapentin (GAB) arouse high concern in previous years. Besides its regulatory medical indications like epilepsy and neuropathic pain, GAB is off-label used for several other indications, such as bipolar disorder (Carta et al., 2003), migraine prophylaxis (Mathew et al., 2001) or restless legs syndrome (Happe et al., 2003). In 2009, according to Lai et al. (2011) 6.7 t of GAB were consumed in Australia. In the same year the consumption in Germany (data for public health insurance, around 85% of the population) was 58.9 t (Schwabe and Paffrath, 2010) with a steady linear annual increase to 73.3 t in 2012 (Schwabe and Paffrath, 2013). GAB represents about 1% of the whole pharmaceutical consumption in Germany (Ebert et al., 2014) and is excreted entirely unchanged (PFIZER PHARMA GmbH (Parke-Davis), 2014). Therefore, GAB is expected to have a high concentration at influents of sewage treatment plants (STPs).

Some pharmaceuticals are not entirely eliminated in STPs, thus being able to reach surface waters (Kasprzyk-Hordern et al., 2009a) or even drinking water (Huerta-Fontela et al., 2011; Zühlke et al., 2004). In the case of GAB, sewage concentrations up to 25 μg L^{-1} and 37 μg L^{-1} were detected in influents of STPs (Kasprzyk-Hordern et al., 2009b), whereas Yu et al. (2006) quantified 1 μg L^{-1}. Ottmar et al. (2010) modeled GAB concentrations at influents of five STPs ranging from 1 μg L^{-1} to 28 μg L^{-1} by means of drug prescription data, which shows that modeled concentrations are very close to measured concentrations. However, there is inconsistent data on the elimination of GAB in STPs. Yu et al. (2006) observed full elimination of GAB in an STP, whereas other studies reported high concentrations of GAB in STP effluents up to 1.7 and 6.5 μg L^{-1}, respectively (De la Cruz et al., 2012; Reungoat et al., 2010). Kasprzyk-Hordern et al. (2009a) in turn compared two STPs with different secondary treatment technologies and concluded that filter bed STPs are not able to eliminate GAB sufficiently, but STPs working with activated sludge are to some degree. The weak elimination of GAB in STPs leads to frequent detection of GAB in surface waters up to 1.9 μg L^{-1} receiving effluent from STPs (Kasprzyk-Hordern et al., 2008; Writer et al., 2013). Morasch et al. (2010) even detected GAB in raw drinking water (0.4 μg L^{-1}) sampled at a drinking water plant receiving river water downstream of an STP. The uptake of GAB via drinking water could be dreadful, because some studies showed teratogenic effects for GAB (Afshar et al., 2007; Prakash et al., 2008).

GAB has a high mobility potential (log p -1.25 (Zhu et al., 2002)) and has been found in every possible aqueous environmental compartment. Therefore, more detailed information about the environmental behavior of GAB should be available.

In connection with the constant release of pharmaceuticals to the environment, advanced treatment processes, like treatment with UV light, are discussed as a feasible method to eliminate pharmaceutical residues from wastewater (De la Cruz et al., 2012). Several possibilities to eliminate GAB after secondary wastewater treatment in STPs have already been studied. Neamţu et al. (2014) identified GAB as one of the most persistent compounds towards UV$_{254}$/H$_2$O$_2$/Fe(II) treatment in ultrapure, lake and wastewater. In comparison GAB was sufficiently eliminated from wastewater by UV/H$_2$O$_2$ treatment as reported by De la Cruz et al. (2012), whereas simple UV treatment leads to the elimination of only 10% (De la Cruz et al., 2012). As UV irradiation is commonly used for drinking water disinfection (Canonica et al., 2008; Hijnen et al., 2006), micro-pollutants like GAB are constantly exposed to UV light and should therefore also be investigated in this respect.

However, the above-mentioned studies only monitored GAB with regard to its primary elimination. An entire mineralization of the parent compound was not compulsive, which means that unknown transformation products (TPs) could be formed. Moreover, TPs formed from GAB during drinking water disinfection with UV light have not been taken into account. The only possibility for identification of TPs is to access intermittent databases (Gómez et al., 2010). Moreover, structural elucidation of TPs leads to the possibility of assessing the potential toxicity and persistence in the environment (Haddad and Kümmerer, 2014; Trautwein and Kümmerer, 2012). Recent studies showed that TPs could have a negative effect on environmental organisms or be more persistent than the parent compound itself (Illés et al., 2014; Trautwein et al., 2014). To the best of our knowledge there have been no literature information on TPs formed from GAB.

In this study the consumption of GAB for a medium-sized city located in the west of Germany with around 40,000 inhabitants was assessed to approximate GAB's potential sewage concentration for the simulation of UV treatment. In a first step, photo-degradation experiments with an initial GAB concentration of 100 mg L^{-1} were performed to generate photolytic mixtures that allowed the identification and characterization of photo-transformation products (PTPs). Photolytic mixtures were analyzed using high-resolution mass spectrometry, further investigated in the Closed Bottle Test (CBT), a modified luminescent bacteria test (LBT) (Menz et al., 2013) and the umu-test to assess changes in biodegradability, antibacterial activity and genotoxicity, respectively. Finally, samples with consecutively lower, i.e. environmentally realistic initial concentrations of GAB were also treated with UV light to check the transferability of the test results to environmental conditions.

2. Data sets, material and methods

2.1. Mass balanced prediction of gabapentin (GAB) in municipal wastewater

For the prediction of GAB influent concentration in an STP, the medium-sized city Dülmen with 46,300 citizens, located in the west of Germany, was chosen. Data of the amount of applied GAB in the allocated hospitals (general hospital (200 beds) and psychiatry (108 beds)) for the year 2012 was collected from the hospital pharmacy. The consumption of pharmaceuticals by the general population was calculated based on drug sales data provided by the local wholesaler for pharmaceuticals and cross checked with data of the annually published report for prescribed drugs in Germany Arzneiverordnungs-Report (AVP) 2013 (based on 2012 data). The predicted influent concentration (PIC), indicating the 'worst-case scenario', with GAB being excreted entirely unchanged, was calculated according to Eq. (1):

$$PIC(GAB) = \frac{\left(A_{hospitals} + A_{domestic}\right) \times f_{nm}}{366 \times P \times V_E} \tag{1}$$

where: $A_{hospitals}$ is the consumption in hospitals, $A_{domestic}$ is the consumption in households (from the wholesaler and AVP, respectively), f_{nm} is the non-metabolized fraction of GAB (100% (Pfizer Pharma GmbH (Parke-Davis), 2014)), P is the number of inhabitants and V_E is the water consumption per capita and day (121 L (Federal Statistical Office DESTATIS, 2015)).

2.2. Chemicals and reagents

GAB (certified purity 99.9%, traceable to USP standard) was purchased from Sigma–Aldrich Chemie GmbH (Steinheim, Germany). Ammonium acetate (HiPerSolv CHROMANORM® for HPLC) and methanol (HiPerSolv CHROMANORM® for HPLC, LC-MS grade) were purchased from VWR International GmbH (Darmstadt, Germany). 2-Propanol (purity ≥99.5%, Ph.Eur.) was purchased from Carl Roth GmbH & Co. KG (Karlsruhe, Germany). Aqueous mobile phase, standard solutions and solutions for photodegradation experiments were prepared with ultrapure water (Q1:16.6 mΩ and Q2: 18.2 mΩ).

2.3. Simulated UV treatment (direct UV photolysis)

The test solutions of GAB with initial concentrations of 100 mg L^{-1}, 20 mg L^{-1}, 5 mg L^{-1}, 1 mg L^{-1} and 0.1 mg L^{-1} were freshly prepared with ultrapure water in order to determine non-purgeable organic carbon (NPOC) and further elucidate PTPs. To obtain information about the photochemical role of reactive oxygen species (ROS) during the photolysis process, an aqueous solution of GAB (100 mg L^{-1}) with 1% 2-Propanol (v/v) was also prepared to undergo photolysis. Additionally, the concentration of dissolved oxygen was measured throughout the experiment by an optical oxygen sensor FDO® 925 (WTW GmbH, Weilheim, Germany). The photolysis experiments were carried out in a 1000 mL batch immersion tube photo reactor using 800 mL of sample volume. Magnetic stirring ensured continuous mixing of the solution. Constant temperature (20 ± 1 °C) was achieved by using a cooling system (WKL230, LAUDA, Berlin, Germany).

The polychromatic irradiation source used in the experiments was a medium-pressure mercury lamp (TQ 150, UV Consulting Peschl, Mainz, Germany). The lamp was surrounded by a cooling jacket separated from the test solution by an ilmasil quartz glass to guarantee unlimited irradiation. The applied UV fluence for experiments is shown in Fig. 1F. Additionally, the measured emission spectrum of polychromatic light after a total operating time of 500 h and the measured absorbance spectrum of GAB is shown in Fig. S1, SM (supplementary material). GAB has a high absorption in the lower wavelength range, and therefore, it is not expected to be mainly eliminated by direct photolysis.

The photolysis experiment was conducted for 128 min. Samples for each test concentration were collected before (0 min), after 2, 4, 8, 16, 32, 64 and 128 min of treatment for LC-MS^n analysis (primary elimination and structural elucidation), as well as for NPOC determination (mineralization). Analogous sampling in the case of 100 mg L^{-1} initial concentration was conducted and subsequently submitted to the LBT and the umu-test. For the CBT, samples (initial concentration 100 mg L^{-1}) were collected at the beginning (0 min), after 32, 64 and 128 min.

2.4. Biodegradation testing according to OECD 301 D (Closed Bottle Test (CBT))

The CBT was performed with little modifications according to the Organisation for Economic Co-operation and Development (OECD) test guidelines (OECD, 1992) using a low content of nutrients (mineral medium) and bacteria to simulate ready biodegradability in the aquatic environment. The concentration of GAB was 2.3 mg L^{-1} corresponding to a theoretical oxygen demand (ThOD) of 5 mg L^{-1}. The final concentration of the photolytic mixtures after 32, 64 and 128 min, respectively, was adjusted according to the remaining NPOC concentration to reach a comparable ThOD.

During the whole test the biochemical oxygen demand (BOD) was monitored by measuring the dissolved oxygen concentration

(Friedrich et al., 2013). According to the test guidelines, degradation of 60%, expressed as a percentage of oxygen consumed in the test bottle, classifies a chemical as readily biodegradable. Additional information regarding the test procedure and validation criteria can be found in Text S1, SM.

Samples from the beginning and the end of the test (after 28 days) were taken for LC-MS^n analysis.

2.5. Analytical conditions

2.5.1. Non-purgeable organic carbon (NPOC) analysis

To monitor the degree of mineralization a Total Organic Carbon Analyzer (TOC 5000, Shimadzu GmbH, Duisburg, Germany) was used. Linear calibration for the measured range was performed with dried potassium phthalate.

2.5.2. Primary elimination of gabapentin (GAB) and structural elucidation of phototransformation products (PTPs)

LC-MS^n analysis was performed on an Agilent 1100 series HPLC system (Agilent Technologies, Waldbronn, Germany) coupled with a Bruker Esquire 6000^{plus} mass spectrometer with an ESI source (Bruker Daltonics, Bremen, Germany) (LC-ITMS), and a Dionex Ultimate 3000 UHPLC system (Dionex, Idstein, Germany) coupled with an LTQ Orbitrap-XL high-resolution mass spectrometer with H-ESI source (Thermo Scientific, Bremen, Germany) (LC-HRMS). Detailed information about chromatographic and mass spectrometric conditions can be found in Text S2, SM.

All photolysis samples with different initial concentrations (100 mg L^{-1}, 20 mg L^{-1}, 5 mg L^{-1}, 1 mg L^{-1} and 0.1 mg L^{-1}) were measured for primary elimination by LC-ITMS injecting 5 μL of the sample volume.

To obtain information about the occurrence of formed PTPs during photolysis experiment, samples with initial concentration of 100 mg L^{-1} GAB were analyzed by LC-ITMS. The peak area A of each newly occurring m/z value with corresponding retention time (t_R) was related to GAB's peak area A_0 at time point 0 min, as the absolute peak area is no indicator for the concentration of unknown PTPs. No correlation between peak area and concentration can be established for unknown compounds because the ionization rate in the MS is different for every compound. The injection volume was 5 μL.

Structural elucidation was performed for every PTP considering a threshold exceeding 2% among the ratio between PTP peak area (A) and GAB's peak area at time point 0 min (A_0) during the photolysis process. A threshold of 2% meets OECD guidelines for the testing of chemicals (OECD, 2010). The guideline recommends the inclusion of PTP >10% on an amount basis for environmental fate assessment, since PTPs in lower occurring amounts do not seem to have a potential effect on the environment. To get further information about the structure of the formed PTPs during the photolysis experiment, PTPs were screened and structural elucidated by ITMS. Structures were ensured with further HRMS fragmentation up to MS^4. Moreover, t_Rs and accurate m/z values of GAB and its PTPs were compared within different initial concentrations to study concentration effects on the formation of PTPs.

Samples from CBT were assessed by LC-ITMS using the recovered peak areas of GAB and its PTPs S/S_0 (S is the peak area of the PTP at day 28 and S_0 is the peak area of the PTP at day 0). Due to lower concentrations of the investigated compounds in CBT compared to the photolysis test, the injection volume was 50 μL.

2.6. Toxicity screening of photolytic mixtures

GAB and the mixtures obtained after photolysis were screened for toxicity using a set of two bioassays. The modified LBT according
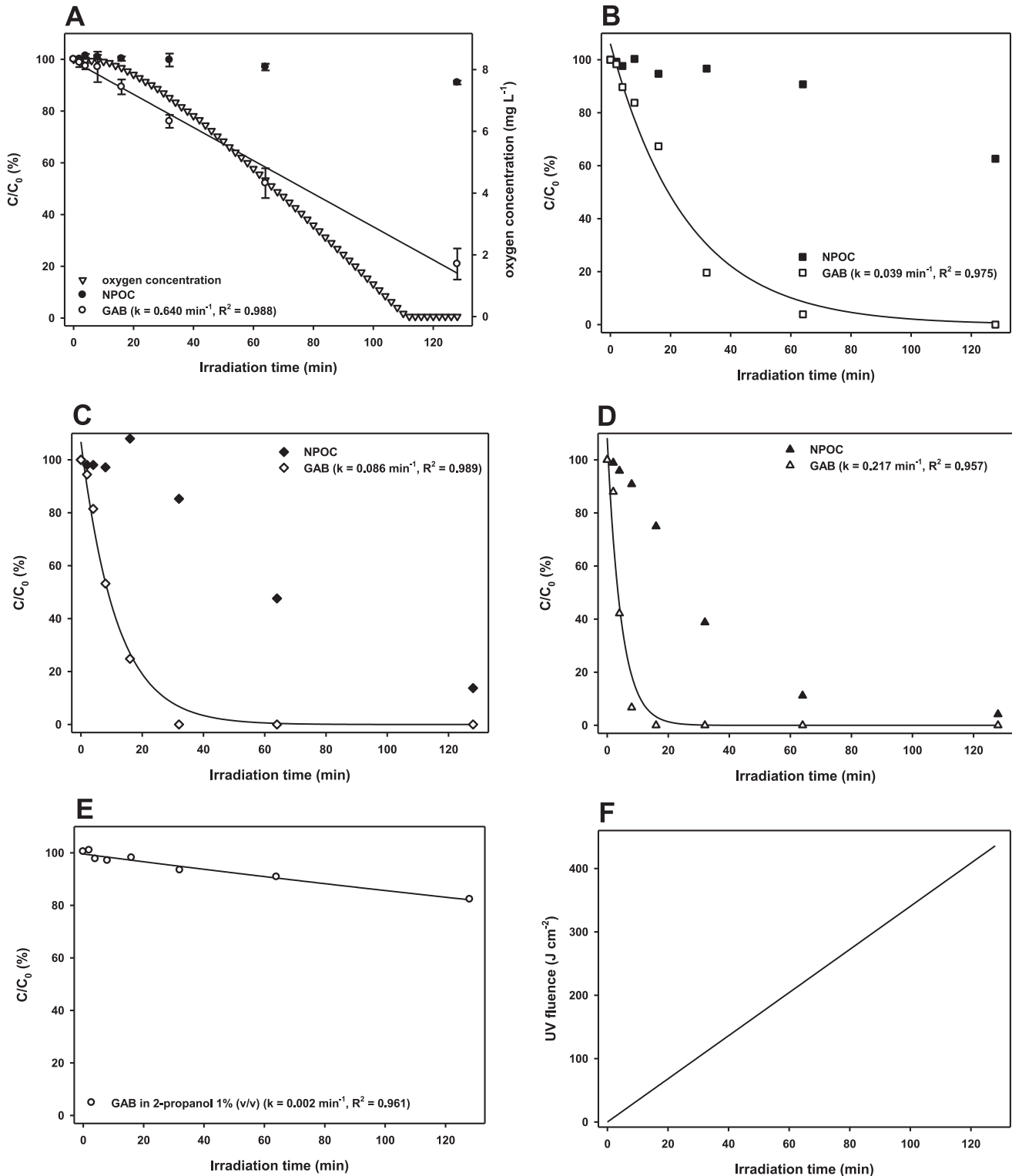
Fig. 1. [A—D] Gabapentin (GAB) and non-purgeable organic carbon (NPOC) elimination during photolysis with different initial concentrations of GAB in ultrapure water (A: 100 mg L^{-1} (n = 4), B: 20 mg L^{-1}, C: 5 mg L^{-1}, D: 1 mg L^{-1}) showing recovery by means of the ratio of the concentration at the specific time point C and the concentration at 0 min C$_0$, the rate constant k, and the correlation R^2 to the kinetic model. [E] GAB elimination during photolysis with initial concentration of 100 mg L^{-1} in aqueous solution of 1% 2-propanol (v/v). [F] Applied UV fluence as a function of irradiation time.

to Menz et al. (2013), using the luminescent bacteria strain Vibrio *fischeri* NRRL-B-11177 (Hach-Lange GmbH), was employed for the combined assessment of short-term luminescence inhibition after 30 min (LI$_{30min}$), long-term luminescence inhibition after 24 h

(LI$_{24h}$) and growth inhibition after 14 h (GI$_{14h}$). Moreover, the photolytic mixtures of GAB were subject to a genotoxicity screening using the umu-test with *Salmonella typhimurium* TA1535 psk 1002 (German Collection of Microorganisms and Cell Cultures GmbH)

according to ISO/FDIS 13829 (ISO/FDIS, 1999). Initial photo-degradation concentration (C_0) of GAB for toxicity screening purposes was 100 mg L^{-1}. More detailed information about testing procedures can be found in Text S3, SM.

3. Results and discussion

3.1. Predicted influent concentration (PIC) of gabapentin (GAB)

In 2012, 48.7 kg (1.052 g capita^{-1}) (estimated data from AVR) and 46.3 kg (1.000 g capita^{-1}) (data from the cognizant wholesaler) of GAB was consumed in households of the city Dülmen (46,300 citizens), respectively. 1.1 kg (3.571 g bed^{-1}) of GAB was consumed in hospitals. Consumption data based on a per capita consumption were slightly different from the Suisse city Lausanne with surrounding communities (221,000 citizens). In Lausanne, in households the consumption was lower with 107.0 ± 53.5 kg year^{-1} (0.484 ± 0.242 g capita^{-1} year^{-1}), and in hospitals also comparably lower with 8.6 ± 4.3 kg year^{-1} (2.429 ± 1.215 g bed^{-1} year^{-1}) (Chèvre et al., 2013).

PIC of GAB obtained from Eq. (1) with the data of AVP 2013 was 24.3 μg L^{-1}. With data from the pharmacies' wholesaler and the domestic hospital pharmacy a value of 23.2 μg L^{-1} was calculated. These calculations were confirmed by information found in literature (Kasprzyk-Hordern et al., 2009b; Ottmar et al., 2010). Considering that GAB is not eliminated in STPs (Morasch et al., 2010), the concentration in the effluent might not change, resulting in high STP effluent concentrations in comparison to other pharmaceutical compounds (Petrie et al., 2015).

3.2. UV photolysis: primary elimination and mineralization

The fate of GAB at UV treatment was evaluated by monitoring the primary elimination and mineralization. For the initial concentration of 100 mg L^{-1} the process was performed four times to cope with experimental uncertainties and potential fluctuation in lamp emission.

Using the initial concentration of 100 mg L^{-1}, 50% of GAB was eliminated after approximately 1 h (Fig. 1A). At the end of the test, the concentration was around 20% of the initial concentration. Due to the high initial concentration, the elimination of GAB followed a zero order kinetics, with a half-life $t_{1/2}$ of 78 min. The NPOC concentration remained unchanged over almost the whole photolysis time. Mineralization started after around 1 h of photolysis and NPOC elimination was only 9% after 128 min. The low degree of mineralization and the primary elimination of 80% indicate the formation of PTPs.

The elimination kinetics for the initial concentrations of 20 mg L^{-1}, 5 mg L^{-1}, 1 mg L^{-1} used in photolysis experiments following a first order model are shown in Fig. 1B–D, respectively. The quantum yields for initial concentrations fitting a first order model were calculated according to Zepp (1978) by means of the respective rate constants, measured lamp irradiance, and the molar extinction coefficient of GAB. Accordingly, the estimated quantum yield was increasing with decreasing initial concentration (20 mg L^{-1}: 0.015, 5 mg L^{-1}: 0.032, 1 mg L^{-1}: 0.082). At an initial concentration of 0.1 mg L^{-1}, GAB concentration was below limit of detection (LOD) after 4 min of photolysis (data not shown) and kinetic fitting was not conducted. Due to insufficient detection limits of the TOC analyzer, NPOC measurement was not carried out for this concentration. The elimination kinetics for GAB and the corresponding NPOC were slower with increasing initial concentration of GAB. Likewise, rate constants were decreasing, because more intermediates and PTPs were formed from UV light with high initial start concentration. These intermediates are in concurrence

to GAB and may absorb UV light partially, before the UV light can pass through the whole solution (Chelme-Ayala et al., 2010; Ding et al., 2013).

As shown in Fig. 1E, the elimination of GAB in aqueous solution with 1% 2-propanol (v/v) was slower compared to the elimination of GAB in ultrapure water (Fig. 1A) fitting a first order kinetic model. It can be assumed that 2-propanol is acting as a radical scavenger, quenching the reaction of ROS, generated from dissolved oxygen, with GAB. As shown in Fig. 1A, the dissolved oxygen concentration was constantly decreasing during the treatment process in ultrapure water. Du et al. (2014) have found that ROS are generated from dissolved oxygen through UV irradiation. In their study the decay of gallic acid was mainly induced by ROS oxidation. Likewise, the elimination of gallic acid through direct photolysis was less important. Therefore, it is expected that GAB's degradation will be predominately caused by ROS. As an explanation, GAB could be transitioned to its excited state by means of UV irradiation energy. Further reaction with dissolved oxygen could lead to the formation of superoxide anions and hydroperoxyl radicals (Du et al., 2014), which induce the elimination of GAB and the formation of PTPs. Additionally, in every taken sample the hydrogen peroxide concentration was measured semi-quantitatively with MQuant Peroxide test strips (Merck Chemicals GmbH, Schwalbach, Germany). At the beginning of the test, no hydrogen peroxide could be determined. After 32 and 64 min, 0–0.5 mg L^{-1} hydrogen peroxide was detected. Whereas, the highest observed concentration range (0.5–2.0 mg L^{-1}) was found after 128 min (test end). The formation of hydrogen peroxide can be explained by the recombination of superoxide anions with hydroperoxyl radicals (Bielski et al., 1985). Before the biodegradation and toxicity tests were performed, the hydrogen peroxide concentration had been measured again. As a result, the concentration was even lower than 0.5 mg L^{-1} in the sample after 128 min probably due to further reaction of hydrogen peroxide with GAB and PTPs.

3.3. Occurrence and structural elucidation of PTPs

Table 1 shows GAB and the PTPs formed during the course of the photolysis at different initial concentrations. 27 PTPs as newly occurring peaks were identified showing eight different m/z values with different t_Rs indicating the formation of isomers. The time course of newly formed PTPs and a tentative photodegradation pathway for initial concentration of 100 mg L^{-1} is shown in Figs. 2 and 3, respectively. Potential chemical mechanisms for the formation of PTPs are described in the following. The fragmentation pattern as well as the fragmentation pathway listed according to m/z values for the PTPs can be found in Text S4, SM.

In this study, most of the PTPs were found to be more polar than GAB. Accordingly to their fragmentation pattern, they were mostly formed by hydroxylation during photolysis (Mahmoud et al., 2013).

PTP 204 and PTP3 160 were renamed to PTP 204a and PTP 204b, as well as to PTP3 160a and PTP160b, respectively. In LC-ITMS PTP 204 and PTP3 160, occurred as one peak. However, in LC-HRMS the PTPs were separated, allowing to propose the formation of constitutional isomers. As LC parameters for both instruments were the same, the better separation resulted from the UHPLC working in LC-HRMS, achieving better peak separation compared to LC-ITMS.

M/z values 128, 154, 168 and 188 were identified as primarily formed PTPs. The course of the curves for these PTPs − except for 168 m/z − showed a steep slope during the beginning of the photolysis experiment (Fig. 2A, C and G). PTPs were immediately formed after starting the experiment, constantly increasing up to 64 min and then decreasing, in favor for the formation of follow-up PTPs or mineralization. PTP 154 was probably formed after the loss of water resulting in the formation of a cyclic lactam ring

Table 1
Gabapentin (GAB) and its phototransformation products (PTPs) in chronologic order according to LC-ITMS retention time (t_R), showing the highest observed A/A_o during photolysis and corresponding LC-HRMS t_R with detected accurate mass in LC-HRMS (A is the peak area of the PTP and A_0 is the peak area of GAB at time point 0 min). PTPs were named with m/z value and numbered according to their t_R. The occurrence of PTPs was compared in different concentrations (showing • for occurrence and ∘ for absence during photolysis process).

PTP/GAB	LC-ITMS t_R (min)	Highest observed A/A_0 (%)	LC-HRMS t_R (min)	Detected mass (m/z)	Occurrence of PTPs during photolysis				
					100 mg L^{-1}	20 mg L^{-1}	5 mg L^{-1}	1 mg L^{-1}	0.1 mg L^{-1}
PTP 204a	1.8	10.2	1.70	204.1224	•	•	•	∘	∘
PTP 204b	1.8	10.2	1.91	204.1224	•	•	•	•	∘
PTP1 188	2.1	1.8	1.95	188.1274	•	•	•	•	∘
PTP 168	2.1	4.9	1.79	168.1014	•	•	•	∘	∘
PTP1 186	2.2	3.1	2.08	186.1121	•	•	•	•	•
PTP1 160	2.2	0.2	2.00	160.0966	•	•	∘	∘	∘
PTP2 188	2.4	1.4	2.25	188.1274	•	•	•	•	∘
PTP3 188	2.6	5.8	2.42	188.1275	•	•	•	•	∘
PTP2 160	3.0	0.3	2.88	160.1326	•	•	∘	∘	∘
PTP1 144	3.1	0.2	2.16	144.1378	•	•	∘	∘	∘
PTP4 188	3.2	1.4	3.05	188.1275	•	•	•	•	∘
PTP2 186	3.3	0.4	3.18	186.1115	•	•	•	•	•
PTP5 188	3.4	1.3	3.35	188.1274	•	•	•	•	∘
PTP3 186	4.0	0.8	4.28	186.1118	•	•	∘	∘	∘
PTP3 160a	4.0	2.5	3.81	160.1327	•	•	∘	∘	∘
PTP3 160b	4.0	2.5	3.98	160.1326	•	•	∘	∘	∘
PTP4 186	4.3	0.6	4.62	186.1115	•	•	∘	∘	∘
PTP2 144	4.9	0.4	3.60	144.1380	•	•	∘	∘	∘
PTP3 144	5.4	0.8	3.90	144.1380	•	•	•	∘	∘
PTP5 186	6.1	3.7	6.63	186.1119	•	•	•	∘	∘
PTP4 160	6.2	0.1	6.12	160.1322	•	•	∘	∘	∘
PTP5 160	6.8	0.6	6.75	160.1325	•	•	∘	∘	∘
GAB	7.9	100	7.34	172.1327	•	•	•	•	•
PTP4 144	10.4	0.1	7.64	144.1385	•	•	•	∘	∘
PTP5 144	11.0	0.3	8.02	144.1384	•	•	•	∘	∘
PTP6 144	11.4	0.4	8.48	144.1016	•	•	•	∘	∘
PTP 128	14.2	5.9	10.81	128.1430	•	•	•	∘	∘
PTP 154	16.1	2.3	13.31	154.1220	•	•	•	∘	∘

(Fig. 3). The proposed lactam structure induced myoclonic and generalized clonic seizures in kindled rats (Potschka et al., 2000). In contrast, the expected effect of an antiepileptic drug, like GAB, is preventing from seizures. The lactam is GAB's main degradation product. Its formation becomes more probable after long-time storage or temperatures higher than room temperature and was reported in several other studies (Ciavarella et al., 2007; Hsu and Lin, 2009; Lin et al., 2010). As the photolysis was carried out at controlled temperature of 20 ± 1 °C, it could also be formed during photolysis.

PTP 128 could be formed by decarboxylation of GAB, which results in a typical loss of 44 Da accounting for the carbonic acid moiety (Fig. 3). Several other studies also reported the loss of a carbonic acid moiety during photolysis processes (Rastogi et al., 2014; Sheu et al., 2003; Wang and Lin, 2012).

160 m/z were probably formed as secondary PTPs. PTP3 160a and PTP3 160b were formed after two hydroxylation steps from PTP 128. Position 4 on the cyclohexane is preferred for hydroxylation, because the other positions are sterically hindered. Fragmentation pattern (Text S4, SM) revealed a second hydroxylation in position 1 on the methenamine chain for PTP3 160b and on the methyl group for PTP3 160a.

PTPs formed with 188 m/z are primarily formed by single hydroxylation (Fig. 2G). As a result of the fast further transformation of mono hydroxylated GAB, only PTP3 188 exceeded the threshold of 2% A/A_0. The most probable position for the hydroxylation was position 4 on the cyclohexane ring, because the other positions are sterically hindered. A further hydroxylation step is proposed to explain the formation of PTP 204a and PTP 204b (Fig. 3) as a follow-up PTP of PTP3 188.

It can be assumed that PTP1 186 and PTP5 186 could stem from an intermediary, not analytically detected, structure with 170 m/z. During UV treatment, 170 m/z would be formed from PTP3 188 after

dehydroxylation in combination with formation of a double bond in the cyclohexane ring (loss of water). Further hydroxylation, as in the case of PTP 204a and PTP 204b, would lead to PTP1 186 and PTP5 186, respectively (Fig. 3).

PTP 168 is proposed to be formed directly from the parent compound GAB and presented an increasing slope during the course of the photolysis (Fig. 2E). The proposed structure comprehends to the formation of a cyano group by dehydrogenation of the aminoethyl side chain (Fig. 3). The PTP 168 has been described as one of GAB's impurities by the United States Pharmacopeia (USP, 2013).

The occurrence of each PTP identified at the initial concentration of 100 mg L^{-1} was checked at consecutively lower concentrations (Table 1) as well, in order to evaluate if the PTPs formed at lower concentrations are identical to the ones identified at higher initial concentration of photolysis. This would allow an estimation of the significance of PTPs and their properties to environmentally relevant concentration levels.

As can be seen, each PTP identified at the initial concentration of 100 mg L^{-1} also occurred during photolysis of 20 mg L^{-1} GAB. In the same way, at initial concentration of 5 mg L^{-1} still more than the half of the PTPs were also identified. On the other hand, at initial concentration of 0.1 mg L^{-1} only 2 PTPs could be identified. As a result, for environmentally realistic concentrations not every PTPs, detected at higher concentrations, could be identified. Possible reasons for this could be (i) a different photodegradation pathway, (ii) non-sufficient detections limits, (iii) faster elimination kinetics of PTPs or (iv) the occurrence of PTPs within determined sampling points. Nevertheless, it cannot be excluded that PTPs formed at higher concentrations are formed at lower concentrations as well, which is why further targeted analysis must be conducted to clarify the relevance of suspected PTPs under environmentally realistic conditions.
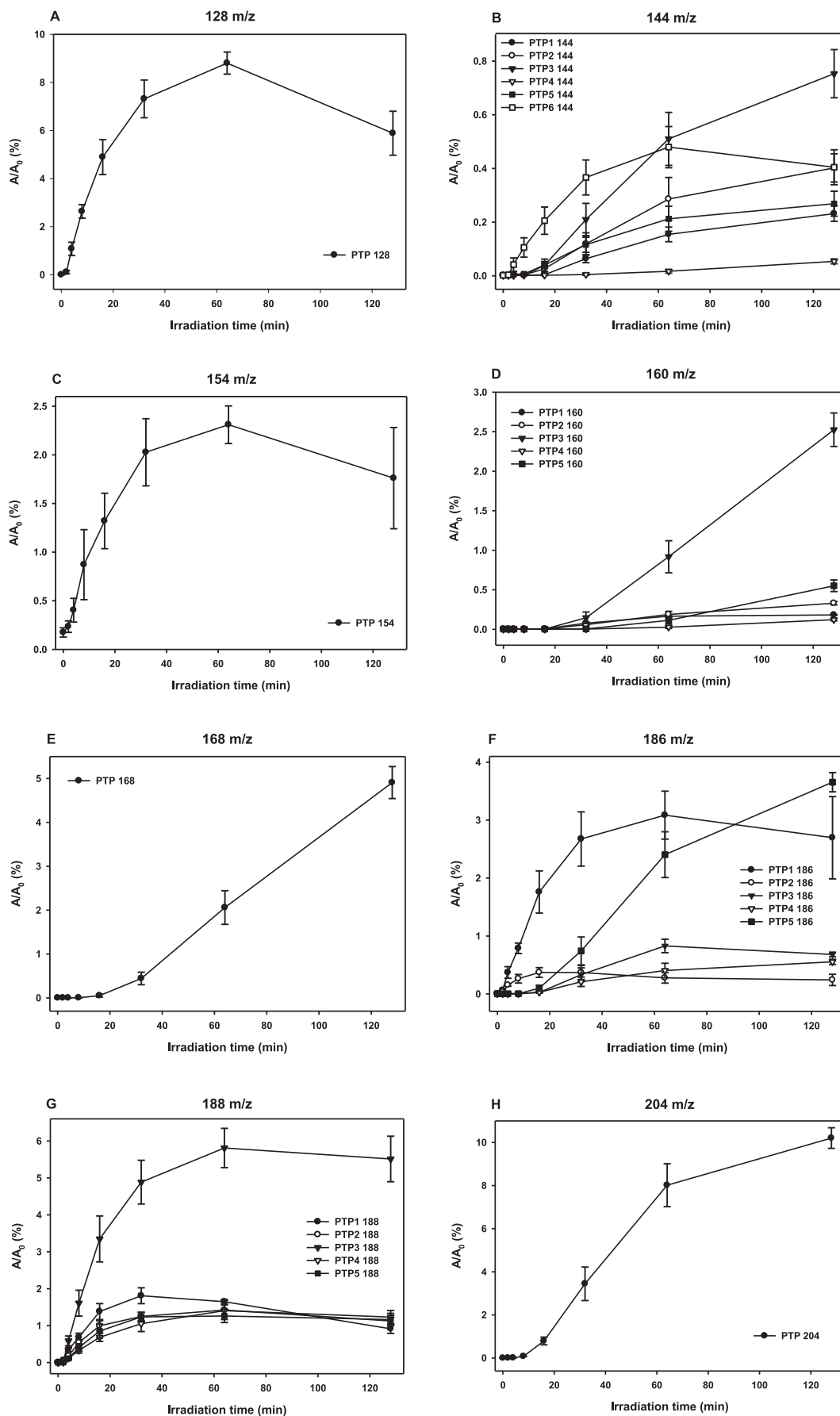
Fig. 2. Relative peak area A/A_0 (%) of phototransformation products (PTPs) during photolysis assigned to their m/z ratio (A is the peak area of the PTP at a specific time point, A_0 is the peak area of GAB at 0 min) (n = 4).
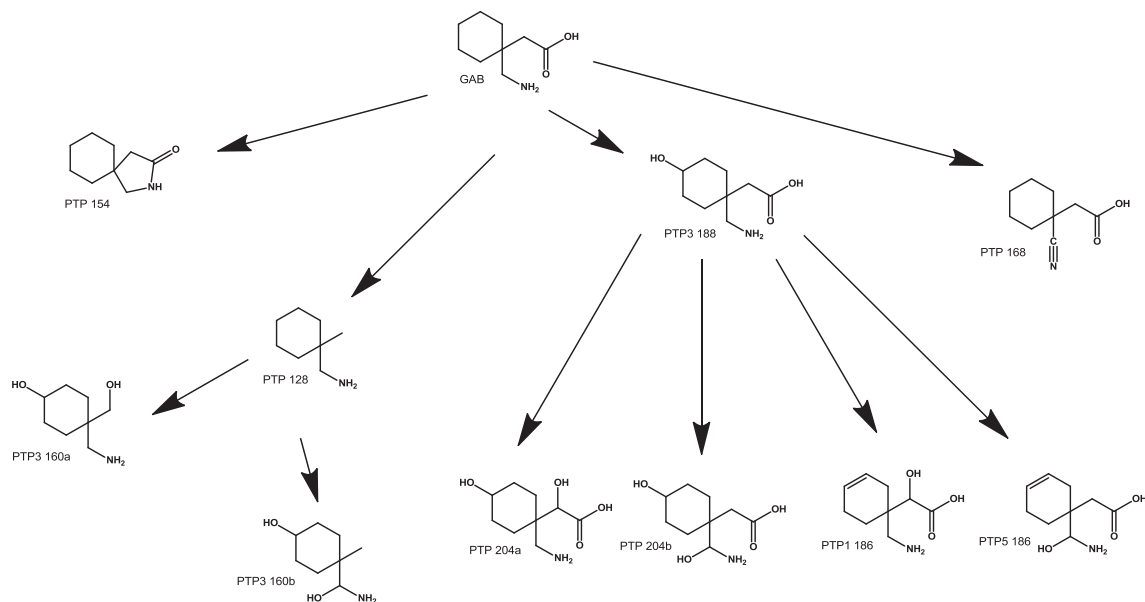
Fig. 3. Tentative phototransformation pathway of structural elucidated phototransformation products (PTPs) during UV photolysis of gabapentin (GAB) with initial concentration of 100 mg L^{-1}.

3.4. Biodegradation testing

All validity criteria of the OECD guideline for CBT were fulfilled.

GAB showed low degradation regarding ThOD achieving $7.9 \pm 3.6\%$ after 28 days. Accordingly, GAB has to be classified as a not readily biodegradable compound (OECD, 1992). The recovery of GAB regarding the peak area ratio S/S_0 was $99.6 \pm 0.13\%$ (n = 4) indicating that no biodegradation TP from GAB was formed. These findings agree with the studies of De la Cruz et al. (2012) and Reungoat et al. (2010), which even showed that GAB was not eliminated in sewage sludge, which contains a much higher density and diversity of bacteria. In conclusion Kasprzyk-Hordern et al. (2008) and Writer et al. (2013) detected GAB in river water. Because of GAB's persistence and increasing consumption, in the future, higher concentrations of GAB in the environment have to be expected.

As the CBT is used to study ready biodegradability for single chemicals with known elementary composition (OECD, 1992), the interpretation on biodegradability for the photolytic mixtures was assessed by means of measured BOD. The BOD time course of ready biodegradable sodium acetate (quality control, control substance), GAB and photolytic mixtures can be seen in Fig. 4A. GAB and photolytic mixtures at time points 32, 64 and 128 min showed no biodegradation, as BOD over the whole test period was very low. In contrast, the BOD for readily biodegradable sodium acetate increased significantly over 28 days. Samples from CBT were also evaluated by means of LC-ITMS. The TIC didn't show any newly occurring peak. Due to comparably low concentrations of the investigated compounds in CBT, only the elimination of GAB and structural elucidated PTPs were taken into account. The peak recovery after 28 days (S/S_0) of GAB and most PTPs was around 100% in every prepared CBT sample (Fig. 4B), thereby excluding any microbial biotransformation. Only PTP 154 showed a slight elimination in the CBT.

3.5. Toxicity screening of photolytic mixtures

In the modified LBT, untreated GAB did not exert a significant effect at the lowest tested dilution level of 1:2 (C$_0$ = 100 mg L^{-1}). In

contrast, photolytic mixtures obtained after photolysis times of 64 and 128 min caused a significant inhibition (>20%) at the same dilution level (long-term luminescence inhibition (LI_{24h}) and growth inhibition (GI_{14h}), respectively, Fig. 5). Analysis of variance (ANOVA) confirmed a significant difference between untreated GAB (0 min) and the photolytic mixtures after 64 and 128 min for all investigated endpoints (P < 0.001).

The strongest inhibitory effects occurred after 128 min of irradiation, indicating an increasing short-term bacterial cytotoxicity (LI_{30min}) and an even more pronounced long-term antibacterial activity (LI_{24h} and GI_{14h}) for the samples collected during UV-photolysis of GAB. This time-dependency argues for a moderate impact on the biological fitness of the bacterial cells that is mainly expressed by a lowered cell multiplication rate. However, it must be assumed that this impact is not limited to specific biosynthetic pathways in prokaryotes because the short-term luminescence inhibition, as an indicator for immediate disturbances of the cell's integrity and physiology, was also significantly affected. Moreover, GAB was already partly mineralized (10% of NPOC-elimination) but not fully primarily eliminated (approx. 13% recovery) after 128 min which contributes to the finding that PTPs of GAB might possess a considerably higher intrinsic toxic potential than the original parent compound. According to the kinetics of PTP formation (Fig. 2), the following structural elucidated PTPs are suspected candidates for the observed effects: PTP 168, PTP5 186, PTP 204a and PTP 204b. Still, as only whole reaction mixtures were tested, synergistic effects (cocktail-effects) between individual PTPs and the parent compound cannot be excluded. Further, reaction by-products such as ROS might also contribute to the observed mixture toxicity. Therefore, suspected candidates should be tested individually in future experiments to confirm their expected toxic potential.

In the umu-test, a significant induction of the umuC gene was neither observed for GAB, nor for the photolytic samples at the lowest applied dilution level of 1:1.5 (C$_0$ = 100 mg L^{-1}). As the growth factor (G) was above 0.5 for all investigated samples, false negative results due to cytotoxicity can be excluded.

It has to be noted that the applied screening tests in this study cannot replace a sound evaluation of environmental toxicity and
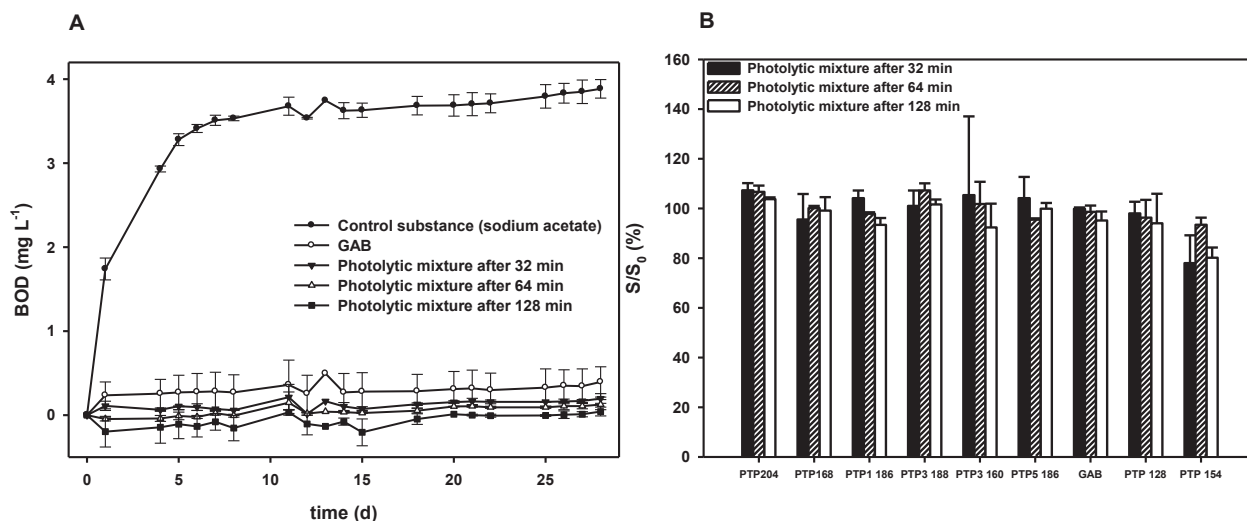
Fig. 4. [A] Time course of biochemical oxygen demand (BOD) over 28 days in the Closed Bottle Test (CBT) showing control substance, gabapentin (GAB) and its photolytic mixtures after 32, 64 and 128 min of UV treatment, respectively (n = 2). [B] Recovery of GAB and its phototransformation products (PTPs) (A/A$_0$ >2%) showing relative peak area S/S$_0$ after 28 days related to day 0 of Closed Bottle Test (CBT) in different photolytic mixtures (n = 2).

possible effects on human health. But they provided clear evidence that PTPs of GAB can have altered (eco)toxicological properties that might be worth receiving further attention. As for the parent compound GAB teratogenicity is already confirmed, the teratogenicity and other possible "side-effects" of environmentally relevant PTPs should also be considered in this context using a read-across approach. However, appropriate testing of all relevant PTPs for teratogenicity and other important toxicological endpoints would require extensive in vitro and in vivo experiments to generate data of ecological relevance. Moreover, the isolation or synthesis of PTPs in appropriate amounts for performance of such assays is difficult, cost-intensive and often not possible. The chemical structures of PTPs were sufficiently elucidated within this study to provide access to powerful in silico tools on the basis of (quantitative) structure-activity relationships ((Q)SARs). Such (Q)SAR-predictions could provide further evidence that might help to develop intelligent strategies for (eco)toxicity testing.

Fig. 5. Toxification of gabapentin (GAB) during UV-photolysis by means of short-term luminescence inhibition (LI_{30min}), long-term luminescence inhibition (LI_{24h}) and growth inhibition (GI_{14h}) in the modified LBT (bars). Initial photolysis concentration (C_0) of GAB was 100 mg L^{-1}. Luminescent bacteria were exposed to photolytic samples in a final dilution of 1:2. Primary elimination and mineralization of GAB during photolysis is shown as percentage of the initial treatment concentration (C/C_0) according to measured peak areas and NPOC concentrations, respectively.

4. Conclusions

GAB was neither entirely eliminated nor fully mineralized by UV photolysis at high elevated concentrations in ultrapure water. Even close to realistic environmental concentrations, a technically long irradiation time was necessary to mineralize GAB. Additionally, newly formed PTPs are not eliminated after biodegradation testing, and therefore could be persistent in the environment. It was demonstrated that some PTPs of GAB might possess altered toxicological properties e.g. toxicity against environmental bacteria. Therefore, further investigations should be conducted regarding the environmental occurrence and the adverse effects of newly formed PTPs of GAB. As for the parent compound GAB teratogenicity is already confirmed, the teratogenicity of PTPs should also be considered in this context.

Moreover, due to the limited sensitivity of (bio)analytical methods, the characterization and elucidation of PTPs should be conducted with initial concentrations that are higher than the environmentally realistic concentrations as to take more formed PTPs into account. However, we recommend to conduct photolysis at different concentration levels to confirm that certain types of PTPs are independent from the initial concentration. Finally, the environmental relevance of PTPs that are suspected to be persistent and/or toxic should be clarified using targeted analysis.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.watres.2015.08.004.

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1   SUPPLEMENTARY MATERIAL

2   FOR

3   IDENTIFICATION OF PHOTOTRANSFORMATION PRODUCTS OF

4   THE ANTIEPILEPTIC DRUG GABAPENTIN: BIODEGRADABILITY

5   AND INITIAL ASSESSMENT OF TOXICITY

6   Manuel Herrmann[ab], Jakob Menz[a], Oliver Olsson[a], *Klaus Kümmerer[a]

7

8   *[a]Sustainable Chemistry and Material Resources, Institute of Sustainable and Environmental*

9   *Chemistry, Leuphana University of Lüneburg, C13, Scharnhorstrasse 1, DE-21335 Lüneburg,*

10   *Germany*

11   *[b]Hospital Pharmacy, Ortenau Klinikum Offenburg-Gengenbach, Ebertplatz 12,*

12   *DE-77654 Offenburg, Germany*

13

14   *Corresponding author*

15   *Postal address: Nachhaltige Chemie und Stoffliche Ressourcen, Institut für Nachhaltige*

16   *Chemie und Umweltchemie, Fakultät für Nachhaltigkeit, Leuphana Universität Lüneburg,*

17   *Scharnhorststraße 1/C13, D-21335 Lüneburg, Germany.*

18   *e-mail: klaus.kuemmerer@leuphana.de*

19   *phone: +49 4131 677 2893; fax: +49 4131 677 2894*

20

21   *e-mail addresses: manuel.herrmann@leuphana.de (Manuel Herrmann),*

22   *jakob.menz@leuphana.de (Jakob Menz), oliver.olsson@leuphana.de (Oliver Olsson),*

23   *klaus.kuemmerer@leuphana.de (Klaus Kümmerer)*

24 **Fig. S1**
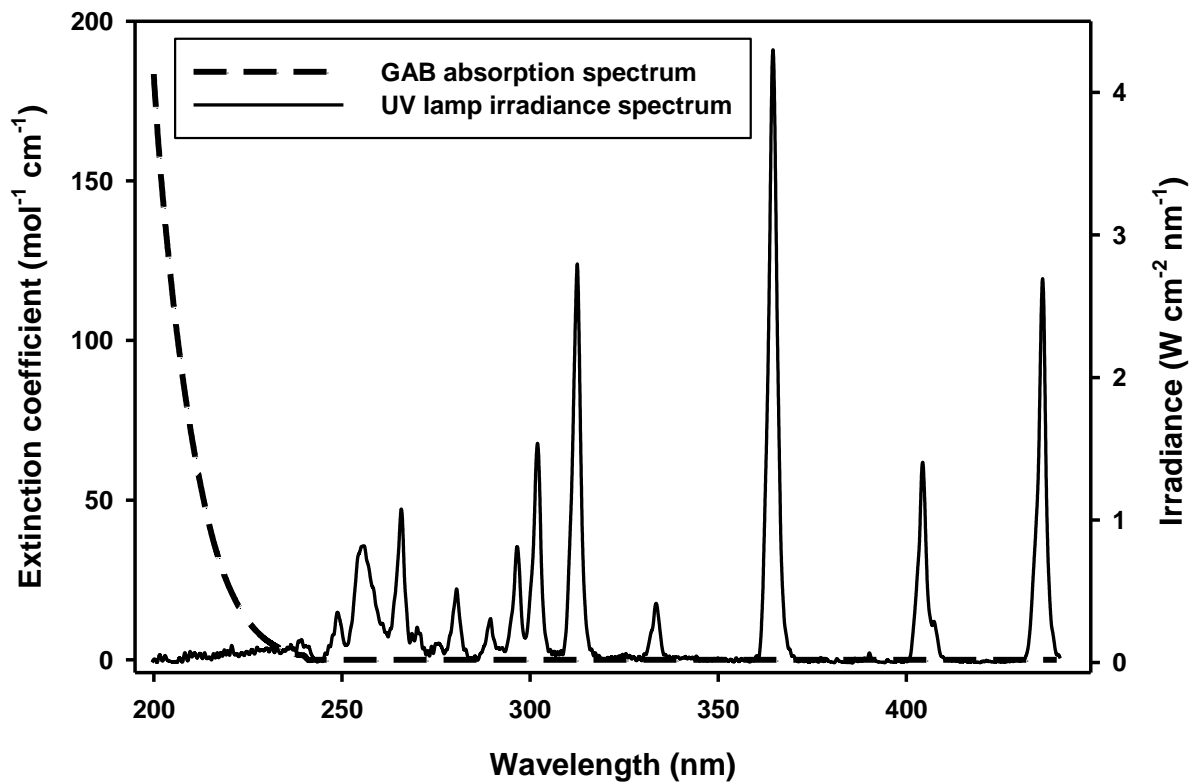
25

26 Fig. S1: Absorption spectrum of gabapentin (GAB) and medium pressure mercury UV lamp

27 irradiance spectrum.

28 **Text S1 Detailed information on biodegradation testing (Closed Bottle Test (CBT))**

29 The Closed Bottle Test (CBT) test was performed in the dark for 28 days at a temperature of

30 20 ± 1 °C. 1 L of mineral medium was inoculated with two drops of effluent after secondary

31 treatment, collected from the sewage treatment plant (STP) in Lüneburg, Germany (144,000

32 population equivalents).

33 Each test consisted of four different series performed in duplicates, respectively. The whole

34 test was repeated two times with respect to variations in the test. The blank sample contained

35 only mineral medium and inoculum from the municipal STP. The quality control consisted of

36 mineral medium, inoculum and readily biodegradable sodium acetate as the only carbon

37 source to be degraded. The test vessels contained gabapentin (GAB) and its photolytic

38   mixtures, respectively, mineral medium and inoculum. The toxicity control consisted of GAB

39   and photolytic mixtures, respectively, mineral medium, inoculum and readily biodegradable

40   sodium acetate in order to monitor toxic effects of GAB on the inoculum bacteria.

41   Different validation criteria had to be fulfilled: (i) the deviation among the duplicates must not

42   exceed 20%; (ii) the toxicity control had to be degraded more than 25% after 14 days (iii) the

43   dissolved oxygen in the blank sample must not decrease more than 1.5 mg L^{-1} within 28 days

44   (iv) the oxygen concentration of the test samples must not be less than 0.5 mg L^{-1} and (v) the

45   quality control has to be degraded at least 60% after 14 days.

46   **Text S2: Detailed information about chromatographic and mass spectrometric settings**

47   **and the primary elimination of Gabapentin (GAB)**

48   **Detailed information about chromatographic and mass spectrometric conditions**

49   Chromatography was performed on a MN Nucleodur® RP-phenyl-hexyl column (EC 125/3

50   mm, 100–3 μm) (Macherey-Nagel, Düren, Germany) applying binary mobile phase consisting

51   of 10 mM ammonium acetate in ultrapure water (A) and methanol (B) at a flow rate of 0.4 mL

52   min^{-1}, oven temperature was 30 °C. The primary elimination was determined using isocratic

53   conditions for 8 min: 70% A and 30% B; retention time (t_R) of GAB was 3.2 min. PTPs were

54   separated in a total run time of 24 min, using a gradient as follows: 0 min: 10% B, 4 min 10%

55   B, 15 min 90% B, 17 min 90% B, 21 min 10% B and 24 min 10% B; t_R of GAB was 7.9 min.

56   Total ion chromatograms (TICs) for low-resolution ion trap mass spectrometer (LC- ITMS)

57   were obtained in positive ionization mode from 90 to 350 m/z with a capillary voltage of 3600

58   V, 30 psi nebulizer pressure, 10 L min L-1 dry gas flow rate at a temperature of 350 °C.

59   TICs for Orbitrap high-resolution mass spectrometer (LC-HRMS) were obtained in positive

60   ionization mode from 90 to 350 m/z with a source voltage of 4000 V, spray voltage of 29 V

61 　　and capillary temperature of 275 °C. Fragmentation to MS^4 was achieved by setting collision-

62 　　induced dissociation (CID) on 35 eV.

63 **Detailed description about primary elimination and detection limits of GAB**

64 　　According to the different initial concentrations (100 mg L^{-1}, 20 mg L^{-1}, 5 mg L^{-1}, 1 mg L^{-1} and

65 　　0.1 mg L^{-1}), the samples were diluted accordingly to meet the linear calibration range. 100 mg

66 　　L^{-1} GAB calibration standards and samples were diluted 1:50 (calibration standards 0.39 -

67 　　100.00 mg L^{-1}, $R^2 = 0.996$, limit of detection (LOD) = 0.09 mg L^{-1}, limit of quantification (LOQ)

68 　　= 0.28 mg L^{-1}). 20 mg L^{-1} and 5 mg L^{-1} GAB calibration standards and samples were diluted

69 　　1:10 (calibrations standards 0.10 - 25.00 mg L^{-1}, $R^2 = 0.996$, LOD = 0.14 mg L^{-1}, LOQ = 0.17

70 　　mg L^{-1}). 1 mg L^{-1} and 0.1 mg L^{-1} GAB calibration standards and samples were not diluted

71 　　(calibration standards 0.05 - 1.56 mg L^{-1}, $R^2 = 0.990$, LOD = 0.05 mg L^{-1}, LOQ = 0.06 mg L^{-1}).

72 **Text S3: Detailed information about toxicity screening test procedures**

73 **Modified luminescent bacteria test (LBT)**

74 　　On the day before testing, a pure culture of the luminescent bacteria strain *Vibrio fischeri*

75 　　NRRL-B-11177 (Hach-Lange GmbH) was prepared in supplemented seawater complete

76 　　(SSWC) media (5% [w/v] Peptone from casein, 0.5% [w/v] Yeast extract, 0.3% [w/v]

77 　　Glycerol, 3% [w/v] NaCl, 44.2 mM NaH_2PO_4, 12.1 mM K_2HPO_4, $MgSO_4$, 0.8 mM 7 H_2O,

78 　　3.8 mM $(NH_4)_2HPO_4$; pH 7). After overnight incubation at 20 °C for 22-24 h, the culture was

79 　　diluted with fresh SSWC media to an initial cell density of 20 formazine turbidity units (FTU)

80 　　and subsequently transferred into a 96-well microplate, adding 100 µL per well. After 30 min

81 　　of preincubation at 15 °C, an initial measurement of luminescence emission and optical

82 　　density (578 nm) was conducted, using a multimode microplate reader (Varioskan Flash,

83 　　Thermo). Subsequently, the test cultures were exposed in triplicates to 100 µL of the

84 　　photolytic samples and a kinetic measurement of luminescence and optical density, was

85 　　carried out for 24 h at 15 °C. In each experiment, 3,5-Dichlorophenol (3,5-DCP, 97%, 591-

86   35-5, Sigma-Aldrich Chemie GmbH) and Chloramphenicol (CAM, 98%, 56-75-7, Sigma-

87   Aldrich Chemie GmbH) were used as positive controls (final assay concentrations: 4.5 mg L^{-1}

88   and 0.05 mg L^{-1}, respectively). Prior to testing, all investigated samples were supplemented

89   with NaCl to a final salinity of 2% (w/v).

90   *Data analysis:* The raw data was normalized to percent inhibition in relation to the negative

91   controls. This was conducted for three different endpoints that are: short-term luminescence

92   inhibition after 30 min (LI_{30min}), long-term luminescence inhibition after 24 h (LI_{24h}) and

93   growth inhibition after 14 h (GI_{14h}).

94   LI_{30min} was calculated according to EN ISO 11348 (DIN 2009). A modified Eq. (1) was used

95   for the calculation of LI_{24h}, which has been described before by Backhaus et al. (1997):

96    $$LI_{24h} = 100\ (I_{NC} - I_t)\ /\ I_{NC} \tag{1}$$

97   LI_{24h} = luminescence inhibition after 24 h (%); I_{NC} = average light intensity of the negative

98   controls after 24 h in relative luminescence units (RLU); I_t = light intensity of the test culture

99   after 24 h (RLU).

100   The measured optical density after 14 h was used for calculation of the growth inhibition (GI)

101   according to Eq. (2):

102   $$GI_{14h} = 100\ (OD_{NC} - OD_t)\ /\ (OD_{NC} - OD_0) \tag{2}$$

103   GI_{14h} = growth inhibition after 14 h (%); OD_t = optical density of the test culture after 14 h;

104   OD_{NC} = average optical density of the negative controls after 14 h; OD_0 = average optical

105   density of the negative controls after sample addition.

106   Inhibition values above 20% were considered as significant. Variations between observed

107   effects at different sampling times were evaluated by one way analysis of variance (ANOVA),

108   following post hoc multiple comparisons (Dunnett's method), in which the untreated samples

109   (0 min) were defined as the control group. ANOVA was performed using the statistical

110   software SigmaPlot 12 (Systat Software).

111   **Umu-test (ISO/FDIS 13829)**

112   The umu-test for genotoxicity was performed according to ISO/FDIS 13829 (ISO/FDIS 1999)

113   with slight modifications. An overnight culture of *Salmonella typhimurium* TA1535 psk 1002

114   (German Collection of Microorganisms and Cell Cultures GmbH) was prepared in TGA-

115   culture medium and incubated for approx. 14 h at 37°C and 250 rpm. Subsequently, the

116   overnight culture was tenfold diluted with fresh TGA-culture medium and incubated for

117   additional 1.5 h to obtain an exponentially growing culture which was used later as inoculum.

118   Then the exposure cultures were prepared by adding the following components into the

119   cavities of a 96-well microplate: 180 µL testing material, 20 µL 10x concentrated TGA-

120   culture medium, 70 µL inoculum and optionally 0.8% (v/v) S9 mix for metabolic activation

121   containing Aroclor 1254-induced rat liver homogenate (Xenometrix AG). Each sample was

122   added as triplicate with an additional reference well containing TGA-culture medium instead

123   of inoculum. The negative control was prepared in nine replicates using ultrapure water

124   instead of testing material. As positive controls 25 ng ml^{-1} of 4-nitroquinoline-1-oxide (4-

125   NQO, Sigma-Aldrich Chemie GmbH) were included in case of testing without metabolic

126   activation and 200 ng ml^{-1} of 2-aminoanthracene (2-AA, Sigma-Aldrich Chemie GmbH) were

127   used in presence of S9 mix. The exposure plate (plate A) was incubated for 2 h at 37°C and

128   250 rpm. Subsequently, the test cultures of plate A were tenfold diluted with TGA-culture

129   medium in another 96-well plate (plate B) and incubated for additional 2 h at 37°C and 250

130   rpm. Thereafter, the optical density at 600 nm (OD_{600nm}) of the samples in plate B was

131   determined using a microplate photometer (Synergy-HT, BioTek Instruments). Then 30 µl of

132   the contents of plate B were placed to a new plate (plate C) containing 120 µL B-buffer,

133   following the addition of 30µL Ortho-Nitrophenol-ß-d-galactopyranoside (ONPG, Carl Roth

134   GmbH) solution. Plate C was incubated for 30 min at 28°C, 250 rpm, after which the reaction

135   was stopped using the stop reagent. Finally, the absorbance at 420 nm (A_{420nm}) of plate C was

136   measured to determine the β-galactosidase activity.

137   *Data analysis:* The growth factor (*G*) and the induction ratio (*IR*) were calculated according to

138   ISO/FDIS 13829 (ISO/FDIS 1999) on the basis of OD_{600nm} and A_{420nm} respectively.

139   **Text S4: Fragmentation pattern of Gabapentin (GAB) and its phototransformation**

140   　　**products (PTPs)**

141   For each m/z value the extracted ion chromatogram (EIC) (initial photolysis concentration:

142   100 mg L^{-1}) obtained by an Agilent 1100 series HPLC system coupled with a Bruker Esquire

143   6000[plus] mass spectrometer (LC-ITMS), and a Dionex Ultimate 3000 UHPLC system coupled

144   with a LTQ Orbitrap-XL high-resolution mass spectrometer (LC-HRMS), respectively, are

145   shown. The MS^4 fragmentation spectrum, obtained by LC-HRMS, is shown for Gabapentin

146   (GAB) and structure elucidated phototransformation products (PTPs) assigned to the

147   corresponding EIC.

148

149   **172 m/z**
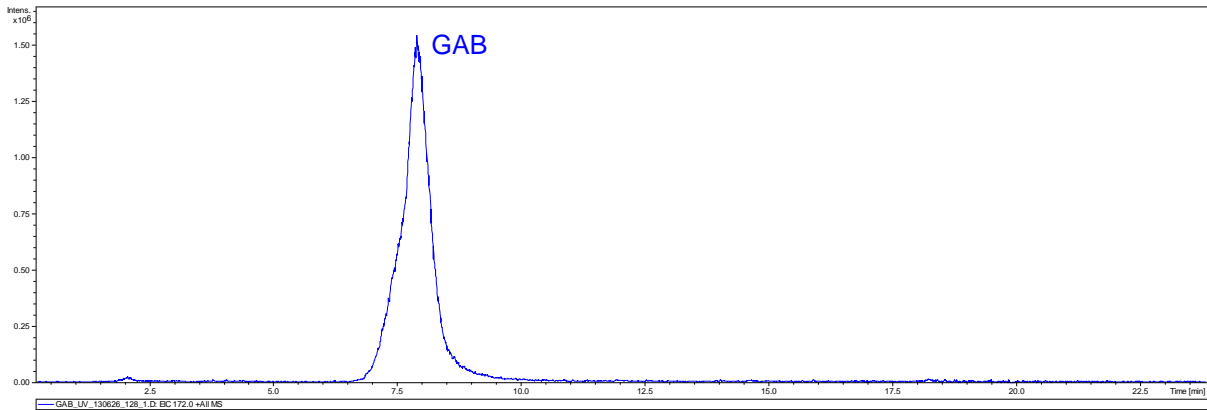
150

151

152   **Gabapentin (GAB)**

153

Chemical Formula: C₉H₁₈NO₂⁺ → Chemical Formula: $C_9H_{18}NO_2^+$
Exact Mass: 172.1332

Chemical Formula: $C_9H_{13}O^+$
Exact Mass: 137.0961

Chemical Formula: $C_{9H16N}O^+$
Exact Mass: 154.1226

Chemical Formula: $C_9H_{11}^+$
Exact Mass: 119.0855

Chemical Formula: $C_9H_{14}N^+$
Exact Mass: 136.1121

Chemical Formula: $C_9H_{13}O^+$
Exact Mass: 137.0961

Chemical Formula: $C_9H_{11}^+$
Exact Mass: 119.0855

Chemical Formula: $C_7H_9^+$
Exact Mass: 93.0699

Chemical Formula: $C_9H_{11}^+$
Exact Mass: 119.0855

Chemical Formula: $C_7H_7^+$
Exact Mass: 91.0542

154

155

156 **128 m/z**

158

159 **PTP 128**

160

Chemical Formula: $C_8H_{18}N^+$
Exact Mass: 128.1434

Chemical Formula: $C_5H_9^+$
Exact Mass: 69.0699

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Exact Mass: 111.1168

161

162

163   **154 m/z**

164

165

166

167   **PTP 154**
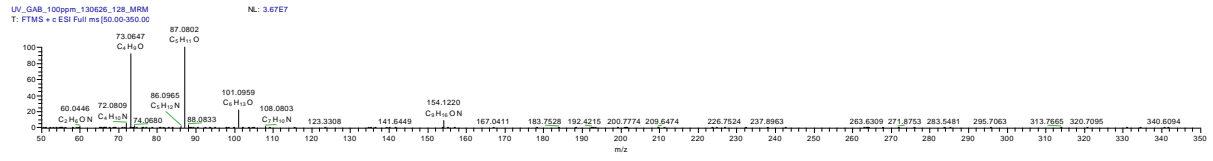
168

169

170   **160 m/z**

171

172

173 **PTP3 160a**

174

175

176

177

178

180

181

182 **168 m/z**

183

184

185 **PTP 168**

186

187

Chemical Formula: C7H7+
Exact Mass: 91.0542

Chemical Formula: C9H14N O2+
Exact Mass: 168.1019

Chemical Formula: C7H10N+
Exact Mass: 108.0808

Chemical Formula: C8H12N+
Exact Mass: 122.0964

Chemical Formula: C9H12N O+
Exact Mass: 150.0913

Chemical Formula: C8H9+
Exact Mass: 105.0699

Chemical Formula: C7H10N+
Exact Mass: 108.0808

Chemical Formula: C8H12N+
Exact Mass: 122.0964

Chemical Formula: C7H9+
Exact Mass: 93.0699

188

189

190   **186 m/z**

191

192

PTP1 186

194

195

197

198

199

200   **188 m/z**

203   **PTP3 188**

Chemical Formula: C₈H₁₃⁺
Exact Mass: 109.1012

Chemical Formula: C₉H₁₈NO₃⁺
Exact Mass: 188.1281

Chemical Formula: C₉H₁₁O⁺
Exact Mass: 135.0804

Chemical Formula: C₉H₁₄NO⁺
Exact Mass: 152.1070

Chemical Formula: C₉H₁₆NO₂⁺
Exact Mass: 170.1176

Chemical Formula: C₉H₁₃O₂⁺
Exact Mass: 153.0910

Chemical Formula: C₇H₉₊
Exact Mass: 93.0699

Chemical Formula: C₉H₁₄NO⁺
Exact Mass: 152.1070

Chemical Formula: C₉H₁₃O₂⁺
Exact Mass: 153.0910

205

206

207 **204 m/z**

208

PTP 204

209

PTP 204a

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60.0445
C2H6ON

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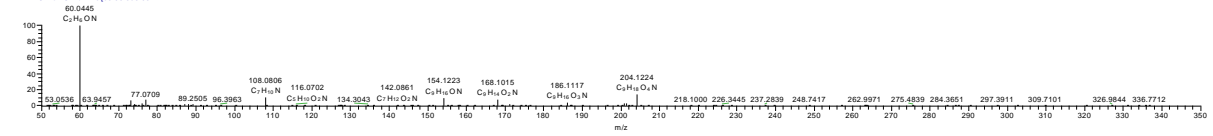
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168.1017
C9H14O2N

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m/z
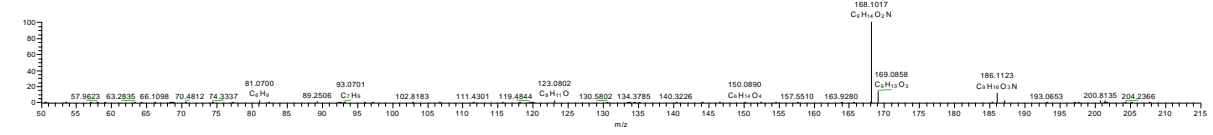
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150.0915
C9H12ON

52.9840 66.1099 70.8858 79.3266 84.4086 89.2505 92.6472 97.8014 103.1558 107.0856 C8H11 115.8733 123.0187 127.7979 136.3806 C8H14ON 140.1078 147.3276 151.7890 158.3091 161.3616 170.8249 175.2027

m/z
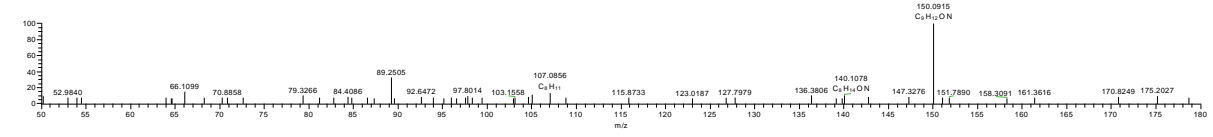
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89.2508
89.7758

52.3407 56.6705 61.9972 66.1099 72.5826 75.1660 80.6532 83.0303 85.0920 92.2753 94.6033 99.1868 106.0439 111.9165 115.9637 120.7872 122.0961 C8H12N 126.9096 136.7905 140.5803 148.2233 157.3658

m/z

Chemical Formula: C7H9+
Exact Mass: 93.0699

Chemical Formula: C9H18NO4+
Exact Mass: 204.1230

Chemical Formula: C8H11O+
Exact Mass: 123.0804

Chemical Formula: C9H13O3+
Exact Mass: 169.0859

Chemical Formula: C9H14NO2+
Exact Mass: 168.1019

Chemical Formula: C9H16NO3+
Exact Mass: 186.1125

Chemical Formula: C8H14NO+
Exact Mass: 140.1070

Chemical Formula: C9H12NO+
Exact Mass: 150.0913

Chemical Formula: C8H11+
Exact Mass: 107.0855

Chemical Formula: C8H12N+
Exact Mass: 122.0964

213 **PTP 204b**

214

215

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Artikel 3

Experimental and *in silico* Assessment
of Fate and Effects of the Antipsychotic Drug Quetiapine
and Its Bio- and Phototransformation Products
in Aquatic Environments

Manuel Herrmann, Jakob Menz, Matthias Gassmann,
Oliver Olsson, Klaus Kümmerer

Experimental and *in silico* assessment of fate and effects of the antipsychotic drug quetiapine and its bio- and phototransformation products in aquatic environments[☆]

CrossMark

Manuel Herrmann [a, b], Jakob Menz [a], Matthias Gassmann [c], Oliver Olsson [a], Klaus Kümmerer [a, *]

[a] Sustainable Chemistry and Material Resources, Institute of Sustainable and Environmental Chemistry, Leuphana University of Lüneburg, C13, Scharnhorststrasse 1, DE-21335 Lüneburg, Germany
[b] Hospital Pharmacy, Ortenau Klinikum Offenburg-Gengenbach, Ebertplatz 12, DE-77654 Offenburg, Germany
[c] Water Quality Management — Modelling and Simulation, Institute of Water, Waste and Environment, Kurt-Wolters-Strasse 3, DE-34125 Kassel, Germany

ARTICLE INFO

ABSTRACT

The antipsychotic drug quetiapine (QUT) has been frequently detected in sewage treatment plants. However, information on the fate of QUT in aquatic environments and its behavior during UV treatment is limited. In this study, QUT is shown not to be readily biodegradable in the Closed Bottle Test and the Manometric Respirometry Test according to OECD guidelines. The main biotransformation product (BTP) formed in the tests, a carboxylic acid derivative, was identified by means of high-resolution mass spectrometry. This BTP is presumably a human metabolite and showed higher detection rates than QUT in a river sampling campaign conducted in northern Germany. UV elimination kinetics of QUT at different initial concentrations (226.5, 45.3, 11.3, and 2.3 μmol L^{-1}) were faster at lower initial concentrations. All seven phototransformation products (PTPs) could be still identified at initial concentration of 11.3 μmol L^{-1}. The photolytic mixture generated after 128 min of photolysis of QUT was not better biodegradable than QUT. Initial UV treatment of QUT led to the formation of several additional BTPs. Four of them were identified. The bacterial cytotoxicity and genotoxicity before and after phototransformation of QUT in a modified luminescent bacteria test (LBT) and the umu-test (ISO/FDIS 13829) showed cytotoxic effects in the LBT for QUT. Furthermore, PTPs had similar cytotoxic effects on luminescent bacteria. The umu-test did not reveal any genotoxic activity for QUT or PTPs. In conclusion, the release of QUT into sewage treatment plants and aquatic environments could result in the formation of a main BTP. Additional UV treatment of QUT would lead to the formation of additional BTPs. Moreover, treatment did not result in lower toxicity to tested organisms. In conclusion, UV treatment of QUT should be considered critically as a potential treatment for QUT in aquatic systems.

1. Introduction

The occurrence of active pharmaceutical ingredients (APIs) in the environment is a well-known issue in environmental research (Al Aukidy et al., 2012; Kümmerer, 2009; Petrie et al., 2015). APIs of various classes, including neurological drugs, have been found in different environmental compartments (Mackulak et al., 2015; Nödler et al., 2010; Subedi et al., 2013). As the worldwide consumption of especially second-generation antipsychotics increased in recent years (Lertxundi et al., 2012; Verdoux et al., 2010), it can be assumed that higher amounts of these kinds of drugs are being discharged into the environment. In particular, quetiapine (QUT) has been used in high amounts for the treatment of psychiatric diseases in England and Canada (Ilyas and Moncrieff, 2012; Pringsheim and Gardner, 2014). Likewise, QUT had the highest prescription volume of all antipsychotic drugs in German

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households with a calculated consumption of 24.9 t in 2014 (Schwabe and Paffrath, 2015).

QUT is almost entirely metabolized in human bodies (AstraZeneca Pharmaceuticals, 2013). However, recent studies have shown that QUT can be found in influents of sewage treatment plants (STPs) at average concentrations of 90 ng L^{-1} (Gurke et al., 2015) and up to 43.6 ng L^{-1} (Subedi and Kannan, 2015). The excreted fraction of unchanged QUT can be primarily eliminated up to 87% by STPs (Subedi and Kannan, 2015). However, other studies observed QUT in even higher concentrations in effluents of different STPs (up to 100 ± 100 ng L^{-1} and 1168 ± 66 ng L^{-1}) (Oliveira et al., 2015; Yuan et al., 2013; respectively).

Comprehensive information on the environmental fate and effects of QUT in general and biodegradation in aquatic systems in particular is not available. There are a few indications for the non-ready biodegradability of QUT (Food and Drug Administration, 2007). Data on possible biotransformation products (BTPs) resulting from incomplete mineralization of the parent compound in STPs or aquatic environments is, however, missing. As the known metabolite QUT carboxylic acid is not active in humans (Food and Drug Administration, 2007), no studies have been conducted regarding the occurrence and fate of this compound in the environment. Mahmoud et al. (2013) already showed that human metabolites and environmental BTPs can be identical. Therefore, it is very likely that the carboxylic acid product of QUT is also formed by bacteria in aquatic systems in oxidation processes. Moreover, some studies have already shown that BTPs can be produced in high amounts by bacteria in surface water (Mahmoud and Kümmerer, 2012; Trautwein and Kümmerer, 2011).

Since QUT can be seen as an API consumed in high amounts at psychiatric hospitals and nursing homes (Herrmann et al., 2015a), the elimination of QUT and its metabolites at the emission source could reduce influent concentrations at STPs. UV irradiation has been discussed as a potential decentralized treatment option (Kovalova et al., 2013). Furthermore, UV radiation is often applied for the finishing of potable water. Different studies have assessed the performance of UV treatment regarding the elimination of APIs from water (Kim et al., 2009; Pereira et al., 2007). However, these decentralized treatment systems have to completely degrade, i.e mineralize substances into non-toxic compounds such as carbon dioxide and water, as incomplete degradation could result in environmental and health problems originating either from the parent compound or its phototransformation products (PTPs). UV treatment of QUT was assessed in the study by Skibiński (2012), who identified five PTPs formed from QUT in methanol by means UV-C irradiation. Data on the fate and effects of these PTPs is, however, still missing. Consequently, an analysis of the degradation efficiency of QUT, the identification of PTPs, and their assessment are required if one is to assess whether UV treatment systems could be used to eliminate QUT from wastewater or potable water finishing (Herrmann et al., 2015b; Mahmoud et al., 2014). Recent studies have shown that PTPs or BTPs could have a negative effect on environmental organisms and be more persistent than the parent compound itself (Gutowski et al., 2015; Illés et al., 2014).

In light of the findings discussed above, the main objectives of this study were (i) to provide new insights concerning the fate of QUT in aquatic environments, (ii) to determine the effect of UV radiation on the behavior of QUT in aqueous solution to evaluate the degradation and mineralization efficiency and suitability of UV treatment, and (iii) to obtain additional information on the formation of BTPs and PTPs. To fulfill these objectives, the biodegradability and biotransformation of QUT in the Closed Bottle Test (CBT) and the MRT according to Organisation for Economic Co-operation and Development (OECD) 301 D and F, respectively, were studied. In addition, selective water sampling was conducted

at six rivers of a rural county in northern Germany for analytical determination of QUT and the BTP that was observed in laboratory testing. Moreover, QUT, at different initial concentrations in aqueous solution, was treated with UV light to investigate elimination kinetics, degree of mineralization and to elucidate the structure of its PTPs. The primary elimination of QUT and structure elucidation was performed by LC-UV-MS^n. Photolytic mixtures of QUT and the generated PTPs were also analyzed in terms of biodegradability, i.e. mineralization and the possible formation of BTPs. The bacterial cytotoxicity and genotoxicity of QUT and the photolytic mixtures were also assessed in a modified luminescent bacteria test (LBT) and the umu-test according to ISO/FDIS 13829, respectively.

2. Materials and methods

2.1. Chemicals and reagents

All tests were conducted with QUT hemifumarate (certified purity 98%) purchased from LGC Standards GmbH (Wesel, Germany). Sodium fumarate dibasic (purity $\geq$ 99%) was purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). Ammonium acetate (HiPerSolv CHROMANORM® for HPLC) and methanol (HiPerSolv CHROMANORM® for HPLC, LC-MS grade) were purchased from VWR International GmbH (Darmstadt, Germany). Aqueous mobile phase, standard solutions, and solutions for photolysis treatments were prepared with ultrapure water.

2.2. Simulated UV treatment

The test solutions were freshly prepared with ultrapure water, and 100, 20, 5, and 1 mg L^{-1} of QUT hemifumarate to reach corresponding initial concentrations of 226.5, 45.3, 11.3, and 2.3 µmol L^{-1} of QUT, respectively. Photolysis experiments were carried out in a 1 L batch immersion tube photo reactor using 0.8 L of sample volume. A medium-pressure mercury lamp (TQ 150, UV Consulting Peschl, Mainz, Germany) surrounded by ilmasil quartz glass was used as a polychromatic radiation source. Information on the relative emission spectrum of the lamp can be found in the SM (supplementary material), Text S1. The actual absolute photon flux of the lamp was determined by chemical actinometry. Using this information and the molar extinction coefficient of QUT, the quantum yield of QUT was calculated (SM, Text S2). Magnetic stirring ensured continuous mixing of the solution. Constant temperature (20 ± 1 °C) was guaranteed by using a cooling system (WKL230, LAUDA, Berlin, Germany).

The photolysis experiments were carried out for 128 min. Samples were collected before treatment, and after 2, 4, 8, 16, 32, 64, and 128 min of treatment for an LC-UV-MS^n analysis (primary elimination and structure elucidation) (see Section 2.4) and dissolved organic carbon (DOC) determination for degree of total mineralization (see Section 2.4). For toxicity screening, samples at initial UV treatment concentration of 226.5 µmol L^{-1} of QUT were collected at the identical time points (see Section 2.6). In addition, photolytic samples after 128 min of treatment at initial concentration of 226.5 µmol L^{-1} were collected for biodegradation testing (see Section 2.3). Kinetic curve fitting was performed with Sigma-Plot 11 (Systat Software, San Jose, USA).

2.3. Biodegradation testing

QUT hemifumarate and the photolytic mixture after 128 min of UV irradiation at initial concentration of 226.5 µmol L^{-1} underwent two biodegradation tests with different contents in terms of test substance, minerals, and inoculum according to OECD guidelines

301 D (CBT) and 301 F (MRT), respectively. All biodegradation tests were performed in duplicates (n = 2). The applied inoculum was collected from the effluent of the municipal STP in Lüneburg, Germany (144,000 population equivalents). In both tests, a chemical is classified as readily biodegradable if the measured biochemical oxygen demand (BOD) reaches at least 60% of the theoretical oxygen demand (ThOD) (OECD, 1992). As the standard substance of QUT was only available as a fumaric acid salt, partial degradation could be attributed to readily biodegradable fumaric acid. To confirm this assumption, the CBT was also conducted with fumaric acid to measure its extent of biodegradability compared to QUT hemifumarate.

Samples taken at the beginning and the end of both tests underwent LC-MS^n analysis (see Section 2.4). In the case of the MRT, a DOC analysis (see Section 2.4) was also performed.

2.3.1. Closed Bottle Test (CBT)

The CBT was performed according to OECD test guidelines (OECD, 1992) using a low content of minerals and inoculum. Likewise, ready biodegradability in aquatic environments was simulated. 1 L of mineral medium was inoculated with two drops of STP effluent. The test was conducted in the dark for 28 days at a temperature of 20 ± 1 °C. The initial concentration of QUT hemifumarate was 2.6 mg L^{-1} (5.9 μmol L^{-1}) corresponding to a ThOD of 5 mg L^{-1}. The final concentration of the photolytic mixture after 128 min was adjusted according to the remaining DOC concentration to reach a similar ThOD. The test concentration of fumaric acid in the CBT was 8.3 mg L^{-1} (71.5 μmol L^{-1}) corresponding to a ThOD of 5 mg L^{-1}. Detailed information is prodived in the SM, Text S3.

2.3.2. Manometric Respirometry Test (MRT)

The MRT was also performed according to OECD guidelines (OECD, 1992). This test has a higher inoculum density than the CBT. 1 L of mineral medium was inoculated with 80 mL of STP effluent. The test was also conducted in the dark for 28 days at a temperature of 20 ± 1 °C. The test concentration of QUT hemifumarate was 15.3 mg L^{-1} (34.7 μmol L^{-1}) corresponding to a ThOD of 30 mg L^{-1}. The final concentration of the photolytic mixture after 128 min was adjusted according to the remaining DOC concentration to reach a similar ThOD. Fumaric acid was not measured in the MRT because it was assumed that it is very likely to be readily biodegradable in the CBT. Detailed information is prodived in the SM, Text S4.

2.4. Analytical conditions

The degree of mineralization for photolysis experiments and the MRT was analyzed by a Total Organic Carbon Analyzer (TOC 5000, Shimadzu GmbH, Duisburg, Germany). Primary elimination by UV absorption at 254 nm in all photolytic samples were tested on a Shimadzu Prominence HPLC (Shimadzu GmbH, Duisburg, Germany) (SM, Text S5).

An LC-MS^n analysis was performed a) for screening on an Agilent 1100 series HPLC system (Agilent Technologies, Waldbronn, Germany) coupled to a Bruker Esquire 6000^{plus} low resolution mass spectrometer with an ESI source (Bruker Daltonics, Bremen, Germany) (LC-ITMS) and b) a Dionex Ultimate 3000 UHPLC system (Dionex, Idstein, Germany) coupled with a LTQ Orbitrap-XL high-resolution mass spectrometer with a H-ESI source (Thermo Scientific, Bremen, Germany) (LC-HRMS) for confirmation of the chemical structure of the transformation products. The chromatographic method described above was also for LC-MS^n analysis (see SM, Text S5).

At initial concentration of 226.5 μmol L^{-1} the peak area A of PTPs were related to the peak area of QUT A$_0$ at time point 0 min. Every PTP exceeding 1% of A/A$_0$ was structurally elucidated. Detailed information on the mass spectrometric method regarding peak area ratios can be found in SM, Text S6. Structures were analyzed using the ITMS in Auto-MS Mode and, to improve the reliability of results, the HRMS up to MS^3 (see SM, Text S7). Moreover, the occurrence of PTPs at different initial concentrations was compared to confirm that the formation of PTPs is not depending on initial concentration.

Samples from both biodegradation tests were analyzed with the help of LC-ITMS using recovered peak areas of QUT and its PTPs S/S$_0$ (S is the peak area of the PTP at day 28, and S$_0$ is the peak area of the PTP at day 0). Structural elucidation was conducted for each BTP at each new peak in the total ion chromatogram of the MRT. Structures were established with the help of ITMS in Auto-MS Mode and verified with the help of HRMS up to MS^3 (see SM, Text S7). In addition, the biotransformation pathway of QUT and PTPs was predicted using Meta software (version 1.8.1, Multicase Inc. Beachwood, USA) and Eawag Biocatalysis/Biodegradation Database (Eawag, 2016) to improve the reliability of the structural elucidation of BTP. Further information on Meta software can be found in the SM, Text S8.

2.5. Sampling site and surface water analysis

QUT and its main biotransformation product BTP 398 were monitored in six tributaries of the Ilmenau River, a tributary to the Elbe River, in the district of Lüneburg, Lower Saxony, Germany. A map is available in the SM, Fig. S4, Text S9. Grab sampling, similar to González Alonso et al. (2010) and López-Serna et al. (2012), was conducted every month at seven locations between October 2014 and February 2015 resulting in a total number of 35 samples, which were then analyzed in triplicates using LC-HRMS. Further information on sample preparation and instrumental analysis can be found in the SM, Text S9. As no analytical standard for BTP 398 was available and the chemical structure was similar, QUT was used as a surrogate for analysis. Likewise, it was assumed that mass spectrometric ionization and the behavior during solid phase extraction of BTP 398 were similar to QUT. The limit of detection (LOQ) and limit of quantification (LOQ) for QUT were 1.3 ng and 3.8 ng L^{-1}, respectively.

2.6. In vitro bioassays

The cytotoxic effect on bacteria was assessed in a modified LBT with *Vibrio fischeri* NRRL-B-11177 (Hach-Lange GmbH, Düsseldorf, Germany) following Menz et al. (2013). This test allows for the combined assessment of short-term (30 min) and long-term (24 h) inhibition of bacterial luminescence emission. In addition, the impact on bacterial cell proliferation was evaluated during the transition from exponential growth to the stationary phase after 14 h of incubation. The exposure cultures were prepared in triplicates (n = 3) and the final sample concentration in the test media was 50% (v/v). A detailed description of the experimental procedure is presented in the SM, Text S10. Concentration-response relationships in the modified LBT were established by fitting the experimental data to a four parametric Hill-function (Eq. (1)),

$$y = min + (max - min) \left/ \left(1 + (x/EC_{50})^{-Hillslope}\right)\right. \tag{1}$$

y is the inhibition in %, min is the bottom of the curve, max is the top of the curve, Hillslope is the slope of the curve at its midpoint, and EC$_{50}$ is the half-maximal effective concentration. Curve fitting was performed with the statistical software SigmaPlot 11 (Systat Software, San Jose, USA).

The genotoxic effect on bacteria was assessed using the umu-

test with *Salmonella typhimurium* TA1535 psk 1002 (German Collection of Microorganisms and Cell Cultures GmbH, Braunschweig, Germany) according to ISO/FDIS 13829. The umu-test is based on the colorimetric measurement of the *umuC* gene induction, which is upregulated in the applied tester strain as response to genotoxic lesions in the DNA (ISO/FDIS, 1999). Therefore, the *umuC* induction ratio (*IR*) provides useful information on the genotoxic potential of the tested sample. The exposure cultures were prepared in triplicates (n = 3) and the final sample concentration in the test media was 66.7% (v/v). A detailed description of the experimental procedure is presented in the SM, Text S10.

3. Results and discussion

3.1. Biodegradation of the parent compound quetiapine (QUT) hemifumarate in the Closed Bottle Test (CBT) and the Manometric Respirometry Test (MRT)

3.1.1. Assessment of ready biodegradability

All validity criteria in the CBT were fulfilled. As degradation rates reached $16.6 \pm 1.5\%$, QUT hemifumarate has to be, according to OECD guidelines (OECD, 1992), classified as not readily biodegradable. Moreover, a partial degradation of QUT hemifumarate of up to 6% can be attributed to fumaric acid (ThOD of fumarate: 0.3 mg L^{-1}), because it was readily biodegradable in the CBT (degradation of $86.1 \pm 1.0\%$). Elimination of QUT was assessed for recovered peak area S/S_0. After 28 days, $80 \pm 4\%$ of the peak area was recovered. Therefore, it can be assumed that BTPs were formed.

In the MRT, all validity criteria were fulfilled. QUT hemifumarate was degraded to an extent of $-1.9 \pm 3.2\%$. Therefore, QUT hemifumarate has to be classified as not readily biodegradable according to OECD test guidelines (OECD, 1992) in the MRT as well. Likewise, the measured DOC was eliminated by $5.5 \pm 1.7\%$. In contrast, QUT was recovered by only $11 \pm 11\%$ according to peak area ratio S/S_0. As no mineralization was observed and QUT was almost entirely eliminated, the formation of BTPs is even more likely in the MRT than in the CBT. In the study by Trautwein and Kümmerer (2012), it was also shown that numerous BTPs were formed in biodegradation tests, especially at tests which are using higher inoculum density and diversity such as MRT compared to CBT.

3.1.2. Biotransformation of quetiapine (QUT)

Only one product with an m/z value of 398 was formed (see SM, MRT chromatogram in Fig. S5A, Text S11). Structure elucidation was conducted from samples of the MRT, as BTP 398 was formed in both biodegradation tests (Table 1). The most likely structure proposed for BTP 398 based on analytical results is that of a carboxylic acid derivative. It was formed as a result of the oxidation of the alcohol group and the formation of a group characterized by a carboxylic function with an aldehyde as an intermediate product. Oxidation via addition of oxygen on the sidechain of QUT was confirmed by MS spectra. However, the exact position could not be proven (SM, Text S12). Meta software also predicted that carboxylic acid derivative is a possible BTP (SM, Text S8, Fig. S2). Suggested mechanisms are oxidation by alcohol dehydrogenase and aldehyde dehydrogenase to form aldehyde and carboxylic acid, respectively. Therefore, oxidation of QUT resulting in the carboxylic acid derivative was considered to be the most likely explanation. Eawag Biodegradation Database did not predict suitable biodegradation patterns. It suggested as a first biotransformation step, cleavage on the nitrogen of the tricyclic ring system. Further transformation did not lead to the observed m/z values and fragmentation patterns in MS spectra.

The same structure is formed in high amounts by oxidation in the human metabolism recovering 14.7% of QUT as carboxylic acid

metabolite in plasma (DeVane and Nemeroff, 2001). According to Food and Drug Administration (2007), 29% of a given dose was excreted as the carboxylic acid metabolite. As the carboxylic acid derivative of QUT is formed in the human metabolism and by bacteria, several biotic processes promote the formation of the compound. Therefore, high detection rates in surface water were assumed to be very likely. To strengthen this hypothesis, a small monitoring campaign for QUT and BTP 398 was conducted.

Table 2 presents the results of the sampling campaign. QUT was not detected in any sample. At two sampling points of the same river (MP3 and MP4), BTP 398 was detected in almost every sample. The data shows that the concentrations in the river increased downstream, indicating additional BTP sources after the first sampling point (MP3). Continuous positive detection of BTP 398 at MP3 and MP4 might be due to STP effluents. Furthermore, the highest detection rates were at sampling points with a high density of small STPs in the catchments, indicating a possible influence of small STPs on the sampled concentrations. However, despite the presence of an STP and the highest density of small STPs in the catchment of MP5, no BTP was detected in these samples. As a result, BTP 398 showed high detection rates compared to QUT. This is very likely because BTP 398 is formed in relatively large quantity during human metabolism, biological wastewater treatment and by bacteria in surface water.

3.2. UV treatment of quetiapine (QUT)

3.2.1. Primary elimination and mineralization

Fig. 1A shows QUT elimination by irradiation with UV light at different initial concentrations in ultrapure water. At an initial concentration of 226.5 μmol L^{-1}, elimination of QUT followed zero-order kinetics with a rate constant k of 0.478 min^{-1} ($R^2 = 0.996$). The calculated half-life $t_{1/2}$ was 101 min. Likewise, QUT was not fully eliminated after 128 min of irradiation. The residual QUT recovery rate was 39%. As DOC was only eliminated for 1% (data not shown), it can be assumed that the loss of QUT is due to the formation of one or several PTPs during the treatment. The color of the test solution constantly changed from clear colorless to clear yellowish. Therefore, QUT was probably transformed to PTPs absorbing light in a comparably higher wavelength range. At an initial concentration of 45.3 and 11.3 μmol L^{-1}, QUT elimination followed first-order kinetics (Fig. 1A), a result that is identical with that by Skibiński (2012), who performed tests at an initial concentration of 26 μmol L^{-1}. After 64 min, QUT was entirely eliminated at an initial concentration of 45.3 μmol L^{-1}. In contrast, DOC was only eliminated for 5% at the end of the test (data not shown). The rate constant k was determined to be 0.0155 min^{-1} ($R^2 = 0.976$) with a half-life of 45 min.

At an initial concentration of 11.3 μmol L^{-1}, QUT was under the LOQ after 32 min of treatment. At the end of the test, approximately 70% of DOC was eliminated. The rate constant k was 0.0692 min^{-1} ($R^2 = 0.973$) with a half-life of 10 min. At an initial concentration of 2.3 μmol L^{-1}, QUT was eliminated after 16 min. Kinetic fitting and DOC measurement was not possible.

It was only possible to calculate the quantum yield for initial concentrations of 45.3 and 11.3 μmol L^{-1} following first-order kinetics. The quantum yield was determined to be 0.0001 and 0.0006, respectively. It is possible that QUT was also eliminated due to indirect photolysis and that this process may have affected the findings concerning the quantum yield. Indirect photolysis may have also contributed to faster primary elimination kinetics (Nick and Schöler, 1995). Likewise, different quantum yields were probably obtained due to different initial photolysis concentrations with a different role of indirect photolytic elimination of QUT. Moreover, the reaction of PTPs might lead to different elimination kinetics as

Table 1

LC-MS parameters and structures of biotransformation products (BTPs) formed after biotransformation of quetiapine (QUT) and phototransformation products (PTPs). Positive determination of BTP (●) or non-detectable BTP (○) are indicated with spots.
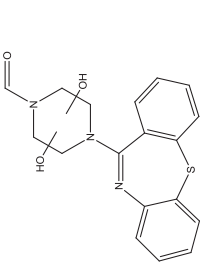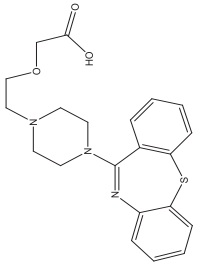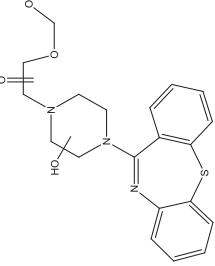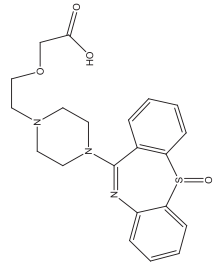
PTPs/QUT	t_R LC-ITMS (min)	BTP identified at the test end of		Presumably formed from	t_R LC-HRMS (min)	Detected mass HRMS (m/z)	Theoretical mass (m/z)	Mass error (Δmmu)	Proposed structure
		CBT	MRT						
BTP 356	16.23	●	●	n/a	13.43	356.1027	356.1063	−3.619	
BTP 398	17.41	●	●	QUT	14.78	398.1536	398.1533	0.271	
BTP 400	16.41	●	●	n/a	13.83	400.1313	400.1326	−1.203	
BTP 414	15.68	●	●	PTP2 400	12.96	414.1451	414.1482	−3.063	

Table 2
Sampling network and corresponding number of STPs (n_{STP}) and density of small STPs (d_{SSTP}) in the corresponding catchments. f_{QTP} and f_{BTP} are the fraction of samples above LOD for QUT and BTP 398, respectively.

Sampling point (MP)	River	n_{STP}	d_{SSTP} km^{-2}	f_{QUT}(%)	f_{BTP}(%)
MP1	Hasenburger Bach	0	0.29	0	20
MP2	Barmbeck-Melbecker Bach	0	0.76	0	0
MP3	Neetze	1	1.21	0	80
MP4	Neetze (mouth)	2	0.95	0	100
MP5	Bruchwetter	1	1.35	0	0
MP6	Marschwetter	0	0.32	0	0
MP7	Roddau	0	0.42	0	40

well (Ding et al., 2013). The number and the concentration of PTPs during UV treatment is usually different at different initial concentrations (Herrmann et al., 2015b).

Ultrapure water was used to study UV elimination kinetics of QUT. Changing the experimental design by using matrices like wastewater, surface water, or potable water containing different amounts of organic matter and minerals could have affected the results found here. Liu et al. (2016) and Neamțu et al. (2014) observed slower UV elimination kinetics in natural waters compared to pure water for their studied organic molecules due to scavenging effects. However, as the amount and the diversity of organic matter and minerals differs greatly in aqueous matrices, suggestions for the specific behavior of QUT cannot be provided.

3.2.2. Peak area profile and structural elucidation of phototransformation products (PTPs)

During UV treatment, several PTPs were formed (see SM, chromatogram Fig. S6, Text S13). Fig. 1B shows the time courses of seven PTPs and QUT at an initial concentration of 226.5 μmol L^{-1}. All of them show A/A_0 ratios lower than 7%, assuming that PTPs were formed in, compared to QUT, low concentrations. Six of them were even lower than 3%. Nevertheless, peak area ratios cannot depict real concentrations, because the ionization and the respective abundance of compounds depend on the chemical structure and can, therefore, only be approximated.

Table 3 shows QUT and all seven PTPs, which are referred to their different m/z values. The fragmentation pattern for each PTP can be found in the SM, Text S14. The proposed photochemical mechanisms and the extent of abundance A/A_0 during UV treatment are described in the following paragraphs.

PTP 251 is likely formed as a secondary PTP. Accordingly, Fig. 1B shows a low slope for PTP 251 at the beginning of the UV treatment. It is possible that PTP 251 is formed due to the cleavage of the moiety on the tertiary amino groups of a PTP with a hydroxylation on the piperazine ring and the subsequent elimination of water.

PTP 296 is probably directly formed from QUT. The ethoxyethanol group is eliminated from the piperazine ring after cleaving at the tertiary amino group which is an often observed pattern. The peak area profile showed a steep slope at the beginning of photolysis with decreasing slope over the entire time course.

PTP 358 also showed a steep slope at the beginning of photolysis (Fig. 1B) with the highest peak ratio A/A_0 after 64 min of treatment. Therefore, it is very likely that PTP 358 is formed as a primary PTP of QUT. It is likely that two carbons are eliminated from the piperazine ring, resulting in the formation of two secondary amines. MS^2 spectrum showed the characteristic 2-(2-(ethylamino)ethoxy) ethanol fragment, a finding supporting the assumption that carbon was eliminated (SM, Text S14).

PTPs can also be formed as a result of multiple hydroxylations on the ring system, the ethoxyethanol sidechain, and the piperazine ring of QUT. PTP1 400 may be formed due to the hydroxylation of the piperazine ring. The fragmentation pattern indicated monohydroxylation on the carbon next to the tertiary amine, which is connected to the dibenzothiazepine ring system. It is likely that PTP3 400 is also formed as a result of hydroxylation of the piperazine ring. In this case, the carbon, which is connected to the tertiary amine and the ethoxyethanol side chain, was hydroxylated.

It can be assumed that PTP2 400 is formed as a result of oxidation of QUT. The preferred atom to be oxidized is the sulfur included in the dibenzothiazepine ring. Skibiński (2012) also identified the sulfoxide formed during photolysis of QUT. QUT sulfoxide is also formed in the human metabolism (Fisher et al., 2012). As PTP2 400 absorbs light in a higher wavelength range than QUT, this PTP could be responsible for the color change of the photolysis test solution from clear colorless to clear yellowish (see Section 3.2.1). PTP2 400 has an additional absorption peak at
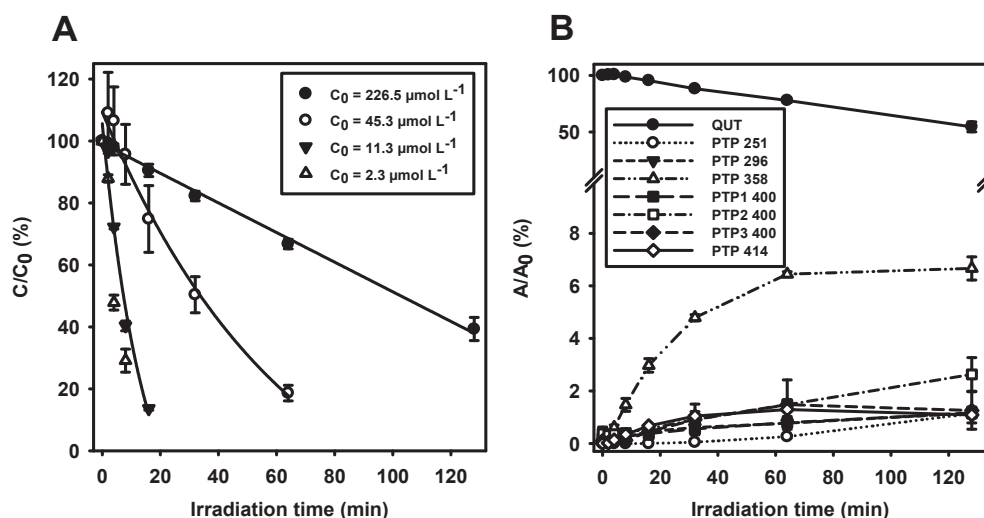

Fig. 1. [A] Kinetic plots of quetiapine (QUT) at different initial concentrations (C_0) (n = 2). C is the concentration at specific time points. QUT at C_0 of 226.5 μmol L^{-1} fits a zero order model. QUT at C_0 of 45.3 and 11.3 μmol L^{-1} fits a first order model. Kinetic fitting at C_0 of 2.3 μmol L^{-1} was not possible. [B] Peak area profile of QUT and phototransformation products (PTPs) at initial QUT concentration of 226.5 μmol L^{-1} (n = 2). A_0 is the peak area of QUT at the beginning of UV photolysis. A is the peak area of QUT and PTPs at specific time points.

Table 3
LC-MS parameters and structures of phototransformation products (PTPs) and quetiapine (QUT). Positive determination of PTP (●) and the time point of highest abundance in parentheses, or non-detectable PTP (○) at different initial concentrations are indicated with spots.

PTPs/QUT	t_R LC-ITMS (min)	Highest abundance for peak (A/A_0) at time point (min) for different initial concentrations				t_R LC-HRMS (min)	Detected mass HRMS (m/z)	Theoretical mass (m/z)	Mass error (Δmmu)	Proposed structure
		226.5 μmol L⁻¹	45.3 μmol L⁻¹	11.3 μmol L⁻¹	2.3 μmol L⁻¹					
PTP 251	18.16	● (128 min)	● (64 min)	● (16 min)	○	15.90	251.0637	251.0637	−0.026	
PTP 296	17.63	● (128 min)	● (64 min)	● (8 min)	● (4 min)	15.11	296.1219	296.1216	0.355	
PTP 358	16.84	● (64 min)	● (32 min)	● (16 min)	● (4 min)	14.33	358.1584	358.1584	0.076	
PTP1 400	16.79	● (128 min)	● (32 min)	● (8 min)	○	14.25	400.1672	400.1689	−1.699	
PTP2 400	17.09	● (128 min)	● (32 min)	● (8 min)	○	14.65	400.1691	400.1689	0.131	
PTP3 400	17.71	● (64 min)	● (32 min)	● (8 min)	○	14.88	400.1688	400.1689	−0.149	
PTP 414	17.79	● (64 min)	● (16 min)	● (8 min)	● (4 min)	15.43	414.1477	414.1482	−0.533	
QUT	18.48	● (0 min)	● (0 min)	● (0 min)	● (0 min)	16.21	384.1749	384.1740	0.876	
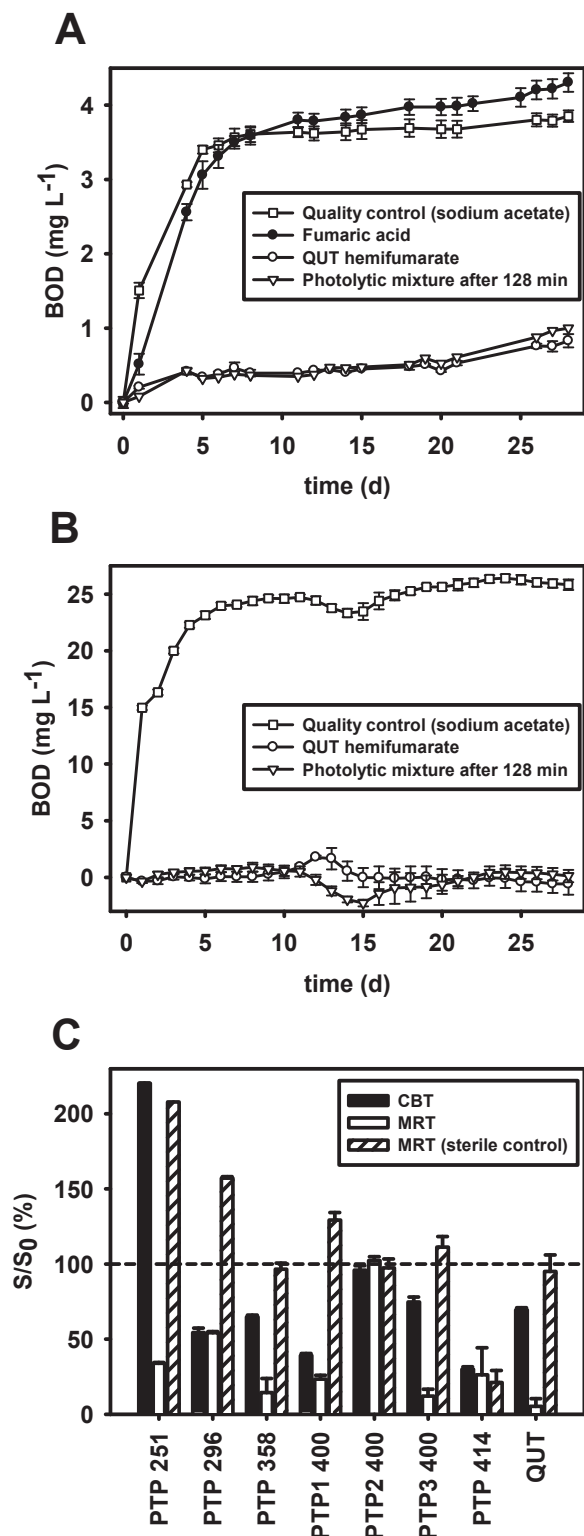
Fig. 2. Biochemical oxygen demand (BOD) as an indicator for biodegradation of sodium acetate, fumaric acid, quetiapine (QUT) hemifumarate and the photolytic mixture created after 128 min of photolysis of 226.5 µmol L^{-1} of QUT in the [A] Closed Bottle Test (CBT) and the [B] Manometric Respirometry Test (MRT) (n = 2). [C] Peak area recovery S/S$_0$ of QUT and selected phototransformation products (PTPs) after 28 days in the CBT and the MRT.

significant change of absorption spectra could be observed compared to QUT. The fragmentation pattern indicated the characteristic elimination of sulfur monoxide (SM, Text S14) due to the formation of a phenantridine ring system. PTP2 400 constantly increased during photolysis.

PTP 414 is most likely quickly formed as a primary PTP of QUT by means of hydroxylation on a position of the piperazine ring that cannot be determined. In addition, the fragmentation pattern suggested oxidation of the carbon next to the ether group. Fig. 1B shows a steep slope for PTP 414 at the beginning of the UV treatment with the highest ratio of A/A$_0$ after 64 min.

All of the seven structurally elucidated PTPs at an initial concentration of 226.5 µmol L^{-1} could also be identified at initial concentrations of 45.3 and 11.3 µmol L^{-1} (Table 3). However, at an initial concentration of 2.3 µmol L^{-1}, only three PTPs were detected. Likewise, it was shown that the formation PTPs is not greatly influenced by the initial photolysis concentration. The possible reasons why they cannot be detected at the lowest initial concentration include a different photodegradation pathway, non-sufficient detection limits, or the faster elimination kinetics of PTPs (Herrmann et al., 2015b). The PTPs identified in this study were formed in ultrapure water by a medium-pressure mercury lamp. The use of UV light is discussed for the elimination of organic pollutants from wastewater (Köhler et al., 2012) and normally used during finishing of potable water (Hijnen et al., 2006). Therefore, it seems reasonable that different systems with a different irradiation time applied, different aqueous matrices containing QUT and different type of lamps could lead to the formation of other PTPs. However, studies indicated that PTPs can be identical when using different aqueous matrices (Cermola et al., 2005; Liu et al., 2009) or different type of lamps (Haddad and Kümmerer, 2014). Likewise, the types of PTPs found in this study are assumed to be the same like in UV treatment facilities.

3.3. Biodegradation of phototransformation products (PTPs) in the Closed Bottle Test (CBT) and the Manometric Respirometry Test (MRT)

3.3.1. Assessment of biodegradability

As can been seen in Fig. 2A, readily biodegradable sodium acetate (quality control) and fumaric acid show a high BOD up to 4 mg L^{-1} in the CBT. In contrast, QUT hemifumarate and the photolytic mixture, after 128 min of photolysis at a concentration of 226.5 µmol L^{-1} QUT, show a low BOD (>1 mg L^{-1}). As a result, photo-treatment did not significantly increase the biodegradability. However, phototransformation occurred. The recovered peak areas S/S$_0$ of PTPs and QUT in the photolytic mixture (sample after 128 min) can be seen in Fig. 2C. The relative peak area of one PTP increased, whereas that of five PTPs decreased during the CBT. The peak area of PTP 251 more than doubled after 28 days in the CBT. This increase might be correlated with the decrease of PTP 358 after the 2-(2-aminoethoxy)ethanol side chain was separated from the rest of the molecule by biological transformation of the molecule. The peak area of QUT sulfoxide (PTP2 400) remained stable. The peak areas of PTP 296, PTP1 400, PTP3 400, PTP 414, and QUT decreased, and it can be assumed that different BTPs could have been formed from these PTPs.

The BOD of photolytic mixture after 128 min of UV light exposure did not show a significant increase compared to that of QUT hemifumarate (Fig. 2B), and in this sense, the results of the MRT were similar to those of the CBT. In the MRT, readily biodegradable sodium acetate showed a BOD of up to almost 26 mg L^{-1}. As a result, QUT and PTPs were turned into BTPs, and this transformation was suggested by the peak areas of QUT and the PTPs (Fig. 2C). QUT was almost entirely transformed by biotic transformation processes.

around 350 nm (SM, Text S15). Therefore, it could be further transformed by sunlight in the environment. For other PTPs, no
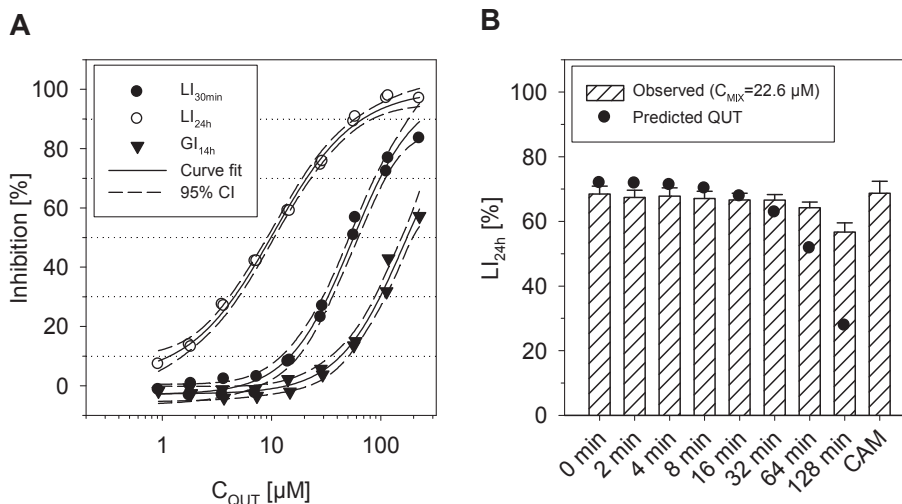
Fig. 3. [A] Concentration-dependent bacterial cytotoxicity of quetiapine (QUT) by means of luminescence inhibition after 30 min (LI_{30min}), luminescence inhibition after 24 h (LI_{24h}) and growth inhibition after 14 h (GI_{14h}). [B] Observed luminescence inhibition after 24 h (LI_{24h}) of the tenfold diluted photolytic mixture of 226.5 μmol L^{-1} QUT ($C_{MIX} = 22.6$ μmol L^{-1}) and the predicted individual effect of measured QUT in the mixture. 0.15 μmol L^{-1} chloramphenicol (CAM) was used as positive control.

PTP 251 and PTP 296 showed a significant decrease in the inoculated test vessel, but increased in the sterile control vessel. Therefore, abiotic transformation processes seem to be the reason for the formation of these PTPs, whereas biotic transformation could be a relevant pathway for their removal. PTP 358 was also transformed by bacteria. As the sterile control vessel showed no change, transformation could only be attributed to bacterial activity. QUT sulfoxide (PTP2 400) did not show a significant change regarding peak area. PTP1 400 and PTP3 400 only decreased in the inoculated vessel, and PTP 414 decreased in the inoculated vessel and the sterile control. Therefore, abiotic transformation of PTP 414 is very likely.

3.3.2. Biotransformation in the photolytic mixture

Each BTP identified in the MRT was also formed in the CBT (see Table 1). Because the MRT yielded higher concentrations, structure elucidation was done from samples of this test. Four BTPs were identified based on new peaks (see SM, chromatogram in Text S12). BTP 398 could also be identified in the sample of the photolytic mixture. It is likely that it was formed from residual QUT; all other BTPs identified here were formed from PTPs (for fragmentation patterns, see SM, Text S12). The proposed structure of BTP 356 could not be traced back to any PTP, but it is possible that it is the result of double hydroxylation on the piperazine ring and degradation of the QUT side chain. BTP 400 was also formed by a PTP. The position of the oxidized carbon on the side chain could not be determined with the help of MS spectra. It is likely that the second hydroxyl group was added to the piperazine ring, but the exact position could not be determined. BTP 414 was probably formed from QUT sulfoxide (PTP2 400). Meta software also predicted BTP 414 as a possible BTP of PTP2 400 (SM, Text S8, Fig. S3). An identical mechanism was also proposed for the formation of BTP 398. The exact position of the carboxylic group could not be determined. It is, however, very likely that BTP 414 is formed after oxidation of the alcohol group. The oxidation of the sulfur was shown by the characteristic elimination of sulfur monoxide.

3.4. In vitro effects of quetiapine (QUT) and photolytic mixtures

The bacterial cytotoxicity before and after the phototransformation of 226.5 μmol L^{-1} QUT was monitored using a modified LBT. QUT was cytotoxic to *V. fischeri* with EC_{50} values of 10.3, 54.3, and 167.3 μmol L^{-1}, depending on the endpoint and the time of exposure (Fig. 3A). The most sensitive endpoint was the luminescence inhibition after 24 h (LI_{24h}), followed by the luminescence inhibition after 30 min (LI_{30min}), and the inhibition of growth after 14 h (GI_{14h}), which was also the case for the photolytic mixtures. Therefore, only LI_{24h} of the tenfold-diluted photolytic mixture ($C_{MIX} = 22.6$ μmol L^{-1}) will be presented as an example here (Fig. 3B). The observed luminescence inhibition of the dark control sample (0 min) was explained by the measured concentration of QUT and the independently determined concentration-response curve, which indicated that QUT itself still had an effect in the photodegradation samples. However, phototransformation of QUT resulted only in a minor decrease of bacterial luminescence inhibition, which did not follow the predicted decrease of the individual effect of QUT. This means that the elimination of QUT during UV irradiation is not necessarily accompanied by a reduction of bacterial cytotoxicity. Moreover, one can expect that most of the PTPs in the photolytic mixture are, in terms of potency, similar to QUT.

The umu-test with and without metabolic activation did not provide any evidence for genotoxic activity of 226.5 μmol L^{-1} QUT before and after the phototransformation at the lowest investigated dilution level ($C_{MIX} = 151$ μmol L^{-1}).

4. Conclusions

In this paper, many new insights concerning the fate and behavior of QUT in aquatic environments were obtained. The results indicate that it is probable that while QUT and its PTPs are not readily biodegradable in aquatic environments, there is some evidence that QUT is transformed in surface water. The main BTP is very likely to be the carboxylic acid derivative, which is also formed by the human metabolism. Because it was observed more often in water samples of the rivers receiving inflows from STPs, one could argue that the BTP is likely to have a greater impact on the environment than QUT. That in turn underlines the necessity to include not just the parent compound into an environmental risk assessment but also possibly formed transformation products.

For the first time, data on the UV elimination kinetics of QUT at different initial concentrations was provided. Moreover, it was

shown that the elimination of QUT from the water cycle by means of UV treatment could result in the formation of multiple hitherto unknown PTPs. Moreover, it was shown that the formation of these main PTPs is not affected by the initial photolysis concentration over two orders of magnitude. Therefore, it was possible to use a comparably high initial concentration of QUT under experimental conditions to identify and determine the characteristic fate and effects of PTPs.

It can be concluded that it is not sufficient to monitor only processes of primary elimination of the parent compound because PTPs may have a similar or even more pronounced toxic effect than the parent compound. Furthermore, primarily formed PTPs can undergo further biological transformation resulting in again different molecules of different fate and effects. In the case of QUT, further research is needed to identify and characterize the PTPs that contributed to the bacteriotoxic effect of the reaction mixture.

Moreover, PTPs were not more biodegradable than QUT. Multiple formation of PTPs even led to a wide range of BTPs, which makes the characterization of resulting transformation products even more difficult. As suggested by these preliminary findings, it seems that UV treatment should not be considered as a possible treatment option for the elimination of QUT from the water cycle.

In general, more information on transformation products of APIs is needed. More specifically, environmental risk assessment studies on metabolites and transformation products have to be conducted because these also have the potential to have an adverse effect on the environment. Furthermore, measures at the source such as proper use as well as better biodegradable molecules should be given more attention to reduce the introduction of pharmaceuticals to the environment at the very beginning.

Acknowledgments

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.envpol.2016.08.040.

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SUPPLEMENTARY MATERIAL

FOR

EXPERIMENTAL AND *IN SILICO* ASSESSMENT OF FATE AND EFFECTS OF
THE ANTIPSYCHOTIC DRUG QUETIAPINE AND ITS BIO- AND
PHOTOTRANSFORMATION PRODUCTS IN AQUATIC ENVIRONMENTS

Manuel Herrmann[ab], Jakob Menz[a], Matthias Gassmann[c], Oliver Olsson[a], *Klaus Kümmerer[a]

[a]*Sustainable Chemistry and Material Resources, Institute of Sustainable and Environmental Chemistry, Leuphana University of Lüneburg, C13, Scharnhorststrasse 1, DE-21335 Lüneburg, Germany*

[b]*Hospital Pharmacy, Ortenau Klinikum Offenburg-Gengenbach, Ebertplatz 12, DE-77654 Offenburg, Germany*

[c]*Water Quality Management - Modelling and Simulation, Institute of Water - Waste and Environment, Kurt-Wolters-Strasse 3, DE-34125 Kassel, Germany*

*Corresponding author

Postal address: Nachhaltige Chemie und Stoffliche Ressourcen, Institut für Nachhaltige Chemie und Umweltchemie, Fakultät für Nachhaltigkeit, Leuphana Universität Lüneburg, Scharnhorststraße 1/C13, D-21335 Lüneburg, Germany.

e-mail: klaus.kuemmerer@leuphana.de

phone: +49 4131 677 2893; fax: +49 4131 677 2894

e-mail addresses: manuel.herrmann@leuphana.de *(Manuel Herrmann),* jakob.menz@leuphana.de *(Jakob Menz),* gassmann@uni-kassel.de *(Matthias Gassmann),* oliver.olsson@leuphana.de *(Oliver Olsson),* klaus.kuemmerer@leuphana.de *(Klaus Kümmerer)*

27 **Text S1: Lamp emission spectrum and quetiapine (QUT) extinction spectrum**

28 The emission spectrum of polychromatic light was measured by the UV-VIS spectrometer

29 Black Comet C25 (StellarNet Inc., Tampa, USA). The molar extinction coefficient of quetiapine

30 (QUT) could be calculated from obtained data of Perkin Elmer LAMBDA 20 UV-VIS spectral

31 photometer (PerkinElmer LAS GmbH, Rodgau, Germany).
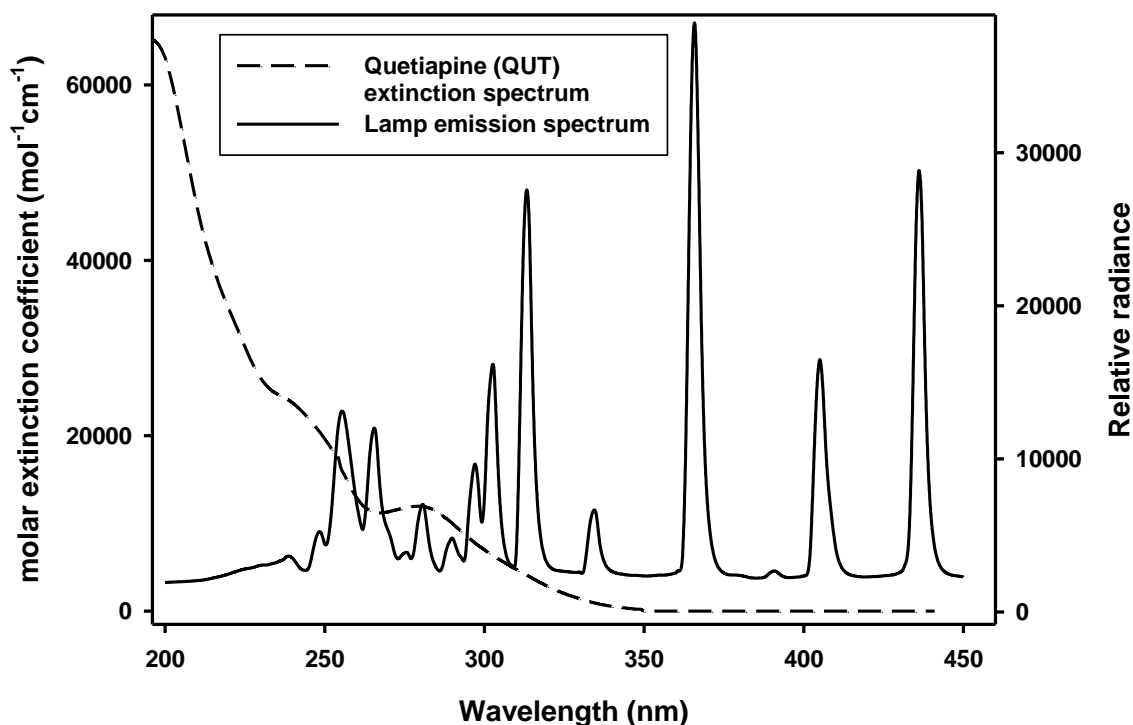
32

33 Fig. S1: Extinction spectrum of quetiapine (QUT) and medium pressure mercury UV lamp

34 radiance spectrum.

35 **Text S2: Calculation of quantum yield**

36 The relative photon flux (relative radiance) J_{rel} was measured in terms of relative counts using

37 a Black Comet C25 UV-VIS spectrometer (StellarNet Inc., Tampa, USA) (see Fig. S1). The

38 conversion factor f was calculated to depict the actual intensity of the lamp. f was determined

39 with the chemical actinometer metamitron (Eq. (1)). Likewise, the metamitron decay was

40   related to quantum yield Φ_{MET} obtained from literature (Palm et al., 1997). As a result, the

41   absolute photon flux, J_{abs}, could be calculated by means of f and J_{rel} (Eq. (2)).

42    $$f = \frac{\frac{dc}{dt}}{\Phi_{MET} \times 2.303 \times c_0 \times l \times \sum_{200\,nm}^{400\,nm} J_{rel} \times \varepsilon} \qquad\qquad (1)$$

43    $$J_{abs} = f \times J_{rel} \qquad\qquad (2)$$

44   f is the conversion factor, dc/dt is the elimination rate of the actinometer, Φ_{MET} is the quantum

45   yield of the actinometer (Palm et al., 1997), c_0 is the initial concentration of the actinometer, l

46   is the path length, J_{rel} is the measured relative photon flux, ε is the molar extinction coefficient

47   of the actinometer, and J_{abs} is the absolute photon flux.

48   The quantum yield of QUT Φ_{QUT} was determined by combining and rearranging Eq. (1) and

49   (2):

50    $$\Phi_{QUT} = \frac{\frac{dc}{dt}}{2.303 \times c_0 \times l \times \sum_{200\,nm}^{400\,nm} J_{abs} \times \varepsilon_{QUT}} \qquad\qquad (3)$$

51   Φ_{QUT} is the quantum yield of QUT, dc/dt is the elimination rate of QUT, c_0 is the initial

52   concentration of QUT, l is the path length, J_{abs} is the absolute photon flux, and ε_{QUT} is the molar

53   extinction coefficient of QUT.

54   **Text S3: Closed Bottle Test (CBT) procedure**

55   The Closed Bottle Test (CBT) consisted of four different test series performed in duplicates.

56   The blank sample contained only mineral medium and inoculum from the municipal STP. The

57   quality control (control substance) consisted of mineral medium, inoculum, and readily

58   biodegradable sodium acetate. The test vessels contained mineral medium, inoculum, and QUT,

59   its photolytic mixtures or fumaric acid. The toxicity control consisted of mineral medium,

60   inoculum, readily biodegradable sodium acetate, and QUT, its photolytic mixtures or fumaric

61 acid. Likewise, possible toxic effects of QUT, photolytic mixtures or fumaric acid on inoculum

62 bacteria could be identified.

63 During the whole test the biochemical oxygen demand (BOD) was monitored by measuring the

64 dissolved oxygen concentration (Friedrich et al., 2013). Degradation of 60% according to

65 Organisation for Economic Co-operation and Development (OECD) test guidelines (OECD,

66 1992), expressed as a percentage of oxygen consumed compared to the theoretical oxygen

67 demand (ThOD), classifies a chemical as readily biodegradable.

68 The following validity criteria had to be fulfilled: (i) the deviation among the duplicates must

69 not exceed 20%, (ii) the toxicity control had to be degraded more than 25% after 14 days, (iii)

70 the dissolved oxygen in the blank sample must not decrease more than 1.5 mg L^{-1} within 28

71 days, (iv) the oxygen concentration of the test samples must not be less than 0.5 mg L^{-1}, and (v)

72 the quality control had to be degraded at least 60% after 14 days.

73 **Text S4: Manometric Respirometry Test (MRT) procedure**

74 The test series were identical as described for CBT with addition of an abiotic control vessel.

75 To this vessel sodium azide was added to obtain sterile conditions. This test series was added

76 to get information about degradation or transformation of the test compound within sterile

77 conditions.

78 The carbon dioxide production of bacteria was measured heads throughout the test. It was

79 measured indirectly by the OxiTop OC110-system (WTW, Weilheim, Germany) sealing the

80 test solutions with pressure heads. In biodegradation processes, oxygen is consumed and carbon

81 dioxide is formed. The carbon dioxide is removed in the pressure heads by reaction with sodium

82 hydroxide under formation of sodium carbonate. Likewise, carbon dioxide is removed from the

83 gas phase. The carbon dioxide production caused by oxygen consumption is, therefore,

84 proportional to the degree of mineralization of the tested substance. Identical to the CBT,

85 degradation of 60% according to Organisation for Economic Co-operation and Development

86 (OECD) test guidelines (OECD, 1992) classifies the tested substance as readily biodegradable.

87 The following validity criteria had to be fulfilled: (i) the deviation among the duplicates must

88 not exceed 20% (ii) the toxicity control had to be degraded more than 25% after 14 days (iii)

89 the oxygen demand in the blank sample must not be more than 60 mg L^{-1} within 28 days and

90 (iv) the quality control had to be degraded at least 60% after 14 days.

91 **Text S5: Analytical conditions**

92 **Chromatography**

93 Chromatography was conducted by means of a MN Nucleodur® RP-phenyl-hexyl column

94 (EC 125/3 mm, 100–3 μm) (Macherey-Nagel, Düren, Germany). QUT was separated from

95 transformation products (TPs) in a total run time of 28 min with the help of a binary mobile

96 phase consisting of 10 mM ammonium acetate in ultrapure water (A) and methanol (B) at a

97 flow rate of 0.4 mL min^{-1}. The oven temperature was 30 °C. The primary elimination was

98 determined using a gradient as follows: 0 min: 10% B, 4 min 10% B, 15 min 90% B, 17 min

99 90% B, 21 min 10% B and 24 min 10% B. (Herrmann et al., 2015a; Herrmann et al., 2015b)

100 **Detection limits for the determination of primary elimination (simulated UV treatment)**

101 The retention time (t_R) of QUT was 17.77 min. Two calibration lines were established with 5

102 and 100 µL of injection volume for higher and lower initial UV treatment concentrations,

103 respectively. For initial concentration of 226.5 µmol L^{-1} and 45.3 µmol L^{-1} QUT calibration

104 standards within 0.77 and 196.72 µmol L^{-1} were used (R^2 = 0.9997). In this case, limit of

105 detection (LOD) was 2.06 µmol L^{-1} and limit of quantification (LOQ) was 2.60 µmol L^{-1}. For

106 initial concentration of 11.3 and 2.26 µmol L^{-1} QUT calibration standards within 0.16 and

107 19.68 µmol L^{-1} were used (R^2 = 0.9984). In this case, LOD was 0.503 µmol L^{-1} and LOQ was

108 0.548 µmol L^{-1}.

Text S6: Mass spectrometric settings for peak area profiles of transformation products (TPs)

Total ion chromatograms (TICs) for ion trap mass spectrometer (LC-ITMS) were obtained in positive ionization mode from 90 to 500 m/z with a capillary voltage of 3600 V, 30 psi nebulizer pressure, 10 L min^{-1} dry gas flow rate at a temperature of 350 °C. The injection volumes for samples of photolysis experiments, the CBT, and the MRT were 5, 25 and 50 µL, respectively.

Text S7: MS^n parameters for fragmentation

LC-ITMS: ESI source parameters were identical to parameters for acquiring TIC data (Text S6). Fragmentation was achieved in AUTO-MS^n Mode up to MS^3. The fragmentation amplitude was set on 1.00 V.

LC-HRMS: The H-ESI source of the Orbitrap high-resolution mass spectrometer (LC-HRMS) was set using following parameters: positive ionization mode, source voltage 4000 V, spray voltage 19 V and capillary temperature 275 °C. Fragmentation up to MS^3 was achieved by setting collision-induced dissociation (CID) on 30 eV.

Text S8: Meta predictions (Multicase Inc.)

The biotransformation pathway was predicted with Meta software (version 1.8.1, Multicase Inc. Beachwood, USA). The software can predict chemical transformations which are occurring under different conditions (aerobic and anaerobic degradation, mammal metabolism, photodegradation). In the present study aerobic degradation products were predicted. Meta software consists of a library with target sequences. TP output is scanned for thermal stability and reactivity. If molecules are considered to be stable, they are given as possible formed TPs. The following biotransformation pathway was predicted by Meta software:

131
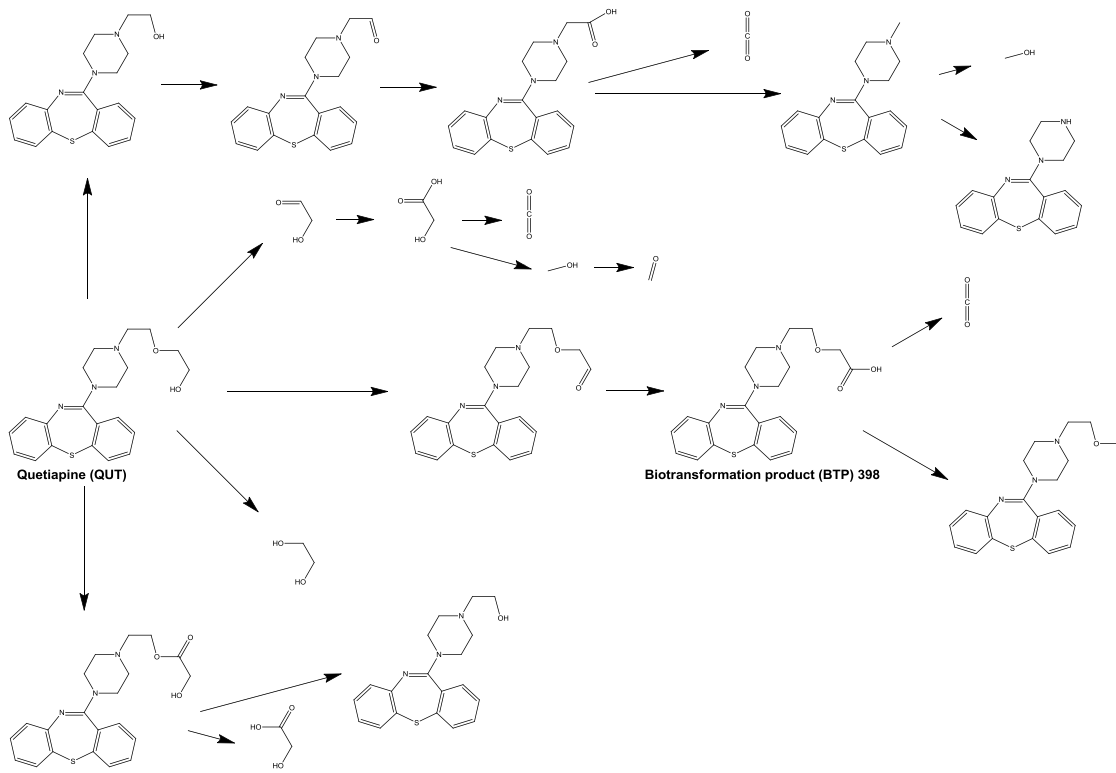
Fig. S2: Predicted biotransformation pathway of quetiapine (QUT) according to Meta software.

133
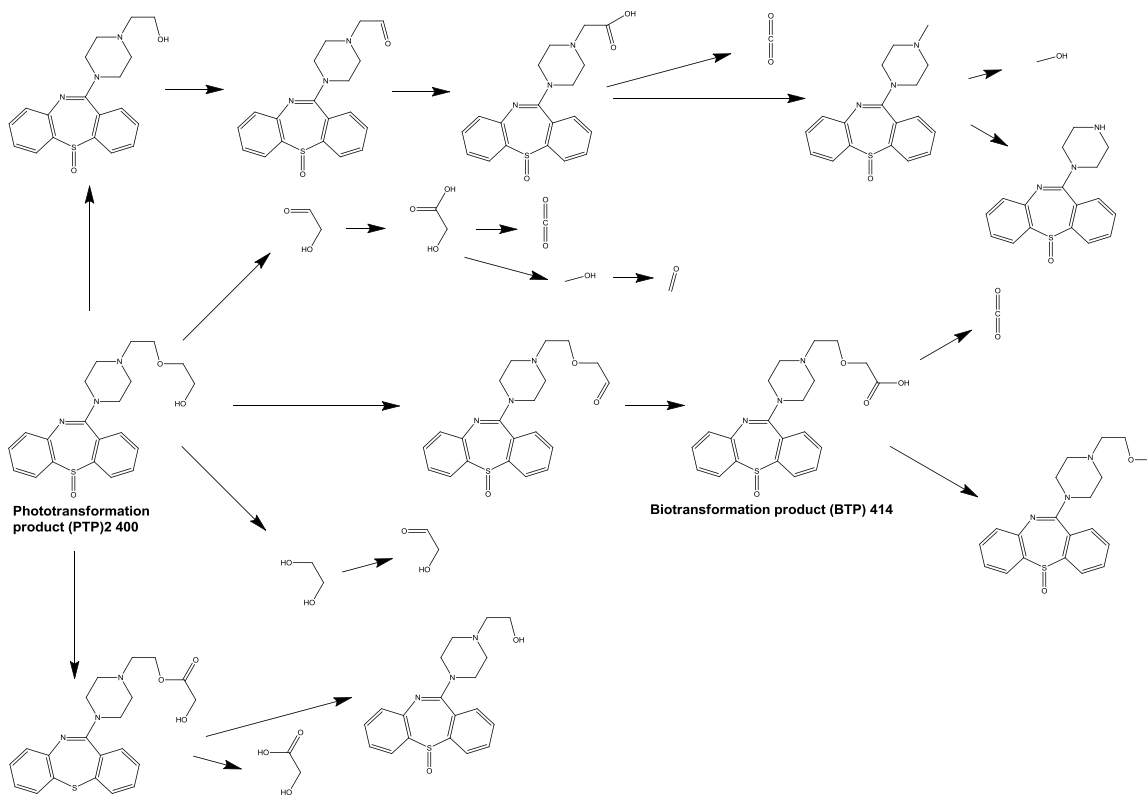
Fig. S3: Predicted biotransformation pathway of phototransformation product (PTP)2 400 according to Meta software.

Text S9: Analytical conditions for surface water analysis

After filtration through a folded filter, the river samples were enriched by means of solid phase extraction (SPE). Therefore, 1 L of each sample was loaded on Oasis HLB 6 mL/200 mg (Waters GmbH, Eschborn, Germany) SPE cartridges. After eluting with methanol and subsequently drying under gentle nitrogen stream, the pellet was reconstituted in 1 mL of methanol.

Matrix effects and SPE recovery were assumed to be similar in every river sample. Therefore, a calibration line for QUT was established from samples of Hasenburger Mühlenbach (MP1) because QUT and biotransformation product (BTP) 398 were not contained in the sampled matrix at sampling point 1 (MP1) (see Fig. S4). A good recovery of 114 ± 4 (n=3) was determined for QUT. Linear calibration range ($R^2 = 0.9976$) was established from 0.4 to 17.4 ng L^{-1}. The LOD and LOQ were determined as follows:

$$LOD = 3.3 \times \frac{SE}{s} \qquad (6)$$

$$LOQ = 10 \times \frac{SE}{s} \qquad (7)$$

SE is the standard error and s is the slope of the calibration line.

The chromatographic method can be found in SM (Chromatography, Text S5). Quantification was conducted by means of LC-HRMS using SIM (single ion monitoring) mode for a range of 383.67 to 384.67 and 397.65 to 398.65 m/z. For identification, in addition, a dependent scan was used to fragment the highest abundant parent ion up to MS^3. QUT and BTP 398 were identified by main fragments 384.1740 → 253.0794 → 221.1073 m/z and 398.1533 → 253.0794→ 221.1073 m/z, respectively, and the retention time.

157
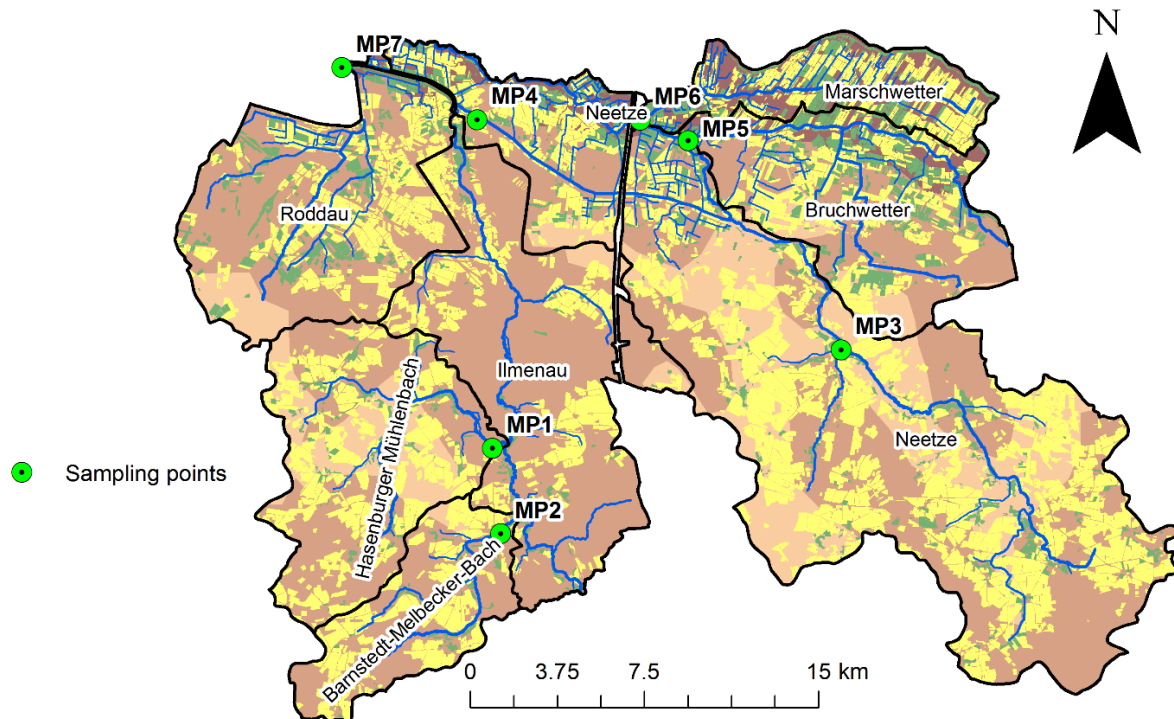
Fig. S4: The six tributaries to the Ilmenau River with seven sampling points (MPs) in the administrative district of Lüneburg.

Text S10: Toxicity screening

Modified luminescent bacteria test (LBT)

An overnight culture of *Vibrio fischeri* NRRL-B-11177 (Hach-Lange GmbH, Düsseldorf, Germany) was prepared in supplemented seawater complete (SSWC) media [0.5% (w/v) peptone from casein, 0.05% (w/v) Yeast extract, 0.3% (v/v) glycerol, 3% (w/v) NaCl, 44.2 mM NaH_2PO_4, 12.1 mM K_2HPO_4, $MgSO_4$, 0.8 mM 7 H_2O, 3.8 mM $(NH_4)_2HPO_4$; pH 7]. After overnight incubation at 20 °C for 22±2 h, the culture was diluted with fresh SSWC media to an initial cell density of 20 formazine turbidity units (FTU) and subsequently transferred to into a 96-well microplate by adding 100 µL to each well. After 30 min of preincubation at 15 °C, an initial measurement of luminescence emission and optical density (578 nm) was conducted, using a Varioskan Flash (Thermo) multimode plate reader. Subsequently, the test cultures were exposed in triplicates to 100 µL of the respective sample and a continuous measurement of luminescence and optical density, respectively, was carried out for 24 h at 15 °C. Prior to

173 testing, all samples were supplemented with NaCl to a final salinity of 2% (w/v). In each

174 experiment, 3,5 Dichlorophenol and Chloramphenicol were used as positive controls (final

175 assay concentrations: 4.5 mg L^{-1} and 0.05 mg L^{-1}, respectively). The short-term luminescence

176 inhibition (LI_{30min}) was calculated according to EN ISO 11348. A modified Eq. (4) was used

177 for the calculation of the long-term luminescence inhibition (LI_{24h}),

178 $$LI_{24h}=100\ (I_{NC}-I_t)/I_{NC} \qquad\qquad (4)$$

179 LI_{24h} is the luminescence inhibition after 24 h (%), I_{NC} is the average light intensity of the

180 negative controls after 24 h in relative luminescence units (RLU) and I_t is the light intensity of

181 the test culture after 24 h (RLU). The measured optical density after 14 h was used for

182 calculation of the growth inhibition (GI_{14h}) according to Eq. (5),

183 $$GI_{14h}=100\ (OD_{NC}-OD_t)/(OD_{NC}-OD_0) \qquad\qquad (5)$$

184 where GI_{14h} is the growth inhibition after 14 h (%), OD_t is the optical density of the test culture

185 after 14 h, OD_{NC} is the average optical density of the negative controls after 14 h and OD_0 is

186 the average optical density of the negative controls after sample addition.

187 **Umu-test**

188 An overnight culture of *Salmonella typhimurium* TA1535 psk 1002 (German Collection of

189 Microorganisms and Cell Cultures GmbH) was prepared in TGA-culture medium and incubated

190 for 16 h at 37 °C and 250 rpm. Subsequently, the overnight culture was tenfold diluted with

191 fresh TGA-culture medium and incubated for additional 1.5 h to obtain an exponentially

192 growing culture for inoculation. The exposure cultures were prepared by adding the following

193 components into a 96-well microplate: 180 µL testing material, 20 µL 10x concentrated TGA-

194 culture medium, 70 µL inoculum and optionally 0.8% (v/v) S9 mix for metabolic activation

195 containing Aroclor 1254-induced rat liver homogenate (Xenometrix AG). The exposure plate

196 (plate A) was incubated for 2 h at 37 °C and 250 rpm. Subsequently, the test cultures of plate

197   A were tenfold diluted with TGA-culture medium in another 96-well plate (plate B) and

198   incubated for additional 2 h at 37 °C and 250 rpm. The optical density at 600 nm (OD_{600nm}) of

199   plate B was determined using a Synergy-HT microplate photometer (BioTek Instruments).

200   30 µl of the contents of plate B were transferred into a new plate (plate C) containing 120 µL

201   B-buffer followed by the addition of 30 µL Ortho-Nitrophenol-ß-d-galactopyranoside (ONPG,

202   Carl Roth GmbH, Germany) solution. Plate C was incubated for 30 min at 28 °C, 250 rpm, after

203   which the reaction was stopped using the stop reagent. Finally, the absorbance at 420 nm

204   (A_{420nm}) of plate C was measured to determine the β-galactosidase activity. The growth factor

205   (G) and the induction ratio (IR) were calculated according to ISO/FDIS 13829 on the basis of

206   OD_{600nm} and A_{420nm} respectively.

207 **Text S11: Total ion chromatograms of QUT and photolytic samples in the Manometric**

208   **Respirometry Test (MRT)**

209 At beginning and at the test end of the MRT, samples for LC-ITMS analysis were taken. The

210 highest abundant m/z for each newly occurring peak was identified as a BTP. Fig. S5 shows the

211 TICs of the test with QUT and the photolytic mixture after 128 min of UV treatment.
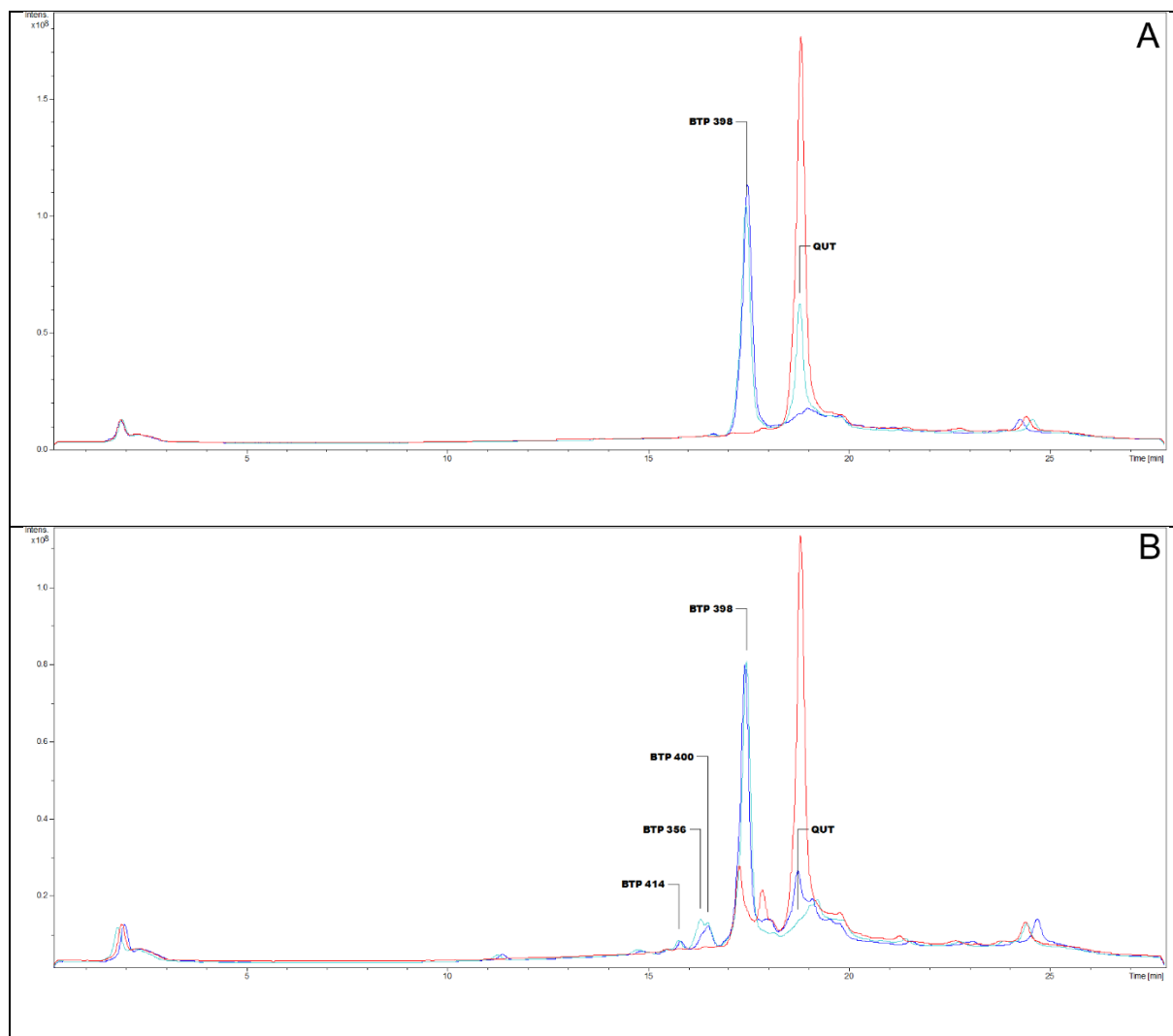
212 Fig. S5: Smoothed total ion chromatograms (TICs) of the MRT (before test start (red) and at

213 test end in duplicates (blue and cyan)). Chromatograms are shown for [A] test substance

214 quetiapine (QUT) and [B] photolytic mixture after 128 min of UV treatment. QUT and

215 biotransformation products (BTPs) are annotated.

Text S12: Fragmentation pattern of biotransformation products (BTPs)

For each occurring peak in the TIC of samples from biodegradation tests (LC-ITMS), the determined m/z was fragmented using above mentioned MS^n parameters (Text S7). The MS^3 fragmentation pattern for each BTP, obtained by LC-HRMS, is shown in the following. The chemical formula and the respective mass in the mass spectrum of the highest abundant product ion is shown in red and framed in red, respectively.
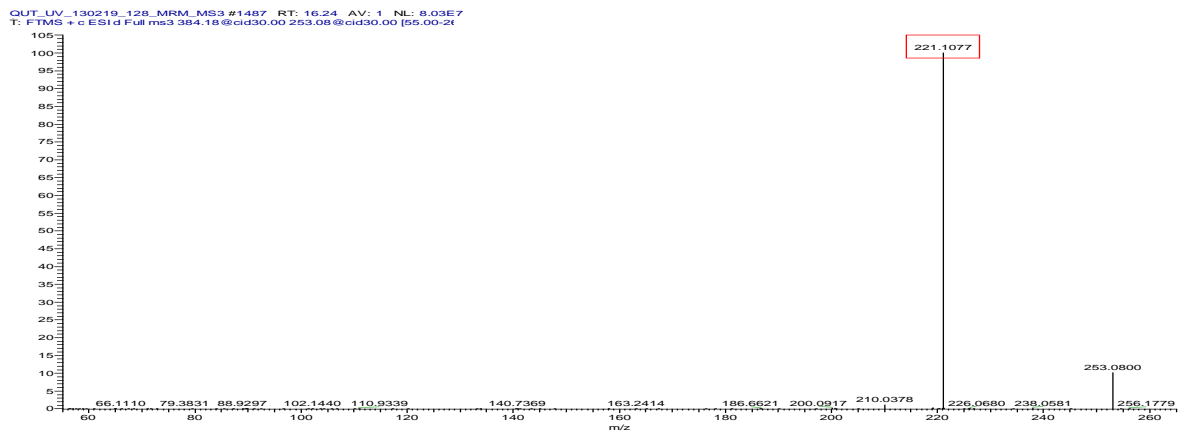
BTP 356

MS^1
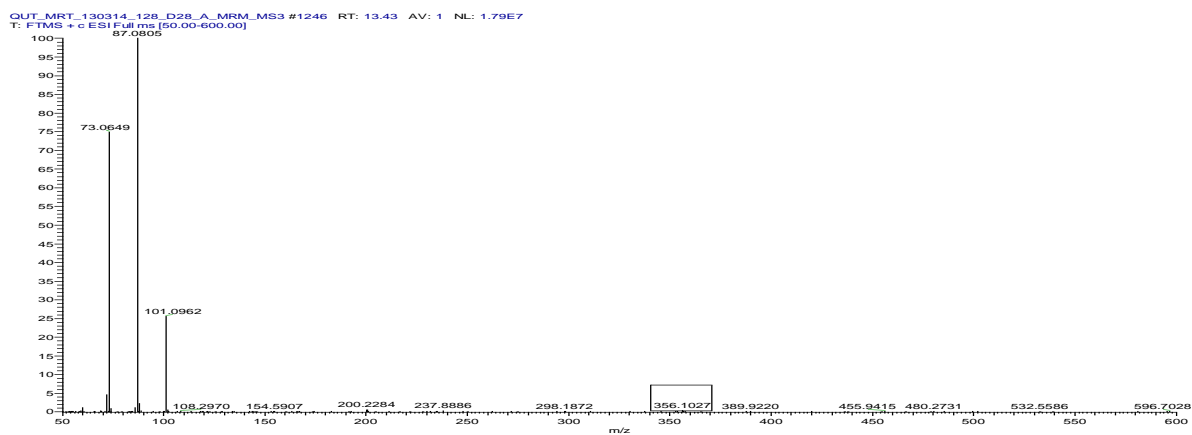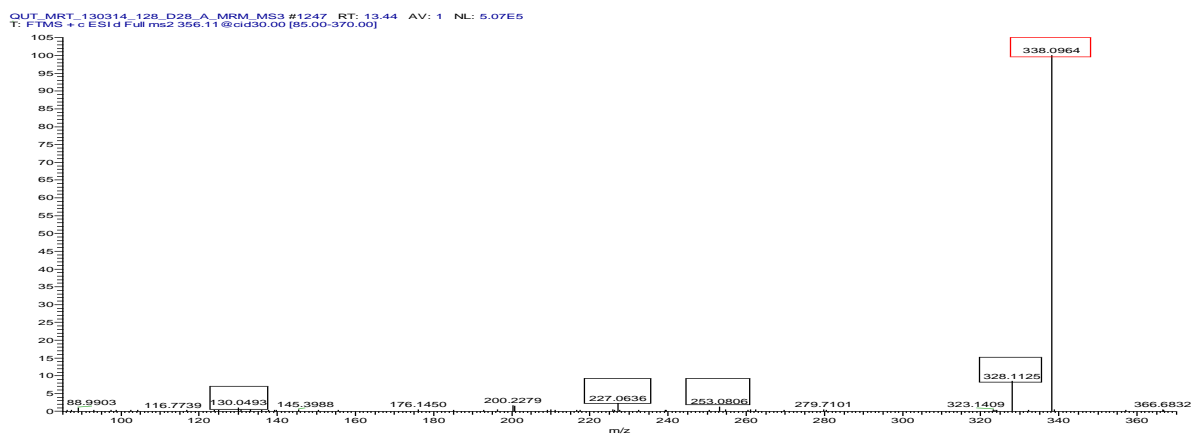
$356.11 \rightarrow MS^2$

232   356.11 → 338.10 → MS^2
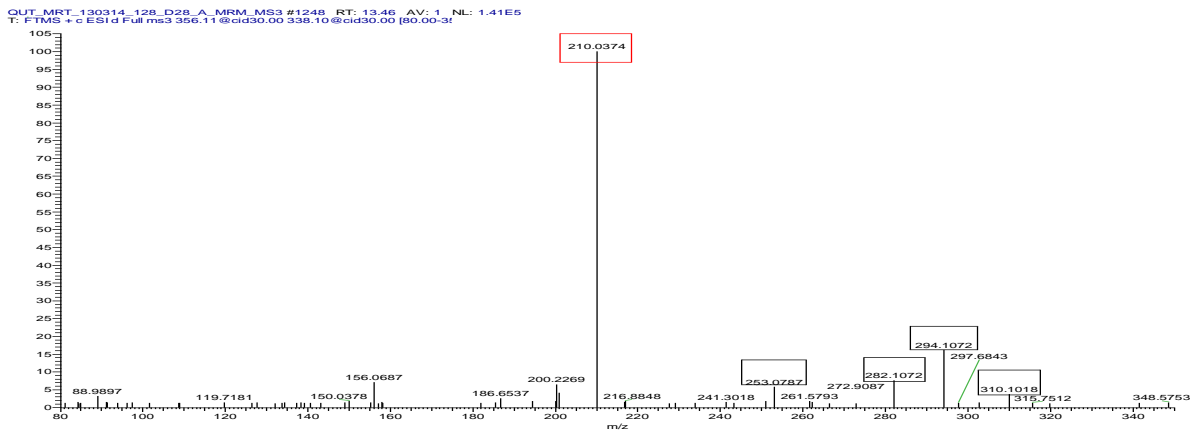
233

234   356.11 → 328.11 → MS^2
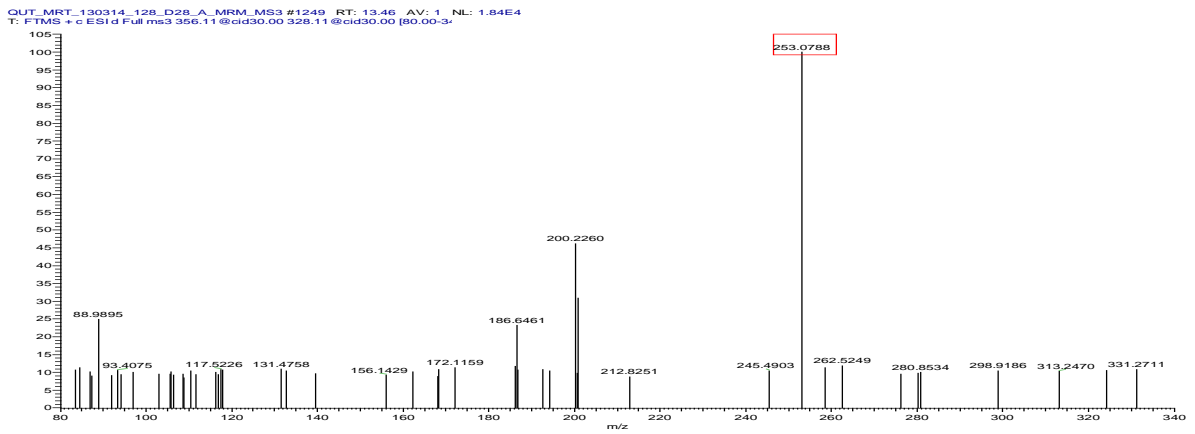
235

236   Fragmentation pattern of BTP 356

237

238 **BTP 398**

239 MS^1

QUT_MRT_130314_128_D28_A_MRM_MS3 #1365   RT: 14.78   AV: 1   NL: 3.19E7
T: FTMS + c ESI Full ms [50.00-600.00]

241 398.15 → MS^2

QUT_MRT_130314_128_D28_A_MRM_MS3 #1366   RT: 14.79   AV: 1   NL: 4.11E7
T: FTMS + c ESI d Full ms2 398.15@cid30.00 [95.00-410.00]

243 398.15 → 253.08 → MS^3

QUT_MRT_130314_128_D28_A_MRM_MS3 #1367   RT: 14.81   AV: 1   NL: 1.96E7
T: FTMS + c ESI d Full ms3 398.15@cid30.00 253.08@cid30.00 [55.00-2(
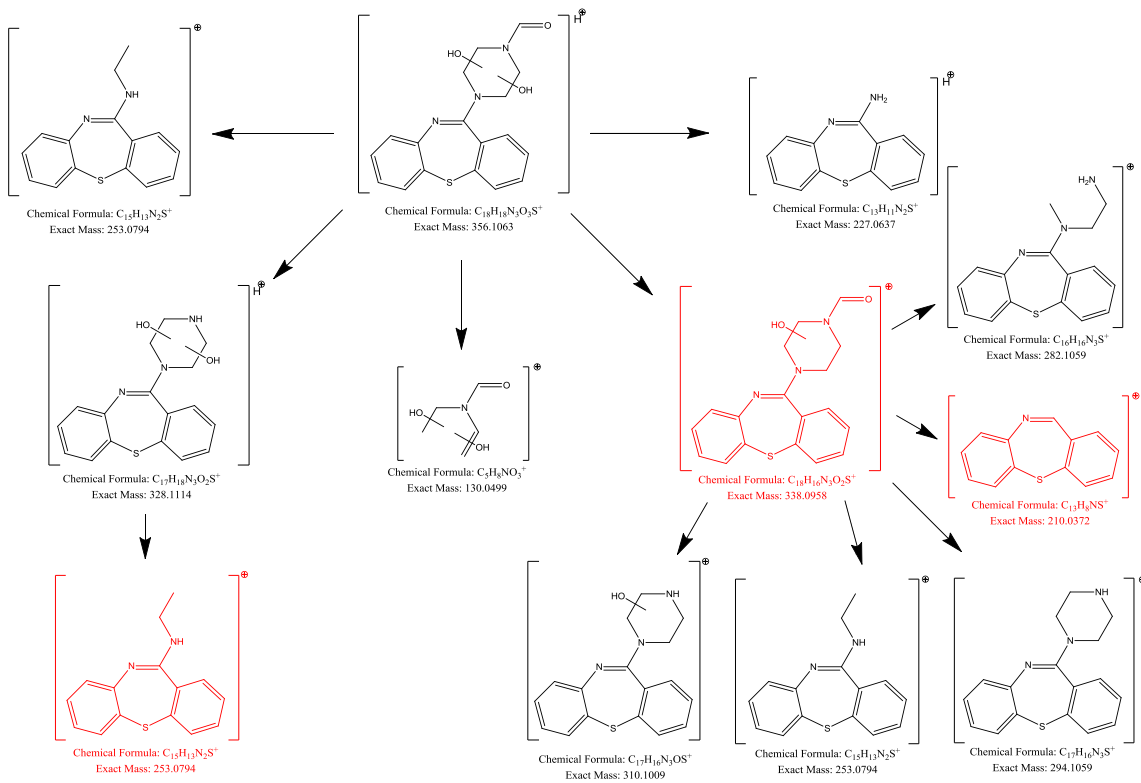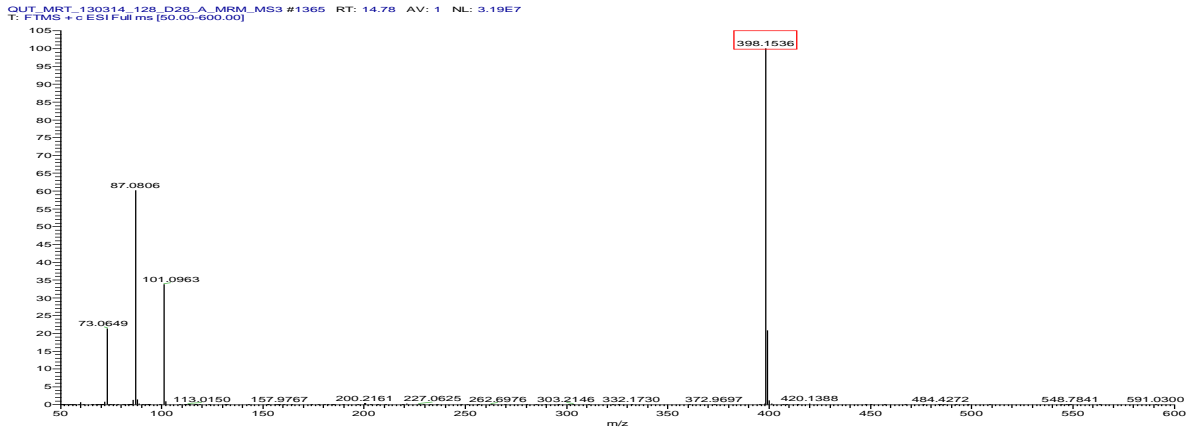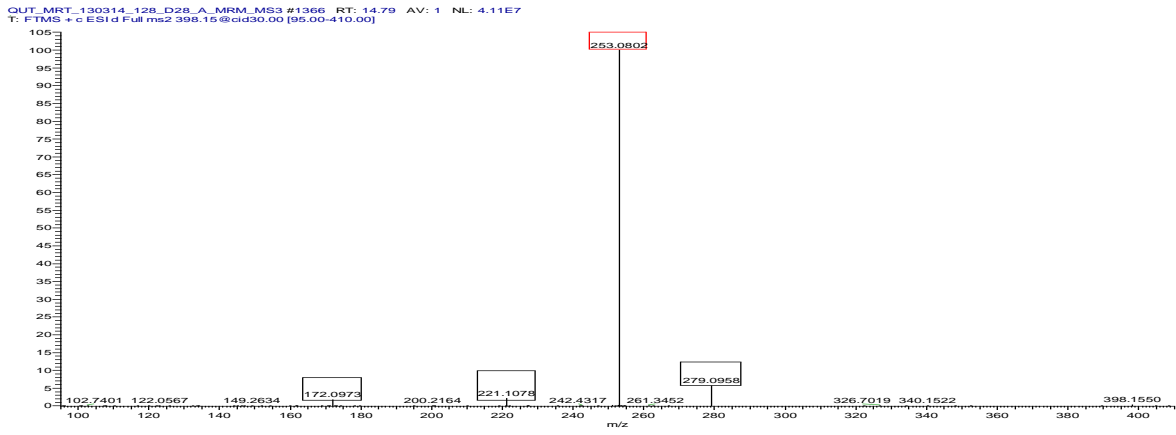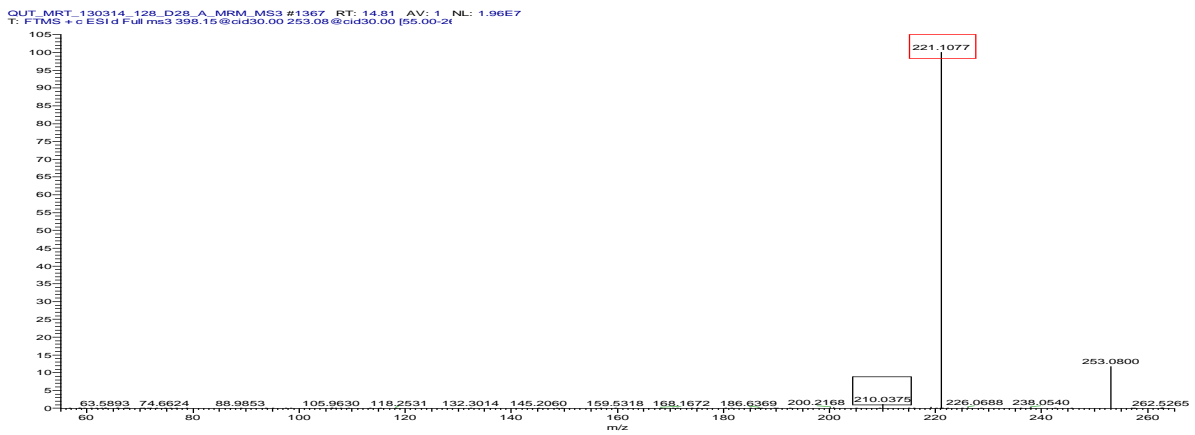
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249   398.15 → 279.10 → MS³
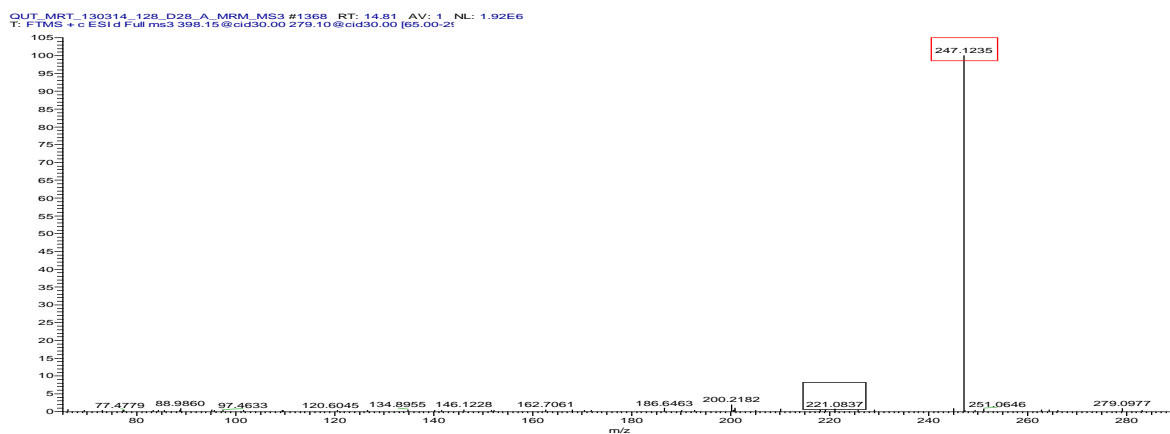
250

251   Fragmentation pattern of BTP 398

252
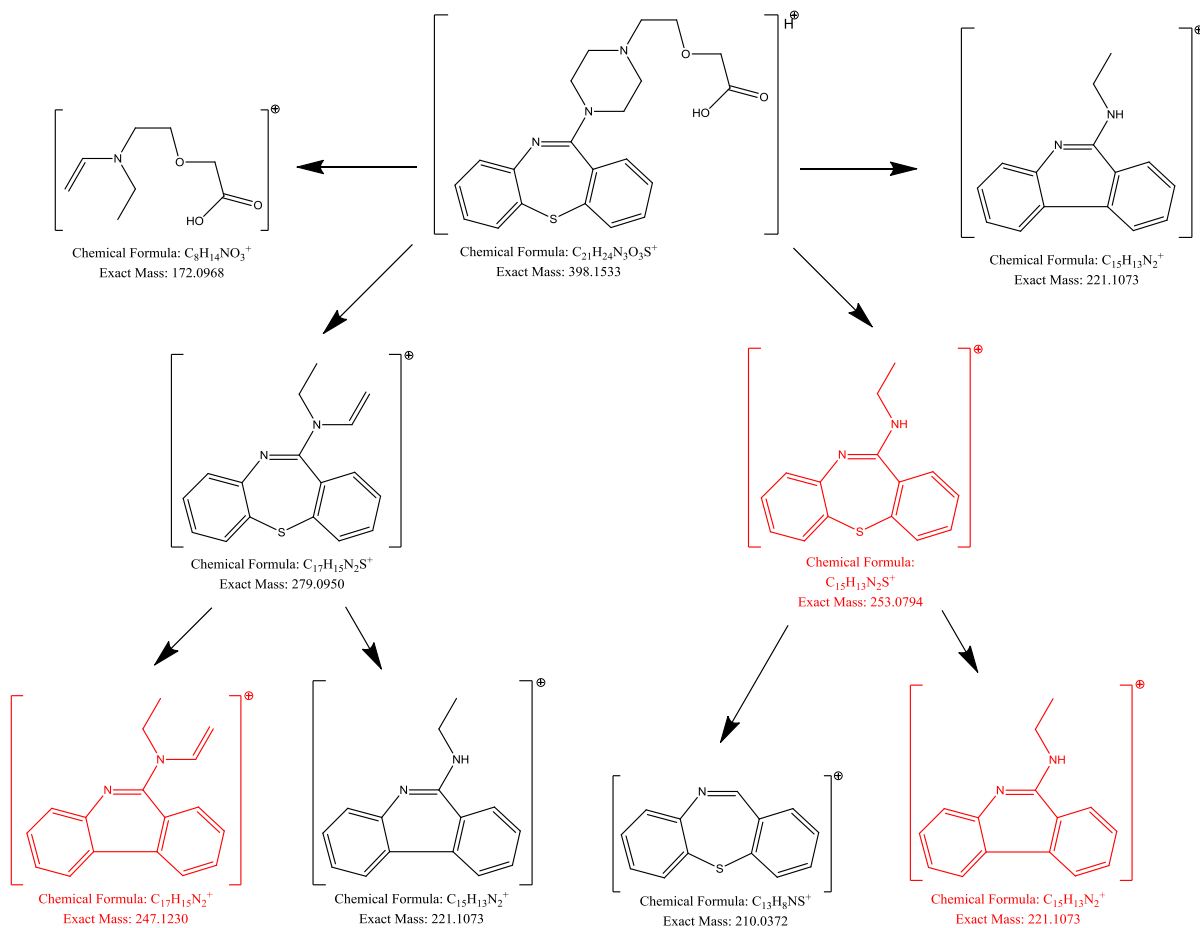
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258 **BTP 400**

259 MS^1
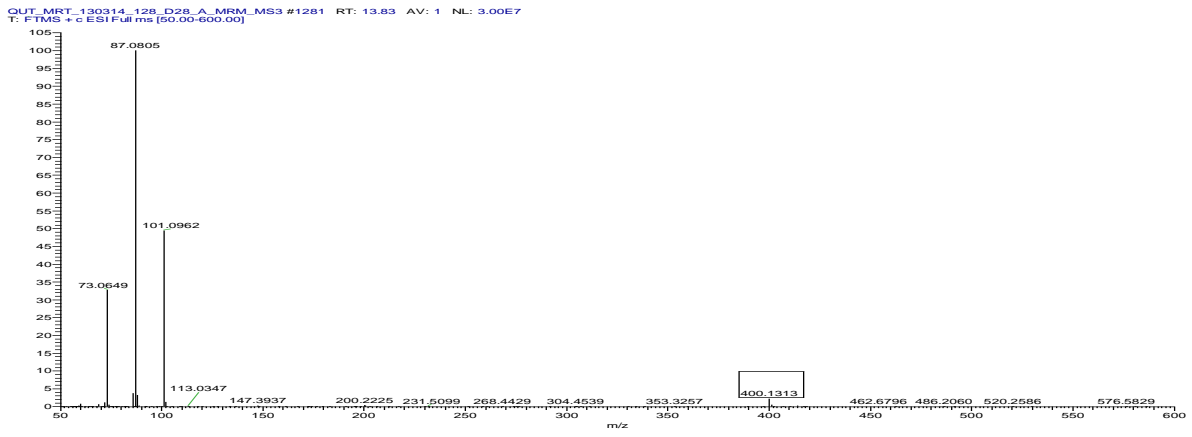
260

261 400.13 → MS^2
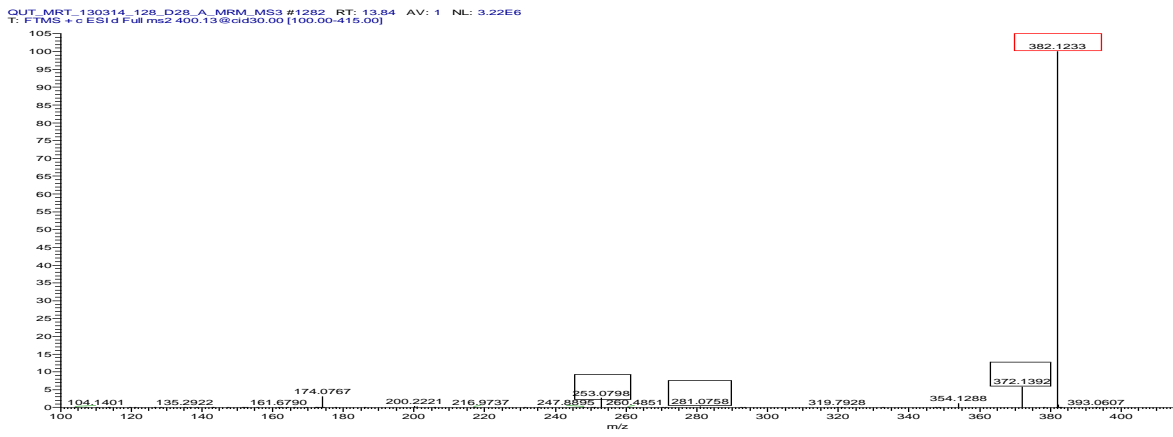
262

263 400.13 → 382.12 → MS^2
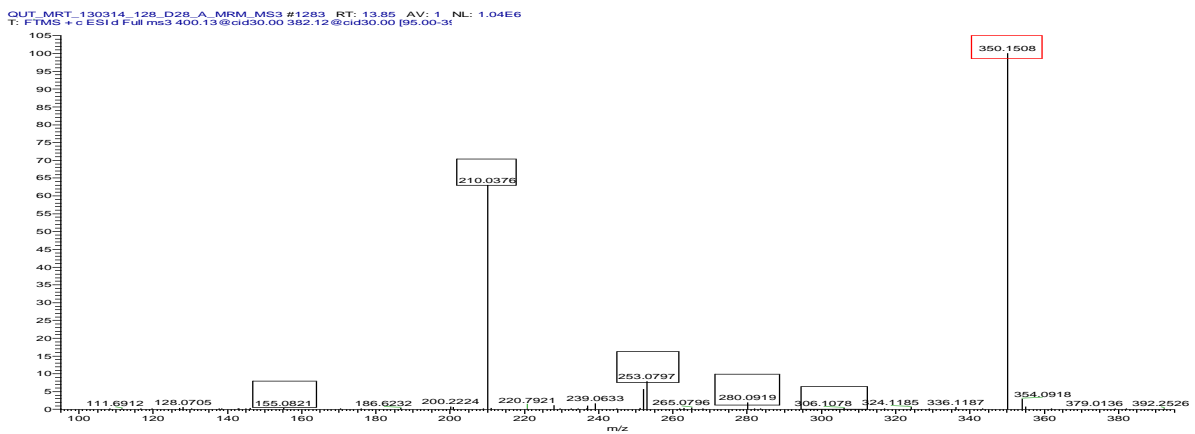
264

265

266

267

268

269   400.13 → 372.14 → MS^2

QUT_MRT_130314_128_D28_A_MRM_MS3 #1284   RT: 13.86   AV: 1   NL: 5.36E4
T: FTMS + c ESI d Full ms3 400.13@cid30.00 372.14@cid30.00 [90.00-3£

270
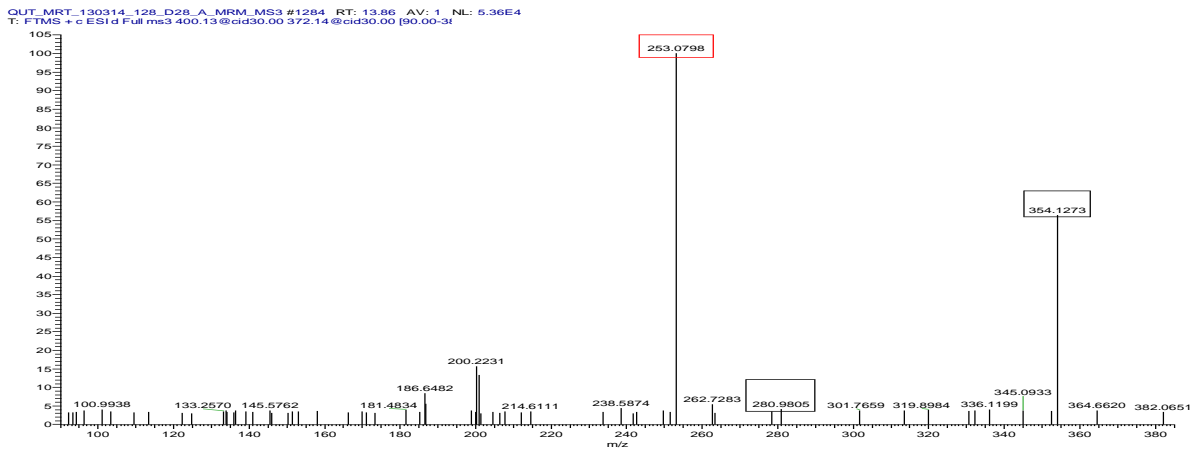
271   Fragmenation pattern of BTP 400

272

273   **BTP 414**

274   MS^1

QUT_MRT_130314_128_D28_A_MRM_MS3 #1204   RT: 12.96   AV: 1   NL: 1.67E7
T: FTMS + c ESI Full ms [50.00-600.00]
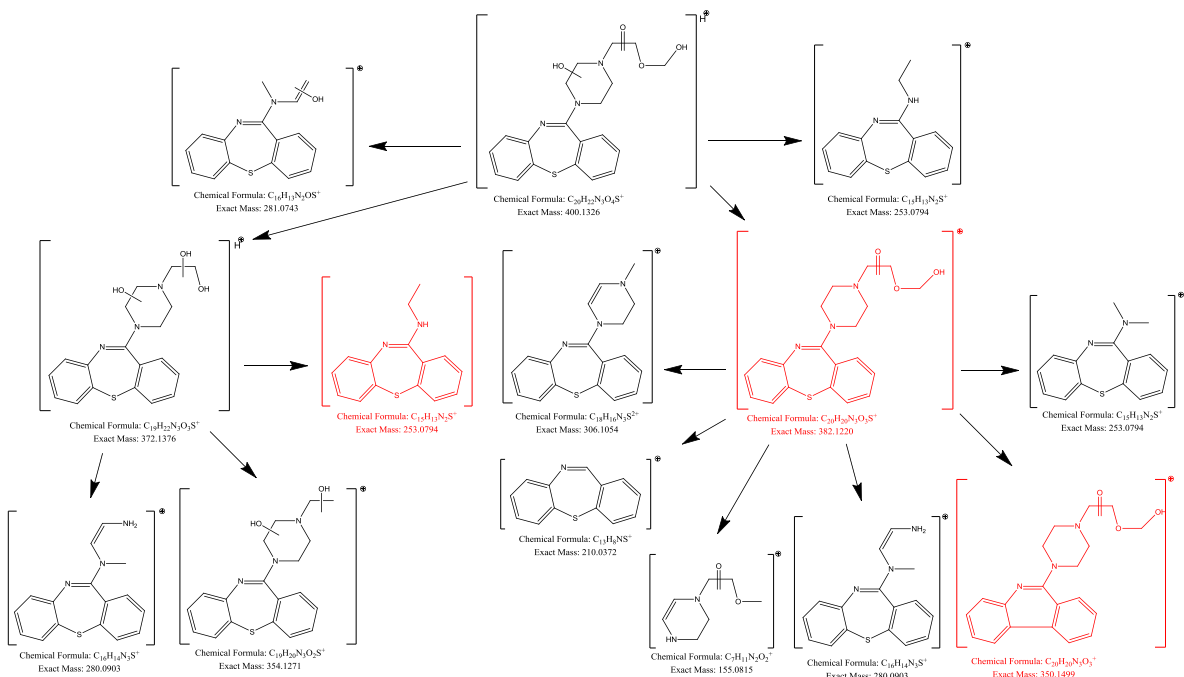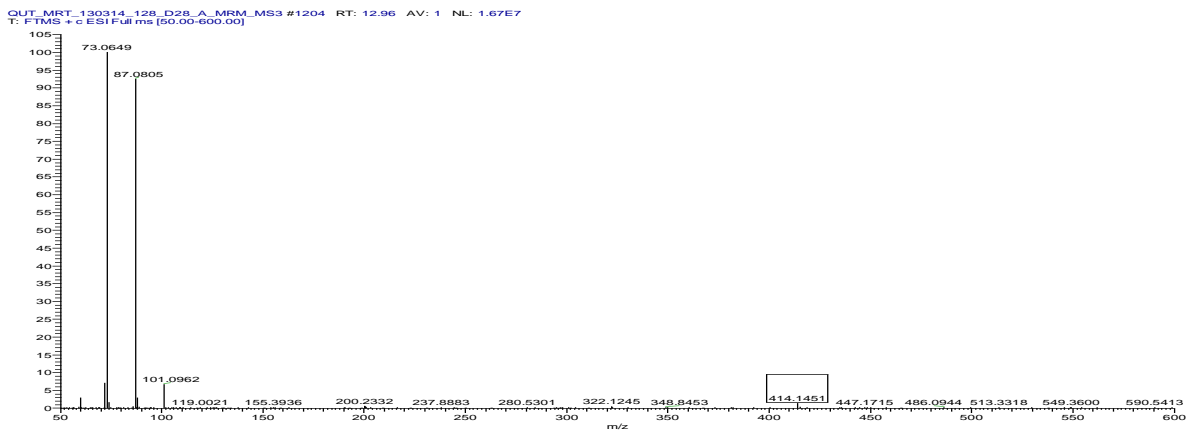
275

276   414.15 → MS²

QUT_MRT_130314_128_D28_A_MRM_MS3 #1205 RT: 12.97 AV: 1 NL: 6.79E5
T: FTMS + c ESI d Full ms2 414.15@cid30.00 [100.00-425.00]
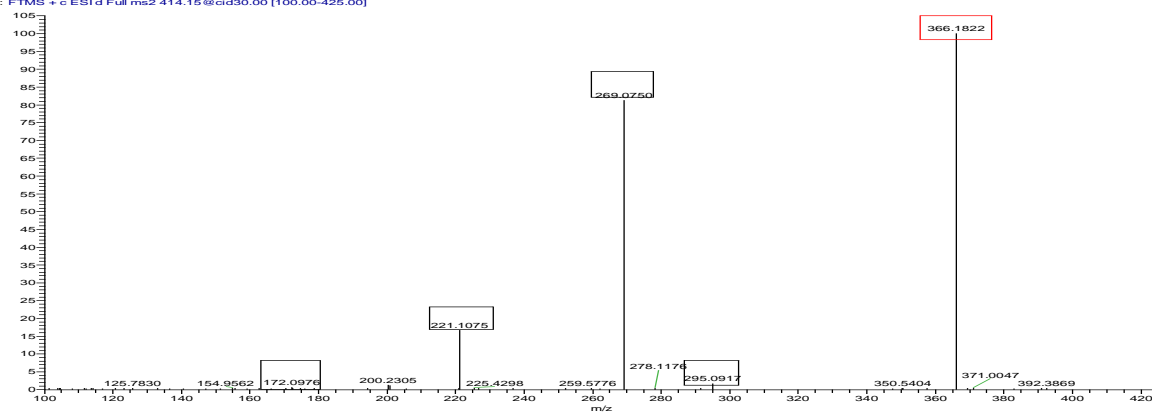
277

278   414.15 → 366.18 → MS³

QUT_MRT_130314_128_D28_A_MRM_MS3 #1206 RT: 12.98 AV: 1 NL: 5.87E5
T: FTMS + c ESI d Full ms3 414.15@cid30.00 366.18@cid30.00 [90.00-3{
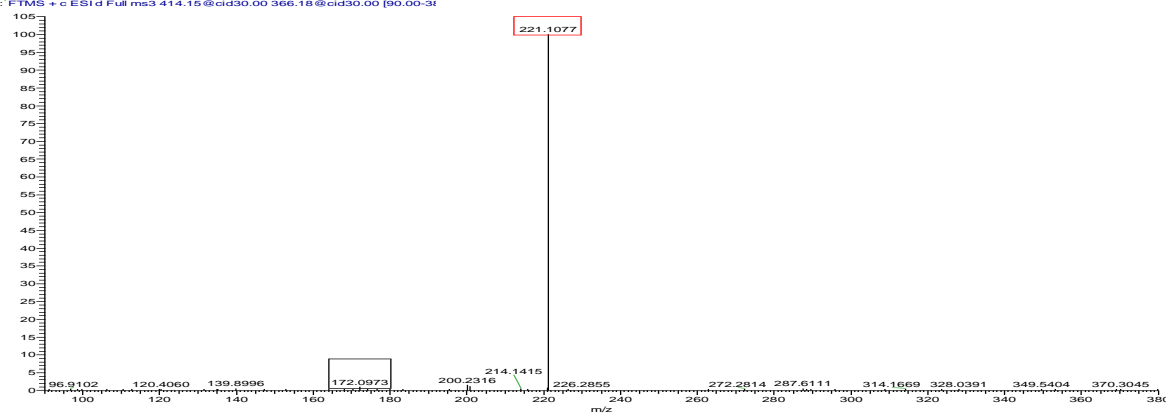
279

280   414.15 → 269.08 → MS³

QUT_MRT_130314_128_D28_A_MRM_MS3 #1207 RT: 12.99 AV: 1 NL: 4.15E4
T: FTMS + c ESI d Full ms3 414.15@cid30.00 269.08@cid30.00 [60.00-2{
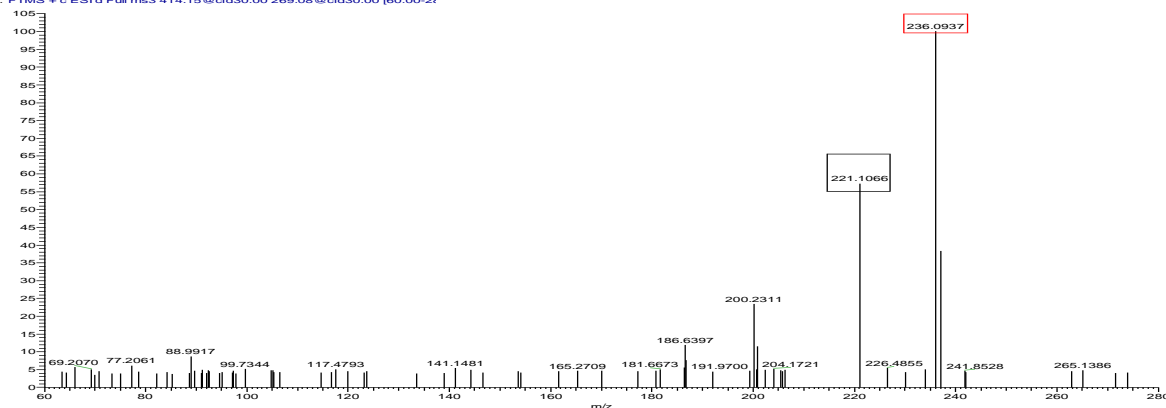
281

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286

287   Fragmenation pattern of BTP 414
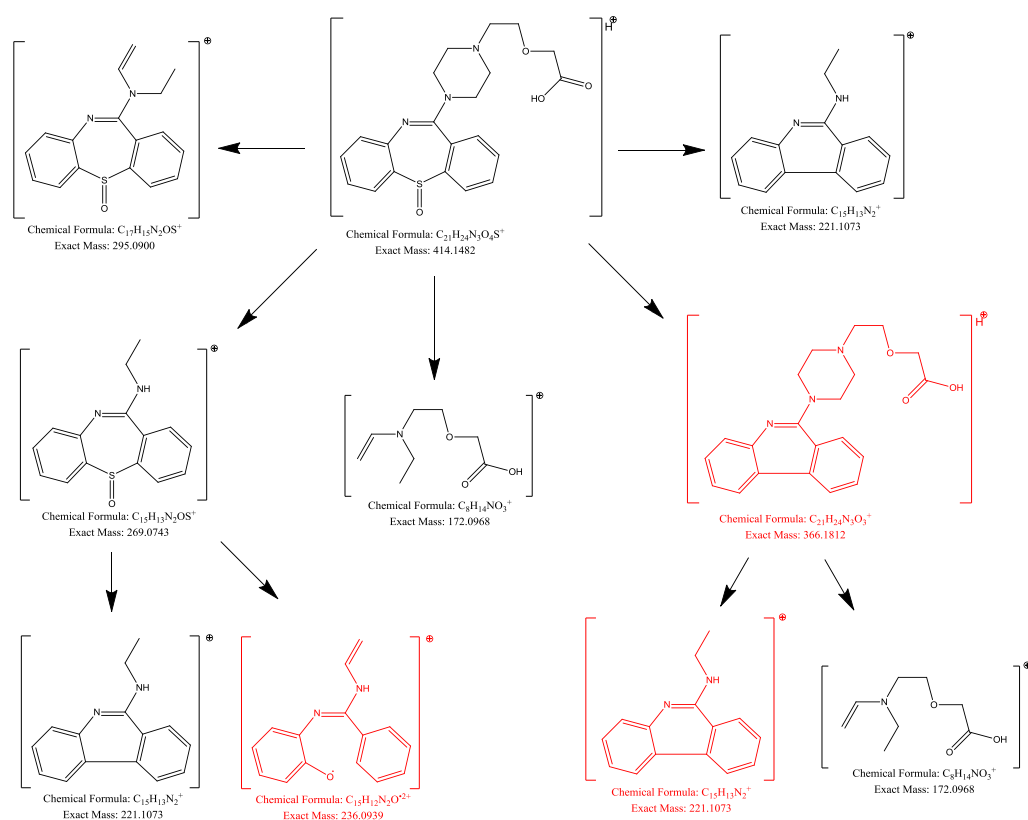
288

289   **Text S13: Total ion chromatograms of photolytic samples throughout treatment**

290   During UV treatment samples were taken for LC-ITMS analysis. Fig. S6 shows the TIC for

291   each sample during one run of UV treatment.
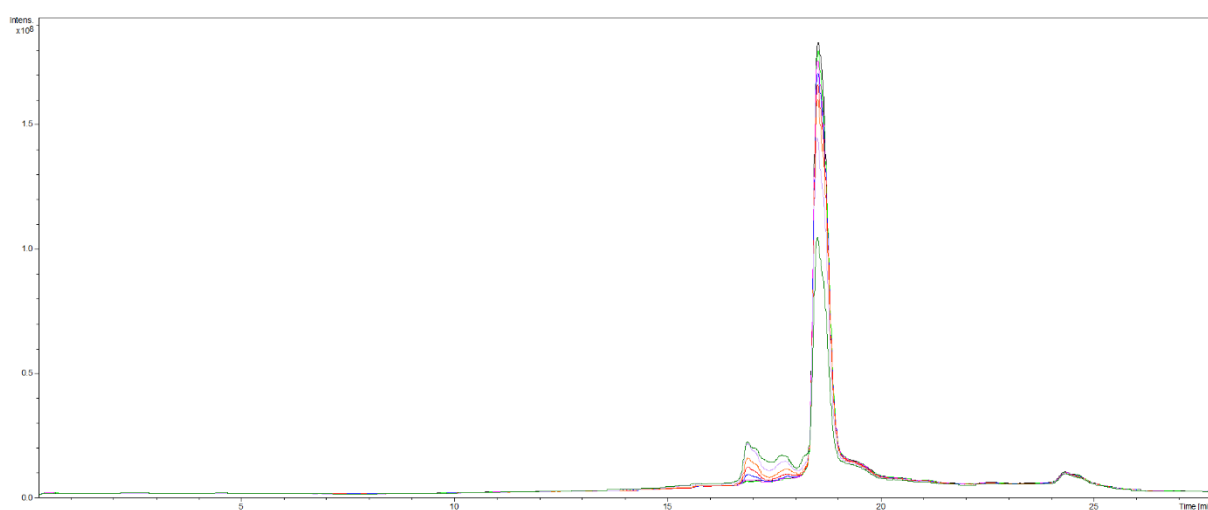
292

293   Fig. S6: Smoothed3 total ion chromatograms (TICs) of photolytic samples (0 min: black; 2 min:

294   lightning green; 4 min: pink; 8 min: blue; 16 min: red; 32 min: orange; 64 min: light purple;

295   128 min: green).

296 **Text S14: Fragmentation pattern of quetiapine (QUT) and phototransformation products**

297 **(PTPs)**

298 PTPs at initial concentration of 226.5 µmol L^{-1} exceeding 1% of A/A$_0$ were structurally

299 elucidated. The determined m/z of PTPs was fragmented using above mentioned MS^n

300 parameters (Text S7). The MS^3 fragmentation pattern, obtained by LC-HRMS, is shown for

301 quetiapine (QUT) and phototransformation products (PTPs) (sample after 128 min of treatment)

302 in the following. The chemical formula and the respective m/z value in the mass spectrum of

303 the highest abundant product ion are shown in red and framed in red, respectively.

304 **Quetiapine (QUT)**

305 MS^1

306

307 384.18 → MS^2

308

309   384.18 → 253.08 → MS^3

QUT_UV_130219_128_MRM_MS3 #1487 RT: 16.24 AV: 1 NL: 8.03E7
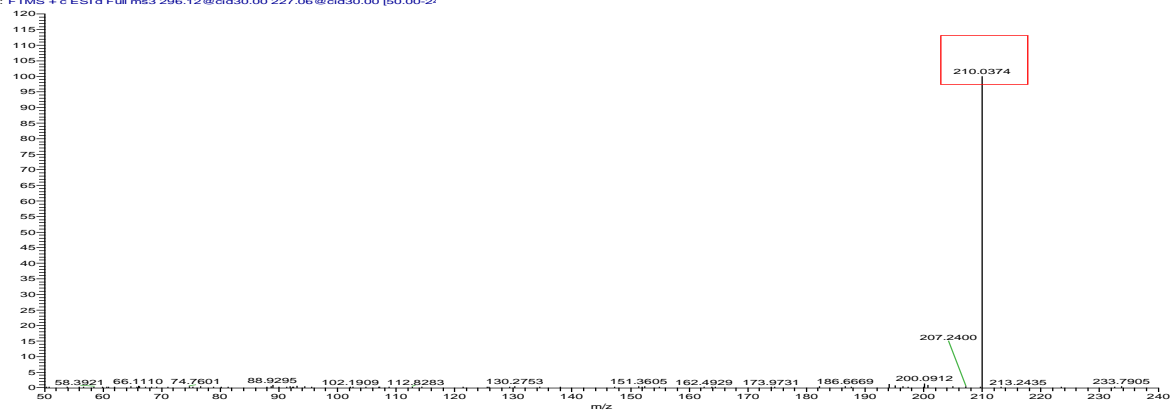T: FTMS + c ESI d Full ms3 384.18@cid30.00 253.08@cid30.00 [55.00-26

310

311   384.18 → 279.10 → MS^3

QUT_UV_130219_128_MRM_MS3 #1488 RT: 16.25 AV: 1 NL: 5.27E6
T: FTMS + c ESI d Full ms3 384.18@cid30.00 279.10@cid30.00 [65.00-29
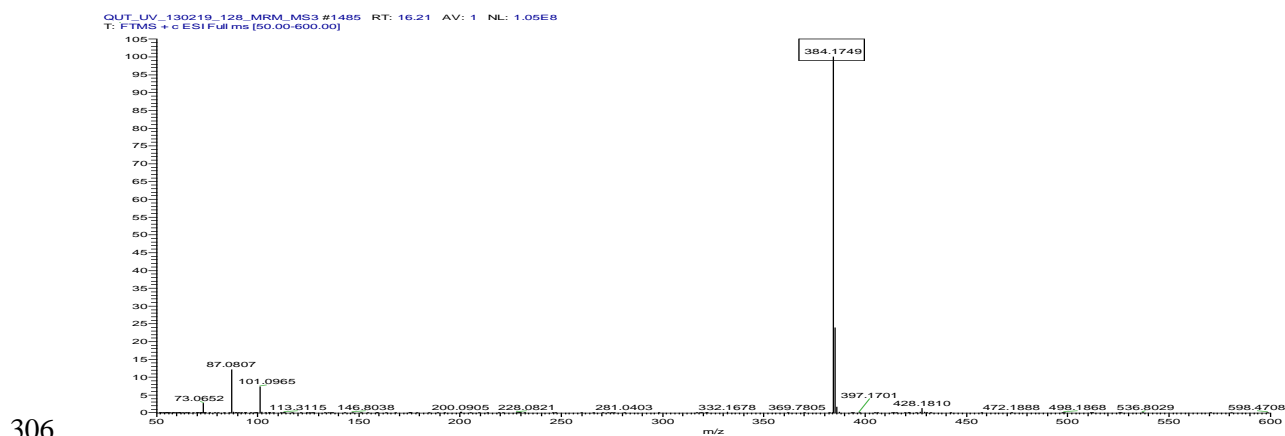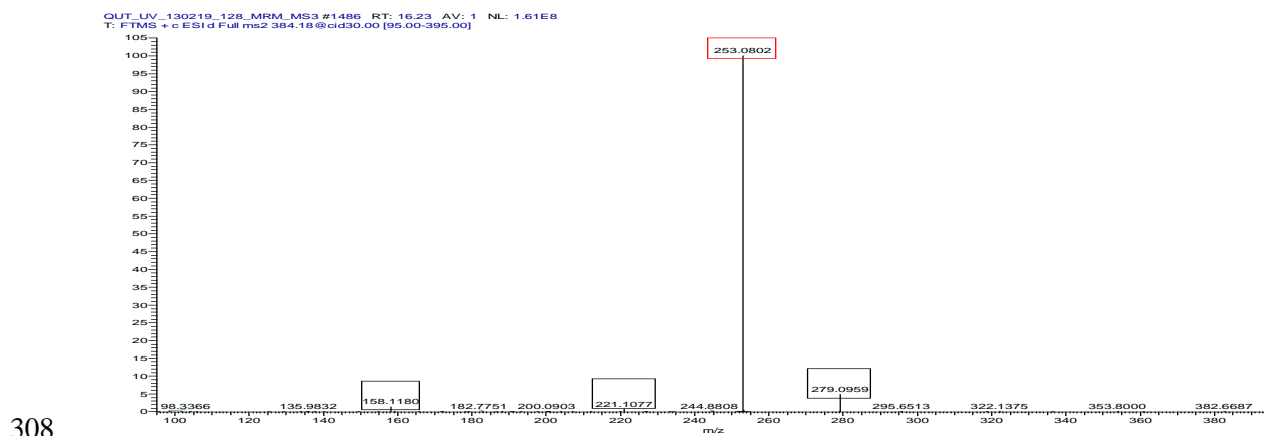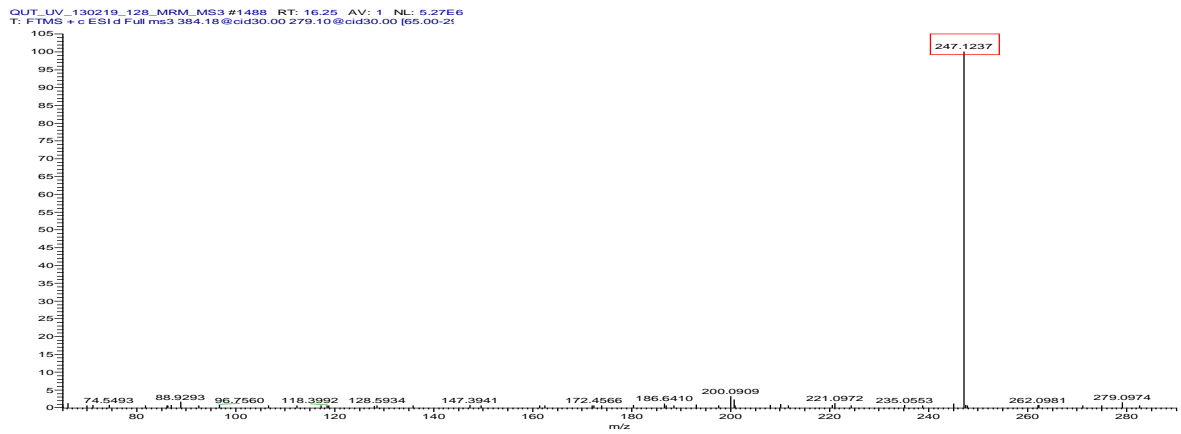
312

313

314   Fragmentation pattern of QUT

Chemical Formula: $C_8H_{16}NO_2^+$
Exact Mass: 158.1176

Chemical Formula: $C_{21}H_{26}N_3O_2S^+$
Exact Mass: 384.1740

Chemical Formula: $C_{15}H_{13}N_2^+$
Exact Mass: 221.1073

Chemical Formula: $C_{17}H_{15}N_2S^+$
Exact Mass: 279.0950

Chemical Formula: $C_{15}H_{13}N_2S^+$
Exact Mass: 253.0794

Chemical Formula: $C_{17}H_{15}N_2^+$
Exact Mass: 247.1230

Chemical Formula: $C_{15}H_{13}N_2^+$
Exact Mass: 221.1073

315

316   **PTP 251**

317   MS^1

QUT_UV_130219_128_MRM_MS3 #1457   RT: 15.90   AV: 1   NL: 1.87E7
T: FTMS + c ESI Full ms [50.00-600.00]

251.0637

87.0805

101.0963

73.0649

115.1114   162.6819   200.0913   231.5135   262.2564   285.3211   332.1740   340.1475   364.1878   384.1533   415.1351   448.3509   501.1176   564.3146   591.1763

m/z

318

319   251.06 → MS^2

320

321   251.06 → 219.09 → MS^3
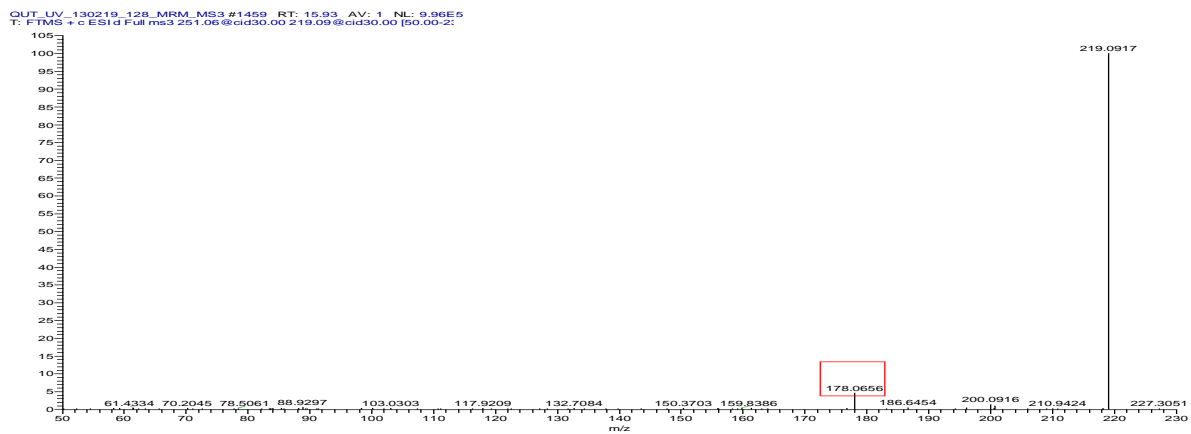
322

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330

331   Fragmentation pattern of PTP 251

Chemical Formula: C$_{15}$H$_{11}$N$_2$S$^+$
Exact Mass: 251.0637

Chemical Formula: C$_{15}$H$_{11}$N$_2$$^+$
Exact Mass: 219.0917

Chemical Formula: C$_{13}$H$_8$NS$^+$
Exact Mass: 210.0372

Chemical Formula: C$_{13}$H$_8$N$^+$
Exact Mass: 178.0651

332

333   **PTP 296**

334   MS^1

QUT_UV_130219_128_MRM_MS3 #1387   RT: 15.11   AV: 1   NL: 1.77E6
T: FTMS + c ESI Full ms [50.00-600.00]

296.1219

228.0453   248.6668   270.7556   276.1959   308.1436   342.1245   358.1546   386.1515   414.1456   430.1663   482.2445   526.2587   544.2630   584.7404

m/z

335

336

337

338

339   296.12 → MS^2

QUT_UV_130219_128_MRM_MS3 #1388 RT: 15.12 AV: 1 NL: 3.38E6
T: FTMS + c ESI d Full ms2 296.12@cid30.00 [70.00-310.00]

253.0801
227.0643
221.1075
279.0957
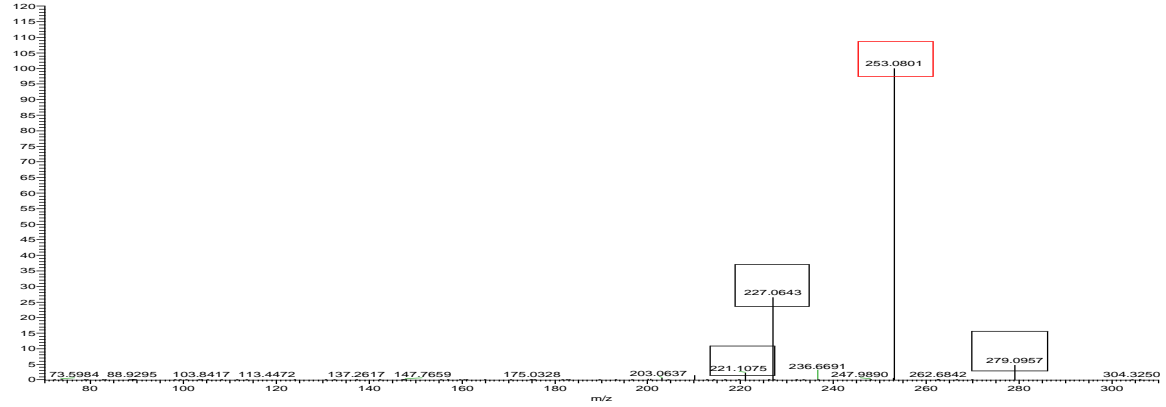
340

341   296.12 → 253.08 → MS^3

QUT_UV_130219_128_MRM_MS3 #1389 RT: 15.14 AV: 1 NL: 2.12E6
T: FTMS + c ESI d Full ms3 296.12@cid30.00 253.08@cid30.00 [55.00-2(

221.1077
226.0663
253.0799
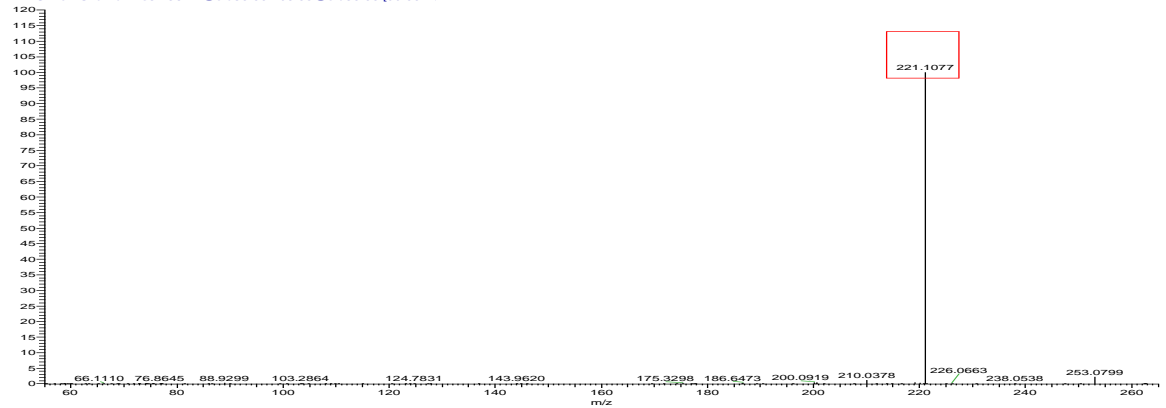
342

343   296.12 → 227.06 → MS^3

QUT_UV_130219_128_MRM_MS3 #1390 RT: 15.15 AV: 1 NL: 6.75E5
T: FTMS + c ESI d Full ms3 296.12@cid30.00 227.06@cid30.00 [50.00-24

210.0374
207.2400
200.0912
213.2435

344

345

346

347   Fragmentation pattern of PTP 296

Chemical Formula: $C_{15}H_{13}N_2^+$
Exact Mass: 221.1073

Chemical Formula: $C_{17}H_{18}N_3S^+$
Exact Mass: 296.1216

Chemical Formula: $C_{17}H_{15}N_2S^+$
Exact Mass: 279.0950

Chemical Formula: $C_{13}H_{11}N_2S^{2+}$
Exact Mass: 227.0632

Chemical Formula: $C_{15}H_{13}N_2S^+$
Exact Mass: 253.0794

Chemical Formula: $C_{13}H_8NS^{2+}$
Exact Mass: 210.0366

Chemical Formula: $C_{15}H_{13}N_2^+$
Exact Mass: 221.1073
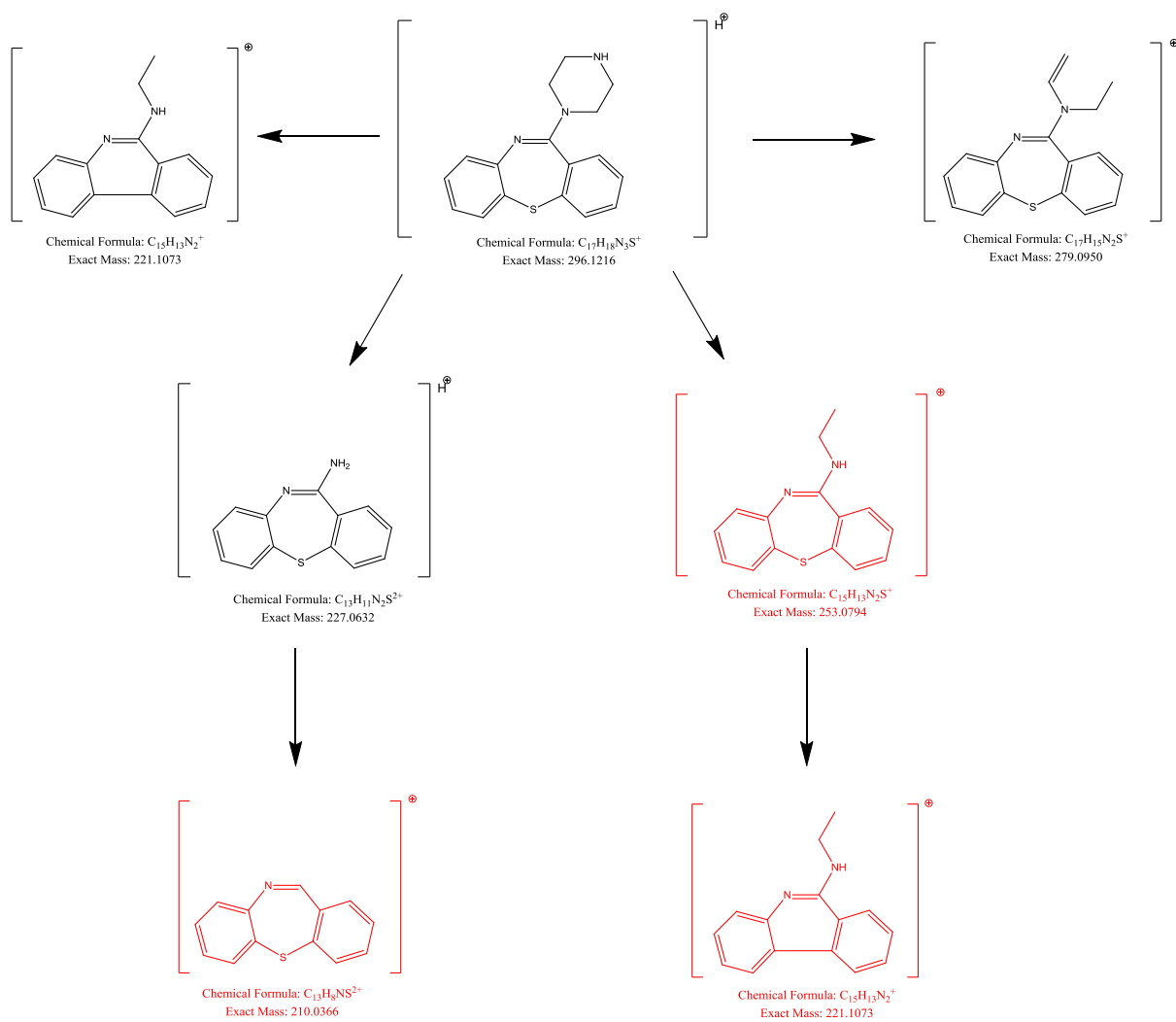
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356 **PTP 358**

357 MS^1

358

QUT_UV_130219_128_MRM_MS3 #1317 RT: 14.33 AV: 1 NL: 2.68E7
T: FTMS + c ESI Full ms [50.00-600.00]

Peaks: 73.0649, 87.0805, 101.0963, 123.9929, 178.1169, 200.0897, 243.0451, 269.0760, 327.1184, 351.8990, 358.1584, 400.1668, 445.1780, 476.7082, 542.8899, 587.9059

359 358.16 → MS^2

360

QUT_UV_130219_128_MRM_MS3 #1318 RT: 14.34 AV: 1 NL: 2.30E7
T: FTMS + c ESI d Full ms2 358.16@cid30.00 [85.00-370.00]

Peaks: 88.9294, 114.0915, 132.1018, 149.2636, 183.9749, 200.0907, 221.1078, 233.1305, 253.0802, 265.1021, 358.1596

361 358.16 → 253.08 → MS^3

362

QUT_UV_130219_128_MRM_MS3 #1319 RT: 14.36 AV: 1 NL: 1.10E7
T: FTMS + c ESI d Full ms3 358.16@cid30.00 253.08@cid30.00 [55.00-26(

Peaks: 99.0000, 110.2890, 124.6990, 142.3372, 155.1457, 165.2914, 176.7907, 186.6561, 200.0898, 210.0369, 219.0926, 221.1077, 226.0681, 238.0541, 246.1405, 253.0799, 259.7583

363   358.16 → 132.10 → MS³

364

365   Fragmentation pattern of PTP 358
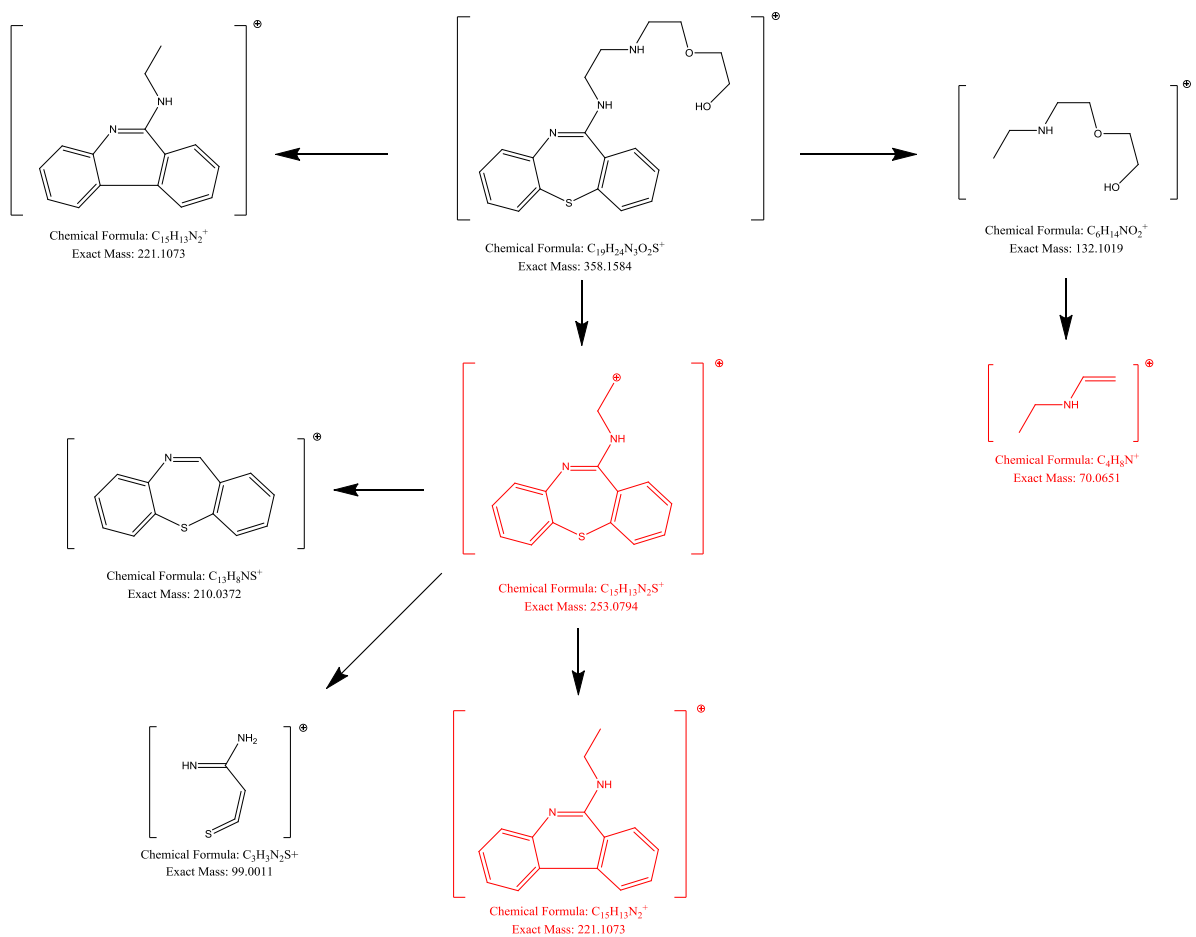
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370 **PTP1 400**

371 MS^1

372

373 400.17 → MS^2

374

375 400.17 → 269.08 → MS^3

376

377

378

379   400.17 → 352.20 → MS³

380

381   Fragmentation pattern of PTP1 400
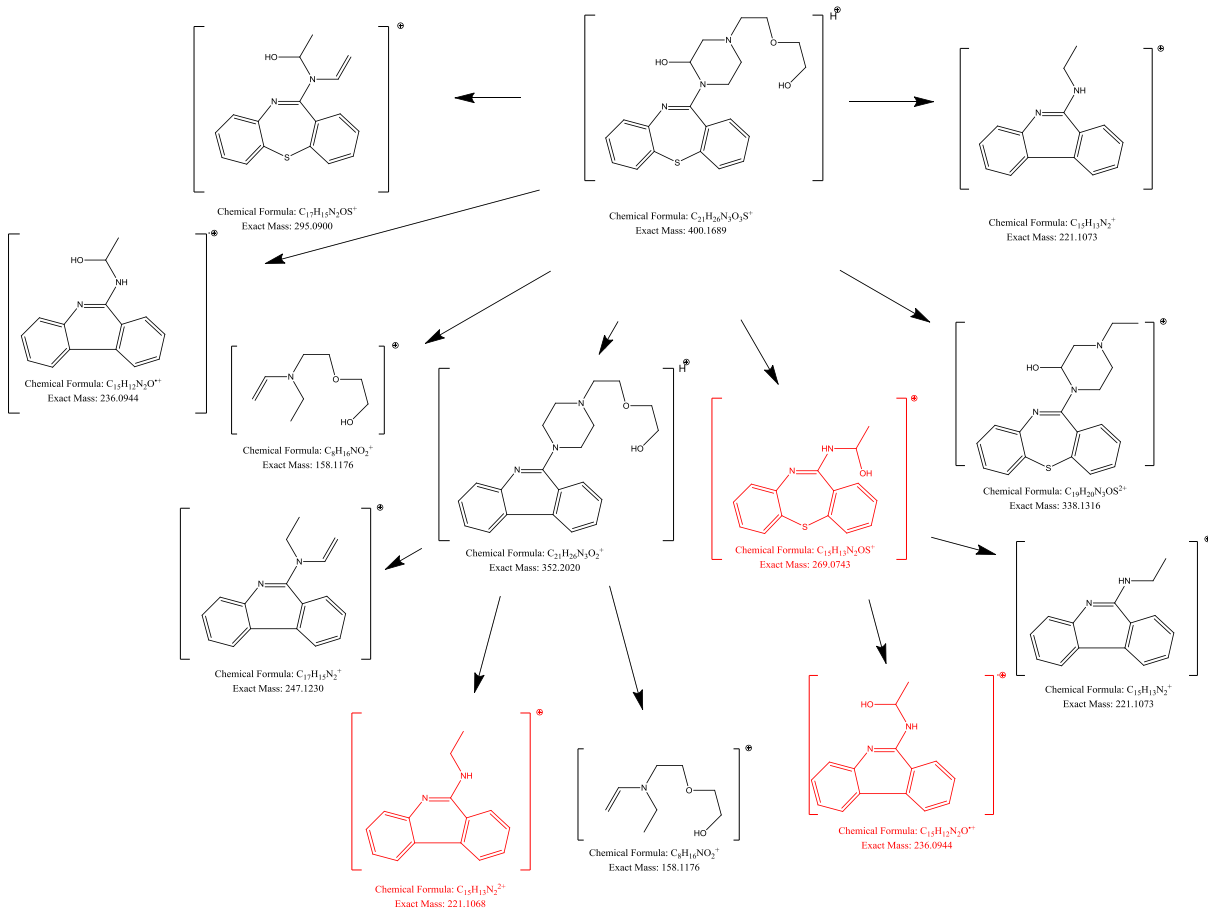
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388 **PTP2 400**

389 MS^1

QUT_UV_130219_128_MRM_MS3 #1345 RT: 14.65 AV: 1 NL: 2.33E7
T: FTMS + c ESI Full ms [50.00-600.00]
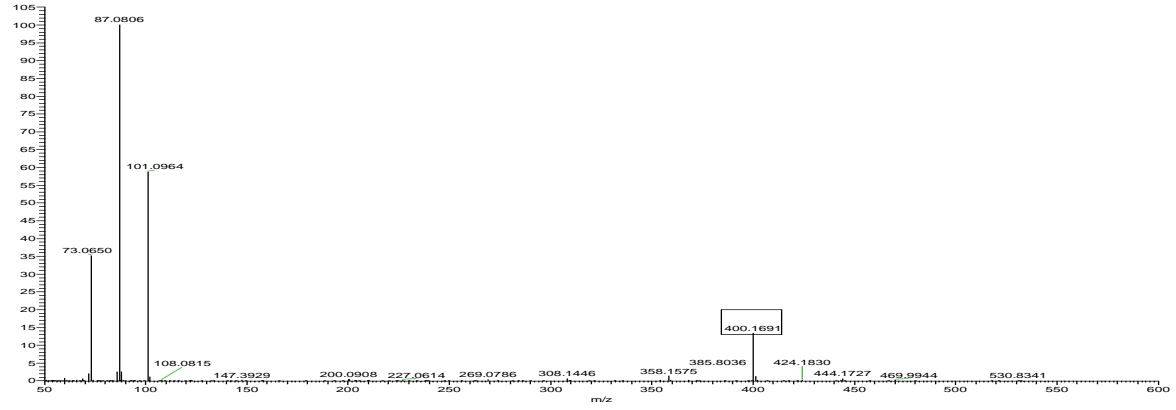
391 400.17 → MS^2

qut_uv_130219_128_mrm_ms3 #1346 RT: 14.66 AV: 1 NL: 4.75E6
T: FTMS + c ESI d Full ms2 400.17@cid30.00 [100.00-415.00]
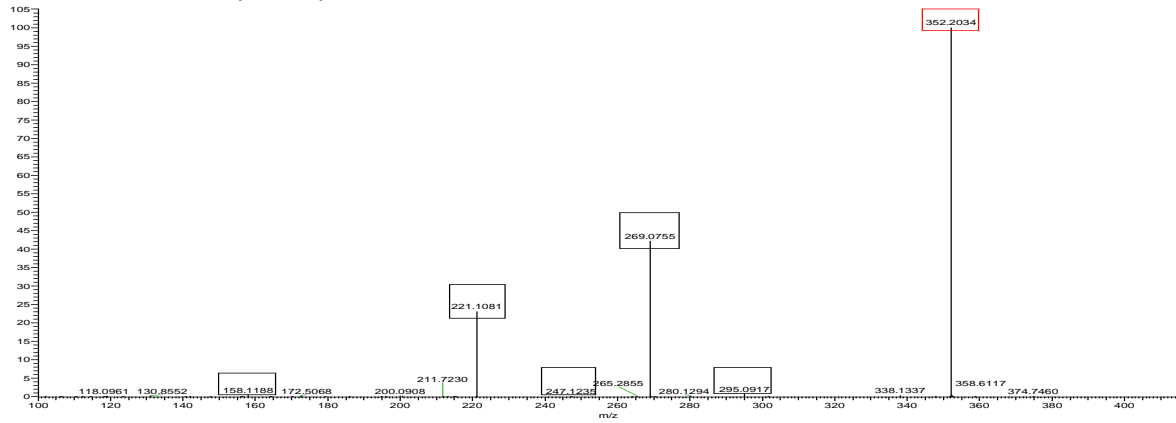
393 400.17 → 352.20 → MS^3

qut_uv_130219_128_mrm_ms3 #1347 RT: 14.67 AV: 1 NL: 4.54E6
T: FTMS + c ESI d Full ms3 400.17@cid30.00 352.20@cid30.00 [85.00-365]
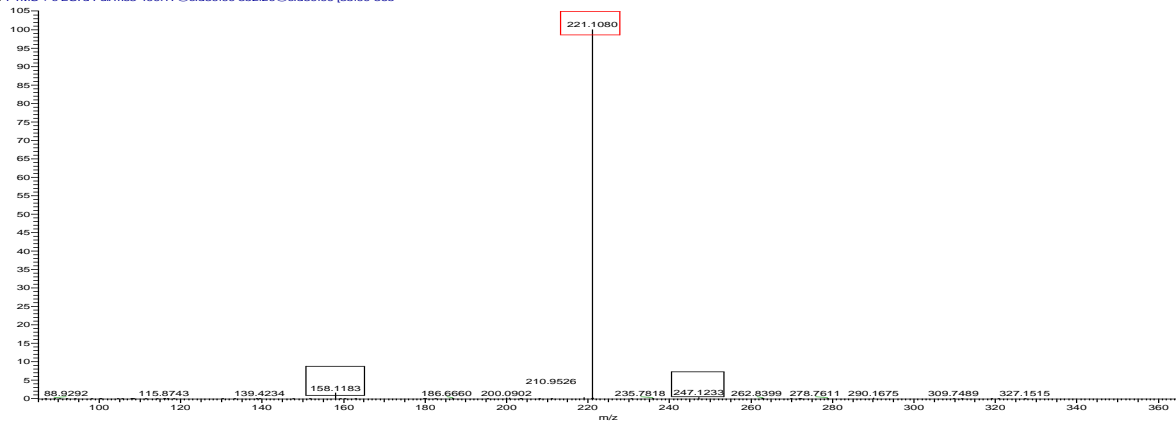
396   400.17 → 269.08 → MS³
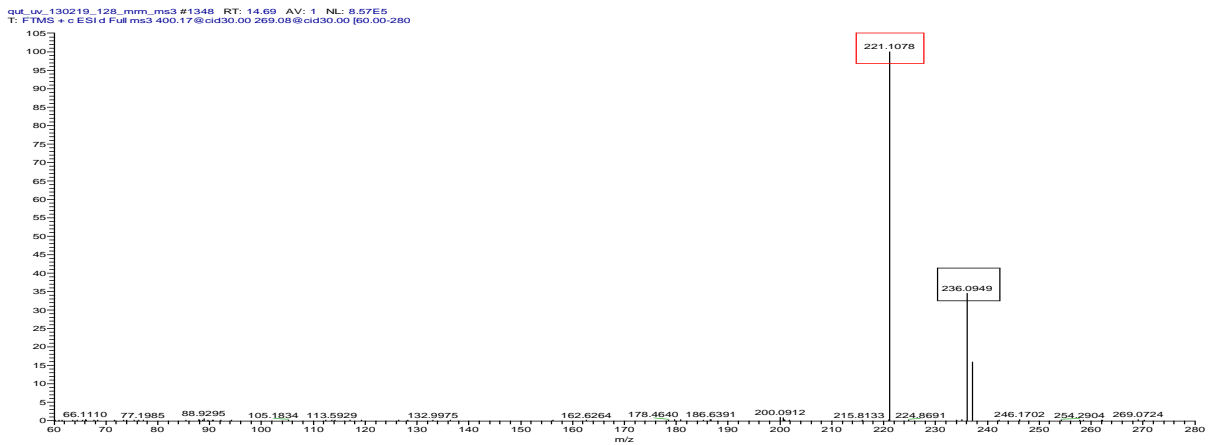
397

398   Fragmentation pattern of PTP2 400
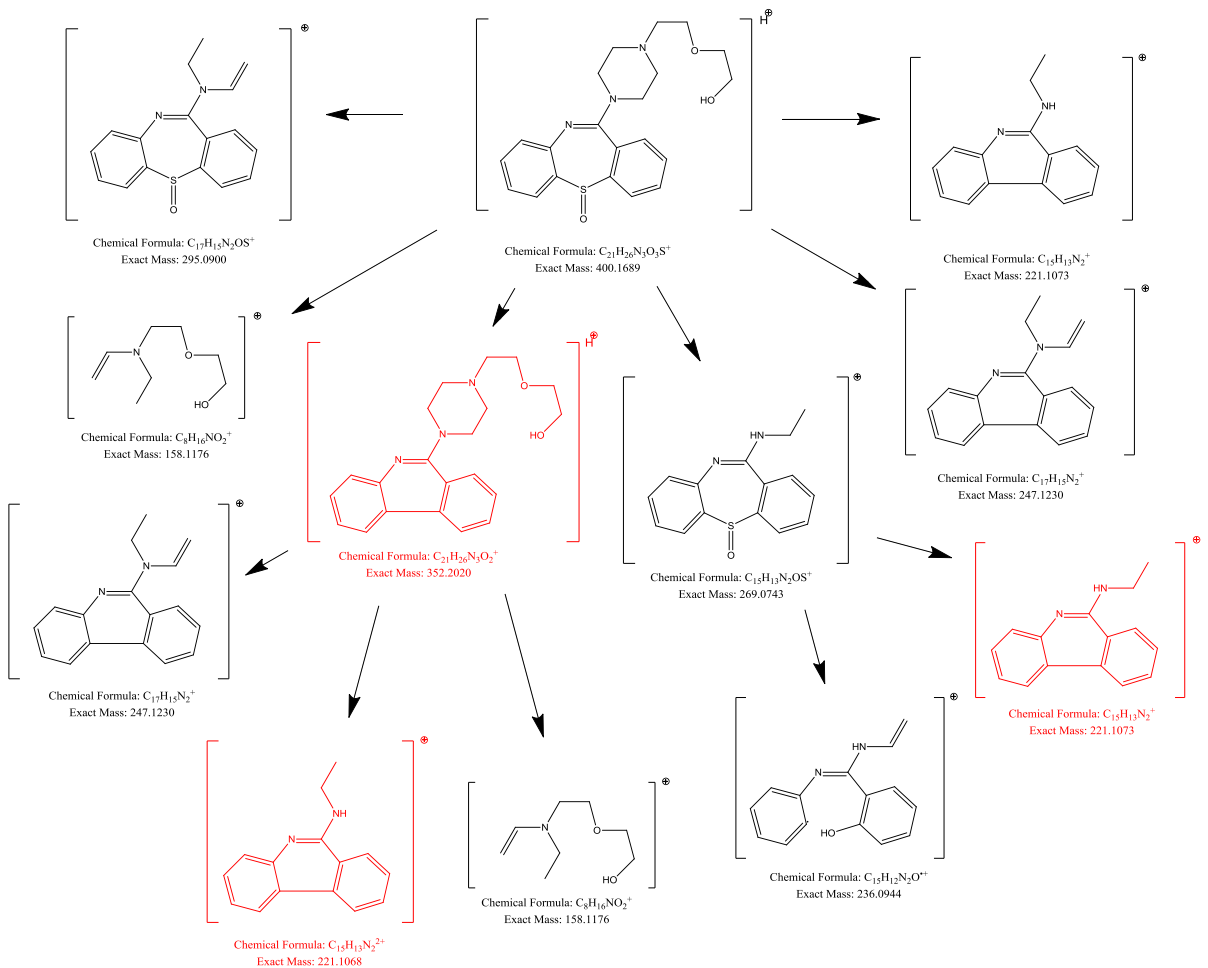
399

400

401

402

403 **PTP3 400**

404 MS^1

QUT_UV_130219_128_MRM_MS3 #1366 RT: 14.88 AV: 1 NL: 1.03E6
T: FTMS + c ESI Full ms [50.00-600.00]
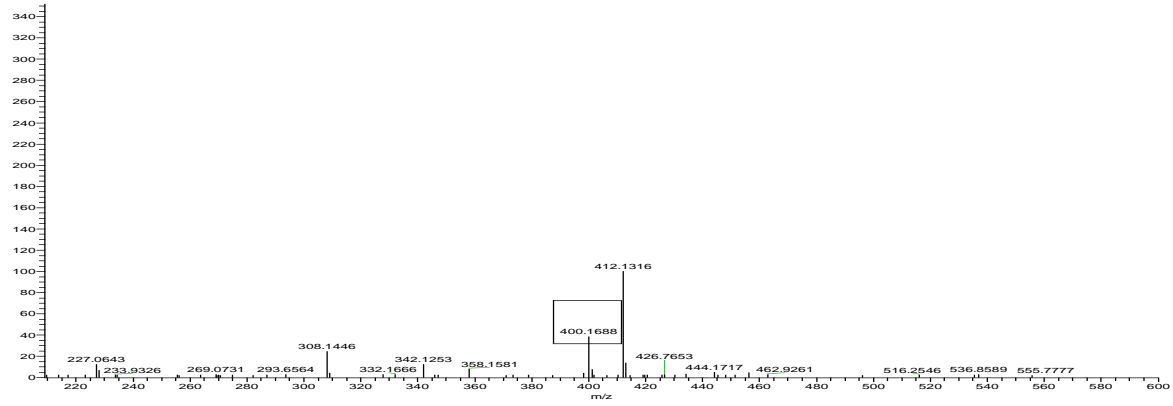
405

406 400.17 → MS^2

QUT_UV_130219_128_MRM_MS3 #1370 RT: 14.93 AV: 1 NL: 1.61E6
T: FTMS + c ESI d Full ms2 400.17@cid30.00 [100.00-415.00]
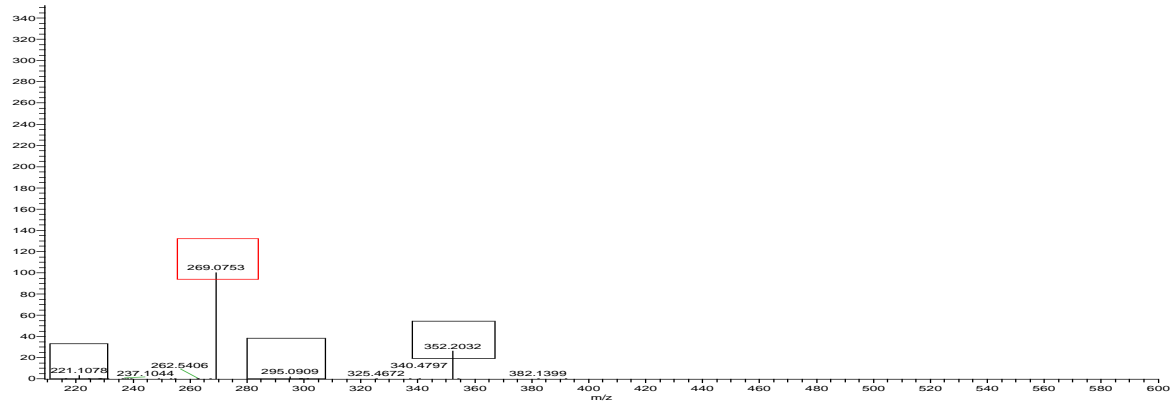
407

408 400.17 → 269.08→ MS^3

QUT_UV_130219_128_MRM_MS3 #1371 RT: 14.94 AV: 1 NL: 5.81E5
T: FTMS + c ESI d Full ms3 400.17@cid30.00 269.08@cid30.00 [60.00-5!
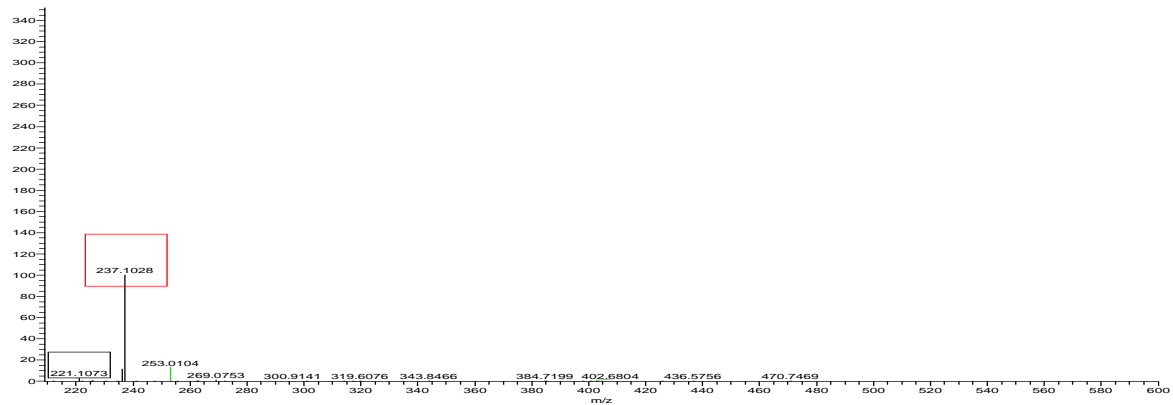
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414   400.17 → 352.20 → MS³

QUT_UV_130219_128_MRM_MS3 #1372   RT: 14.95   AV: 1   NL: 3.72E5
T: FTMS + c ESI d Full ms3 400.17@cid30.00 352.20@cid30.00 [85.00-7'
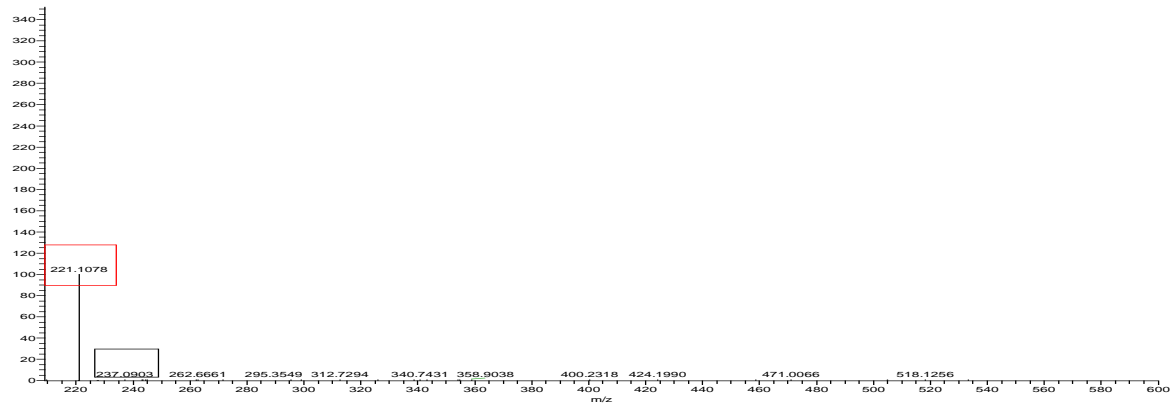
415

416   Fragmentation pattern of PTP3 400
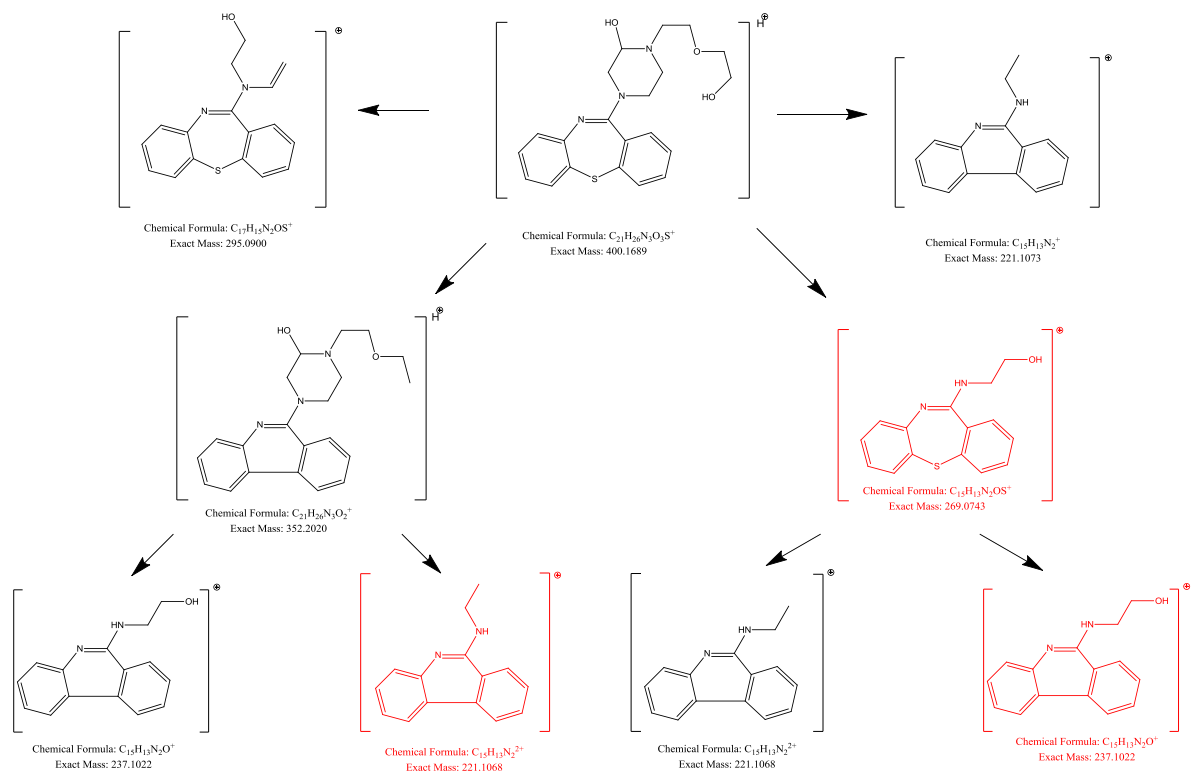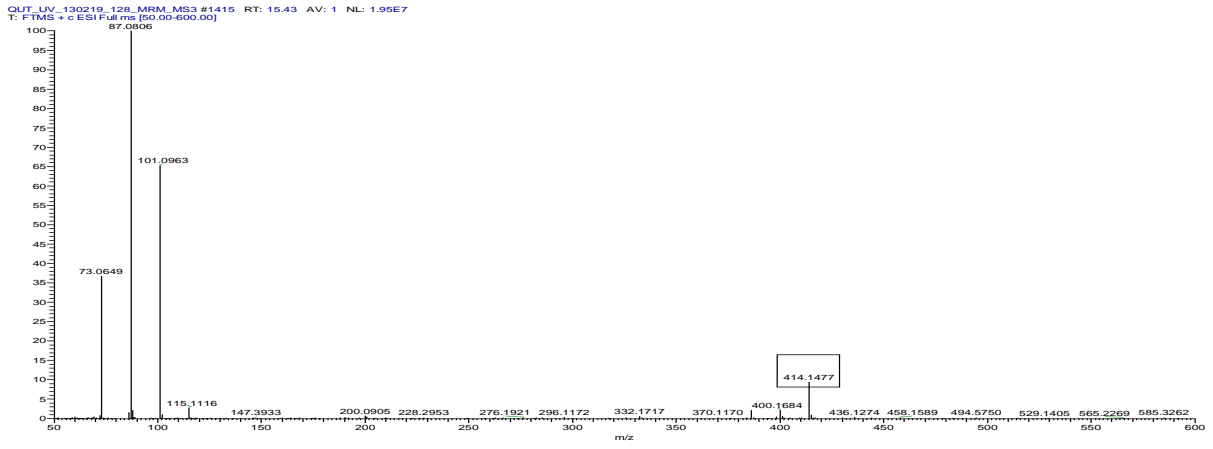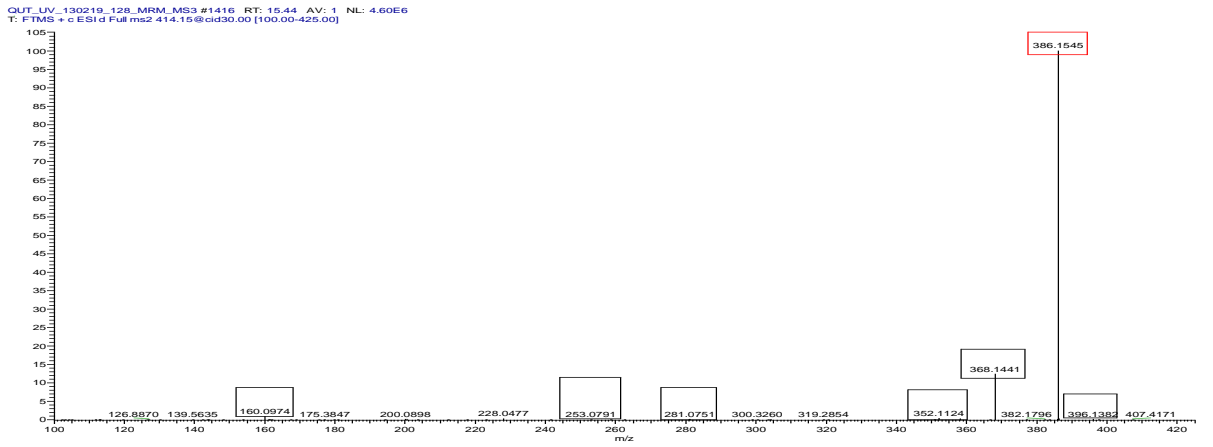

417
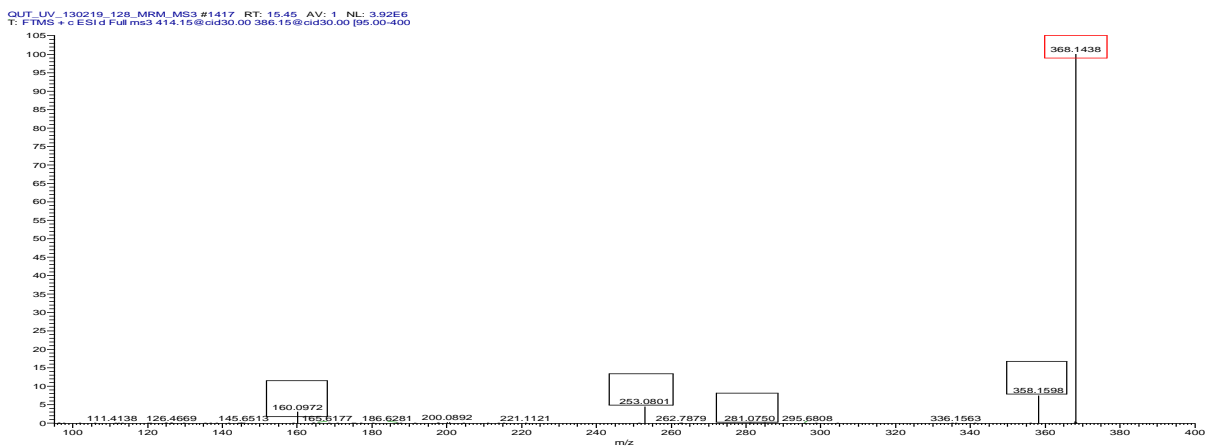
418

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425 **PTP 414**

426 MS^1

427

428 414.15 → MS^2

429

430 414.15 → 386.15 → MS^3

431

432

433

434

435   414.15 → 368.14 → MS³

436

437   Fragmentation pattern of PTP 414


438

439

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443

444 **Text S15: Calculated absorption spectra for quetiapine (QUT) and phototransformation**

445 **product (PTP)2 400**

446 For QUT and all PTPs, the absorbance spectrum was calculated by ChemBio3D Ultra

447 14.0.0.117 (PerkinElmer Inc., Waltham, USA). For PTP2 400, an additional absorbance peak

448 was observed at around 350 nm (see Fig. S8). All other PTPs did not show any significant

449 change regarding absorbance maxima (data not shown).
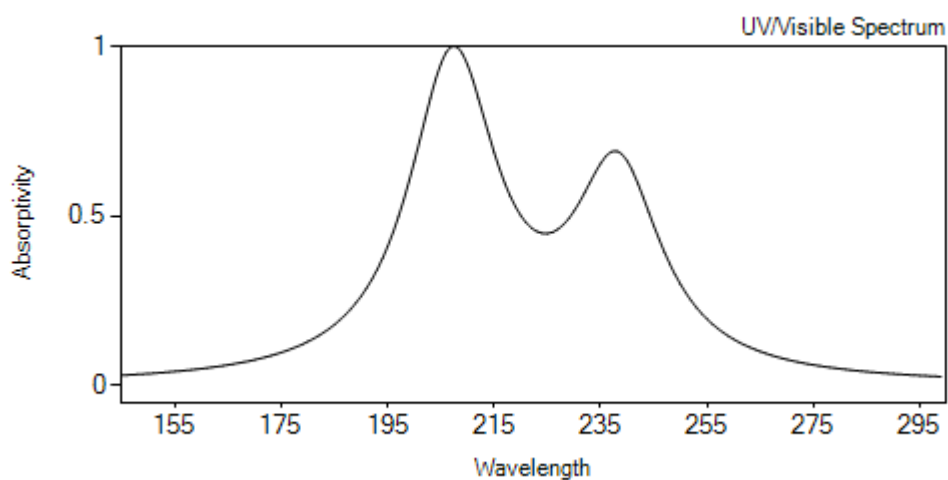
450

451 Fig. S7: Calculated absorbance spectrum for quetiapine (QUT).
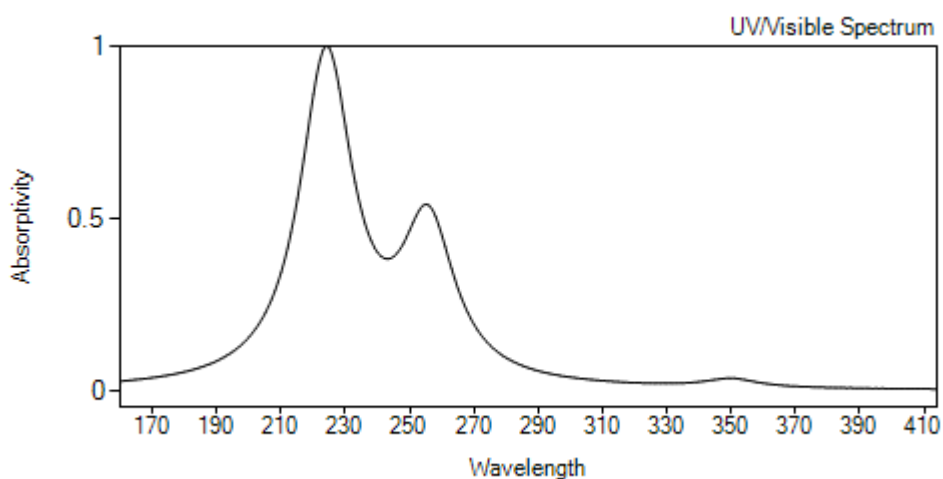
452

453 Fig. S8: Calculated absorbance spectrum for PTP2 400.

454

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