Testing selected micro-contaminants for their applicability as water quality indicators

Dissertation
zur Erlangung des Doktorgrades
der Mathematisch-Naturwissenschaftlichen Fakultäten
der Georg-August-Universität zu Göttingen

vorgelegt von

Karsten Nödler

aus Witzenhausen

Göttingen 2013

D 7

Referent:	Professor Dr. Martin Sauter (Georg-August-Universität Göttingen)
Korreferenten:	1. PD Dr. Traugott Scheytt (Technische Universität Berlin)
	2. Dr. Tobias Licha (Georg-August-Universität Göttingen)
Tag der mündlichen Prüfung:	31. Juli 2012

Abstract

The use of anthropogenic micro-contaminants such as pharmaceuticals, lifestyle products, biocides, and pesticides as water quality indicators has aroused great interest in the scientific community and there is a high potential of using these compounds as indicators for process optimization, source delineation, and to evaluate the extent of a contamination (e.g. the amount of wastewater in surface- and groundwater). The presented work is the successful and consequent continuation of ongoing research activities on the evaluation of selected micro-contaminants as water quality indicators, their occurrence and fate in the environment, and their redox-dependent transformation.

In order to use a compound as an indicator the availability of sensitive and selective analytical methods is essential. In this thesis, the development of a multi-residue analytical method based on high-performance liquid chromatographic separation and electrospray ionization with tandem mass spectrometric detection (HPLC-ESI-MS/MS) for the simultaneous analysis of 46 basic, neutral, and acidic compounds covering a wide range of polarity (log K_{OW} <0–5.9) and potential contamination sources is described. The main feature of the method is the simultaneous solid phase extraction (SPE) of all analytes followed by the simultaneous separation and detection by HPLC-MS/MS with electrospray ionization in both positive and negative polarization within the same chromatogram. Method quantitation limits (MQL) for river- and seawater are in the low ng L^{-1} range. Furthermore, the high flexibility of the method (inserting additional analytes and adaptation to other water types) is demonstrated.

The following part of the thesis presents the results from extensive river monitoring with focus on the correlation of 41 micro-contaminants with potassium (K^+) and its temporal and spatial variation. Depending on the (geogenic) K^+ background concentration, urine can be a significant source of K^+ for surface waters. Accordingly, a positive correlation of concentrations of wastewater-related micro-contaminants and K^+ is to be expected in receiving waters of wastewater treatment plant (WWTP) effluents. This correlation was found for compounds, which meet the following criteria: 1) WWTP effluent is the dominating source of the compound; 2) Variability of its mass flux in the WWTP is negligible; and 3) The compound is persistent in WWTPs and in the environment. Among other compounds, carbamazepine, sulfamethoxazole, and tolyltriazole demonstrate the best correlations. K^+-equivalents of the individual

micro-contaminants obviously depends on land use and population structure of the investigated river section. A correlation with K^+ indicates that the concentration of the respective micro-contaminant depends only on river discharge. Following this assumption, the prediction of micro-contaminant concentrations at certain river locations could be substantially simplified. Regarding bank filtration and the interaction of surface water and groundwater in general, it may be possible to derive input functions of the correlating micro-contaminants. This would allow for a better evaluation of the attenuation potential at individual filtration sites. Furthermore, the approach can be used for contamination source delineation.

Additionally, an approach to estimate the volume of untreated wastewater entering karst aquifers through rapid recharge is presented. Contamination from untreated wastewater leakage and related bacterial contamination poses a serious threat to drinking water quality and public health. However, a quantification of the magnitude of leakage is difficult. For this purpose a balance approach was adapted. It is based on the mass flow of caffeine in spring water, the load of caffeine in untreated wastewater, and the daily water consumption per person in a spring catchment area. The methodology was applied to estimate the amount of leaking and infiltrating wastewater to a well-investigated karst aquifer (Gallusquelle, Germany) on a daily basis.

Also presented is a microcosm study on the transformation of the antibiotic sulfamethoxazole (SMX) under denitrifying conditions. A selective reaction pathway with the under denitrifying conditions produced nitrogen species nitrogen oxide (NO) and nitrite (NO_2^-) was suspected and the evaluation of two hypothesized transformation products (TP) 4-nitro-N-(5-methylisoxazol-3-yl)-benzenesulfonamide (4-nitro-SMX) and N-(5-methylisoxazol-3-yl)-benzenesulfonamide (desamino-SMX) was confirmed in a denitrifying water/sediment batch experiment. Furthermore, the compound 4-nitro-SMX has the potential to retransform to its parent compound SMX. The TPs were also detected in environmental samples demonstrating the high relevance of this study regarding the occurrence and fate of SMX in the environment and for water quality monitoring. Furthermore, TPs, which are specific for certain redox conditions, could be potentially used as redox indicators regarding reactive transport.

Zusammenfassung

Die Verwendung anthropogener organischer Spurenstoffe wie beispielsweise Pharmazeutika, Lifestyle-Produkte, Biozide und Pestizide als Indikatoren für die Bewertung der Wasserqualität hat großes Interesse in der Wissenschaftsgemeinde geweckt, und die Verwendung dieser Substanzen als Indikatoren für die Prozessoptimierung, Quellzuordnung und zur Abschätzung des Ausmaßes einer möglichen Kontamination (z. B. den Abwasseranteil von Oberflächen- und Grundwasser) besitzt ein sehr großes Anwendungspotential. Die hier präsentierte Arbeit ist die erfolgreiche und konsequente Weiterführung bestehender Forschungsaktivitäten zur Eignung ausgewählter Spurenstoffe als Indikatoren für die Bewertung der Wasserqualität, ihrem Vorkommen und Verhalten in der Umwelt sowie ihrer Redox-spezifischen Transformation.

Um eine Substanz als Indikator verwenden zu können, müssen sensitive und selektive Analysenmethoden verfügbar sein. In der vorliegenden Arbeit wird die Entwicklung einer Multimethode für den Nachweis von 46 basischen, neutralen und sauren Analyten mittels der Hochleistungs-Flüssigchromatographie und Elektronenspray-Ionisation (ESI) mit anschließender Tandem-Massenspektrometrie (HPLC-ESI-MS/MS) beschrieben. Das ausgewählte Analytenspektrum deckt einen weiten Bereich hinsichtlich der Polarität der Stoffe (log K_{OW} <0–5,9) sowie ihrer repräsentierten Kontaminationsquellen ab. Die Besonderheit der entwickelten Methode stellt die simultane Festphasenanreicherung (SPE), Trennung und Detektion aller Analyten dar. Um dieses realisieren zu können, wird das ESI-Interface in beiden möglichen Operationsmodi (+/−) verwendet, so dass pro Probe nur eine Injektion notwendig ist. Die Bestimmungsgrenzen der Methode in Fluss- und Meerwasser liegen im Bereich weniger ng L^{-1}. Im weiteren Verlauf der Arbeit wird die hohe Flexibilität der Methode (Integration zusätzlicher Analyten und Anpassung an andere Wassertypen) demonstriert.

Im darauf folgenden Abschnitt werden die Ergebnisse eines intensiven Fluss-Monitorings vorgestellt. Der Fokus liegt dabei auf der Korrelation von 41 Spurenstoffen mit Kalium (K^+) und deren räumlichen und zeitlichen Varianz. Da Urin je nach K^+-Hintergrundkonzentration des Gewässers eine signifikante K^+-Quelle darstellen kann, ist in Gewässern mit hohem Abwasseranteil eine positive Korrelation von abwasserbürtigen Stoffen und K^+ zu erwarten. Diese Korrelation ist für Stoffe mit folgenden Charakteristika bestätigt worden: 1) Kläranlagenabläufe sind die Hauptquelle der Substanz; 2) Die Fracht der

Substanz in der Kläranlage ist nur geringen zeitlichen Schwankungen unterworfen; und 3) Hohe Persistenz der Verbindung bei der Abwasserbehandlung und in der Umwelt. Neben anderen Spurenstoffen zeigen Carbamazepin, Sulfamethoxazol und Tolyltriazol die beste Korrelation. Darüber hinaus sind die K^+-Äquivalente der einzelnen Stoffe offensichtlich abhängig von Landnutzung und Bevölkerungsstruktur im Einzugsgebiet des untersuchten Flussabschnitts. Eine Korrelation mit K^+ zeigt, dass die Konzentration des korrelierenden Spurenstoffs nur vom Abfluss des Fließgewässers abhängig ist. Nach diesem Konzept könnte die Vorhersage der Konzentration entsprechender Spurenstoffe an bestimmten Flussabschnitten erheblich vereinfacht werden. Analog zu den genannten Charakteristika 1–3 kann der Ansatz zur Quellidentifizierung neu auftretender/identifizierter Substanzen genutzt werden. Darüber hinaus könnten Eintragsfunktionen für die korrelierenden Spurenstoffe hinsichtlich Oberflächenwasser/Grundwasser-Interaktion hergeleitet werden. Dies würde eine realistischere Bewertung der Reinigungsleistung von Anlagen zur (künstlichen) Grundwasseranreicherung ermöglichen.

Anschließend wird eine Methode präsentiert, mit Hilfe derer sich das Volumen von schnell transportiertem, unbehandeltem Abwasser in einem Karstaquifer abschätzen lässt. Eine Kontamination mit unbehandeltem Abwasser und die damit verbundene bakterielle Belastung stellen eine ernsthafte Bedrohung für die Trinkwasserqualität und die öffentliche Gesundheit dar. Das Ausmaß einer Kontamination quantifizieren zu können ist allerdings meist problematisch. Daher wurde ein bereits bekannter Massenbilanzansatz der aktuellen Fragestellung angepasst. In die Berechnung der Abwassermenge fließen ein: Die Coffein-Fracht an der Quelle, die übliche Coffein-Belastung in unbehandeltem Abwasser und der tägliche durchschnittliche Trinkwasserverbrauch pro Person im beobachteten Quelleinzugsgebiet. Der entwickelte Ansatz wurde zur Berechnung der täglich zuströmenden Abwassermenge an einem bereits gut charakterisierten Karstaquifer (Gallusquelle, Deutschland) angewendet.

Weiterhin werden die Ergebnisse einer Mikrokosmos-Studie zur Transformation des Antibiotikums Sulfamethoxazol (SMX) unter denitrifizierenden Bedingungen vorgestellt. Ein selektiver Reaktionsmechanismus mit den unter denitrifizierenden Bedingungen gebildeten N-Spezies Stickstoffmonoxid (NO) und Nitrit (NO_2^-) ist die zugrunde liegende Arbeitshypothese und die Bildung der daraus abgeleiteten Transformationsprodukte (TP) 4-Nitro-N-(5-methylisoxazol-3-yl)-benzenesulfonamid (4-Nitro-SMX) und N-(5-methylisoxazol-3-yl)-benzenesulfonamid (Desamino-SMX) während des zeitlichen Verlaufs eines Wasser/Sediment-Batchversuchs wird dargestellt. Beide TPs können auch in Umweltproben nachgewiesen werden. Unter geeigneten Reaktionsbedingungen kann das TP 4-Nitro-SMX zudem zu SMX retransformiert werden. Dies zeigt die hohe Relevanz der vorliegenden Arbeit hinsichtlich des Vorkommens und Verhaltens dieses Antibiotikums in der Umwelt und für das Monitoring der Wasserqualität. Darüber hinaus können Redox-spezifische TPs als Indikatoren für den reaktiven Stofftransport verwendet werden.

Danksagung

An erster Stelle möchte ich mich bei Professor Dr. Martin Sauter und Dr. Tobias Licha für die Betreuung der Arbeit und der Bereitstellung des interessanten Themenfeldes bedanken. Bei Dr. Tobias Licha möchte ich mich dabei ganz besonders für dessen beständige Diskussionsbereitschaft, seine Hilfe und Ratschläge bei konkreten Fragen und das hohe Maß an Vertrauen sowie wissenschaftlicher Freiheit, was er mir während meiner Arbeit entgegengebracht hat, bedanken. Dies alles war sicherlich nicht selbstverständlich und ich habe die daraus entstandene Arbeitsatmosphäre sehr genossen. Herzlichen Dank dafür! Daneben möchte ich mich selbstverständlich auch bei den weiteren Mitgliedern der Prüfungskommission Dr. Michael Hoppert, Dr. Chicgoua Noubactep, Professor Dr. Hans Ruppert und Dr. Traugott Scheytt für die Übernahme dieser Aufgabe bedanken.

Meinen Bürokollegen Martin, Mario und Enrico danke ich herzlich für ihre Diskussionsbereitschaft und die stets angenehme und produktive Atmosphäre in Büro, Labor und darüber hinaus. Anke von Gaza und Mechthild Rittmeier danke ich vielmals für die Messung der An- und Kationen und die generelle Unterstützung im Labor. Olav Hillebrand danke ich vor allem für seine umfangreiche Hilfe bei Probenahme und Probenvorbereitung aber auch für seine oftmals ansteckend gute Laune. Beka Peters-Kewitz und Brigitte Hinz danke ich vielmals für die Unterstützung bei der Überwindung bürokratischer Hürden.

Erfolgreiche wissenschaftliche Arbeit kann nur in guter und intensiver Zusammenarbeit mit anderen engagierten Wissenschaftlerinnen und Wissenschaftlern entstehen. Daher möchte ich mich neben den bereits genannten Personen auch ausdrücklich bei allen weiteren Koautoren und Koautorinnen der in dieser Arbeit vorgestellten Veröffentlichungen bedanken. Der Weg hin zu einer Publikation kann lang und steinig sein – aber er lohnt sich.

Den Abschluss meiner Doktorarbeit möchte ich zum Anlass nehmen, auch den wichtigen Menschen abseits der Georg-August-Universität zu danken, die, vielleicht ohne es zu wissen, am Erfolg meiner Arbeit beteiligt waren. Ganz besonders möchte ich mich bei meiner Familie und meinen Freunden bedanken, die mich immer und ehrlich bei allem unterstützt haben – vor allem, als ich im Alter von 25 Jahren doch eher spät den Entschluss gefasst habe, ein Studium zu beginnen. Meiner lieben Maria möchte ich vor allem für ihre liebevolle Unterstützung in besonders schwierigen Phasen danken. Den Initiatoren des Studiengangs „Water-Science“ an der Universität Duisburg-Essen bin ich ebenfalls zu großem Dank verpflichtet. Dank dieses abwechslungsreichen und interdisziplinären Studiums fühlte ich mich perfekt auf diese Doktorarbeit vorbereitet.

“In the book of life, the answers aren't in the back.”

Charlie Brown

Table of Contents

List of Figures

List of Tables

Chapter 1

1 Introduction

1.1 Motivation and background

The term 'water quality' is used to describe the biological, chemical, and physical characteristics of water. However, a clear and distinctive quality classification of water strongly depends on the particular type and purpose of the water and the underlying national and international quality standards (European Commission, 2000; WHO, 2011a). Fecal contamination bears a serious threat to water quality and public health, which emphasizes the need for sensitive and selective methods to detect a potential contamination. The link between fecal contamination of drinking water sources and the outbreak of infectious diseases was already discovered in the late 19th century and the first concept of using microorganisms such as *Escherichia coli* (*E. coli*) as selective fecal indicators was introduced (Koch, 1893; Tallon *et al.*, 2005). Today, according to the current guidelines for drinking water quality of the WHO (2011a) the criteria determined for microbial fecal indicators are as follows.

The organism should:

(1) not be pathogenic itself;

(2) be universally present in feces of humans and animals in large numbers;

(3) persist in water in a similar manner to fecal pathogens;

(4) be present in higher numbers than fecal pathogens;

(5) respond to treatment processes in a similar fashion to fecal pathogens;

(6) be readily detectable by simple, inexpensive culture methods.

According to the criterion (1) listed above it is important to highlight that the indicator itself can - but does not necessarily - be a pathogen provided that the pathogens are sufficiently “represented” by the indicator organism regarding the source of contamination and/or the technical process. Moreover, considering occupational health and safety the cultivation of non-pathogens shall always be given preference before working with pathogenic organisms. In fact, only a small group of *E. coli* strains causes diseases in humans (Tallon *et al.*, 2005). Criterion (5) reflects the assumption that the organism is also a suitable indicator for treatment and/or process efficiency. However, it is widely accepted that one indicator organism alone usually cannot provide all indicator criteria. As an example regarding the evaluation of the efficiency of disinfection and physical removal processes for viruses and protozoan parasites during drinking water treatment preference should be given to the bacteria *Clostridium perfringens* instead of *E. coli* (WHO, 2011a).

Alternatives to microorganisms as indicators for fecal contamination are chemical compounds such as fecal sterols (Glassmeyer *et al.*, 2005; Savichtcheva and Okabe, 2006). Apart from slight modifications most of the indicator criteria given above are also valid for chemical compounds: Fecal sterols are non-hazardous substances, high concentrations are universally present in feces of mammals, and the compounds can be sensitively detected and quantified by a common analytical technology (gas chromatography - mass spectrometry; GC-MS) (Isobe *et al.*, 2002 and 2004; Savichtcheva and Okabe, 2006).

It becomes obvious that the introduced indicator concept can be further generalized and expanded: The optimal indicator or set of indicators (chemical compounds or organisms) is very selective and highly sensitive regarding the indicated source of contamination and/or the technical or environmental process. It is thus essential that selective and sensitive analytical methods are available.

During the last decades the field of analytical sciences progressed rapidly. This progress led to the fact that numerous mobile trace organic compounds of anthropogenic origin (micro-contaminants) such as pharmaceuticals, personal care products, biocides, pesticides, and lifestyle products are present in virtually all parts of the water cycle (Bergmann *et al.*, 2008; Buerge *et al.*, 2006 and 2009; Halling-Sørensen *et al.*, 1998; Kolpin *et al.*, 2002; Schwarzenbach *et al.*, 2006; Ternes, 2007, Wittmer *et al.*, 2011). Significant entry routes of micro-contaminants are demonstrated in Figure 1.1.

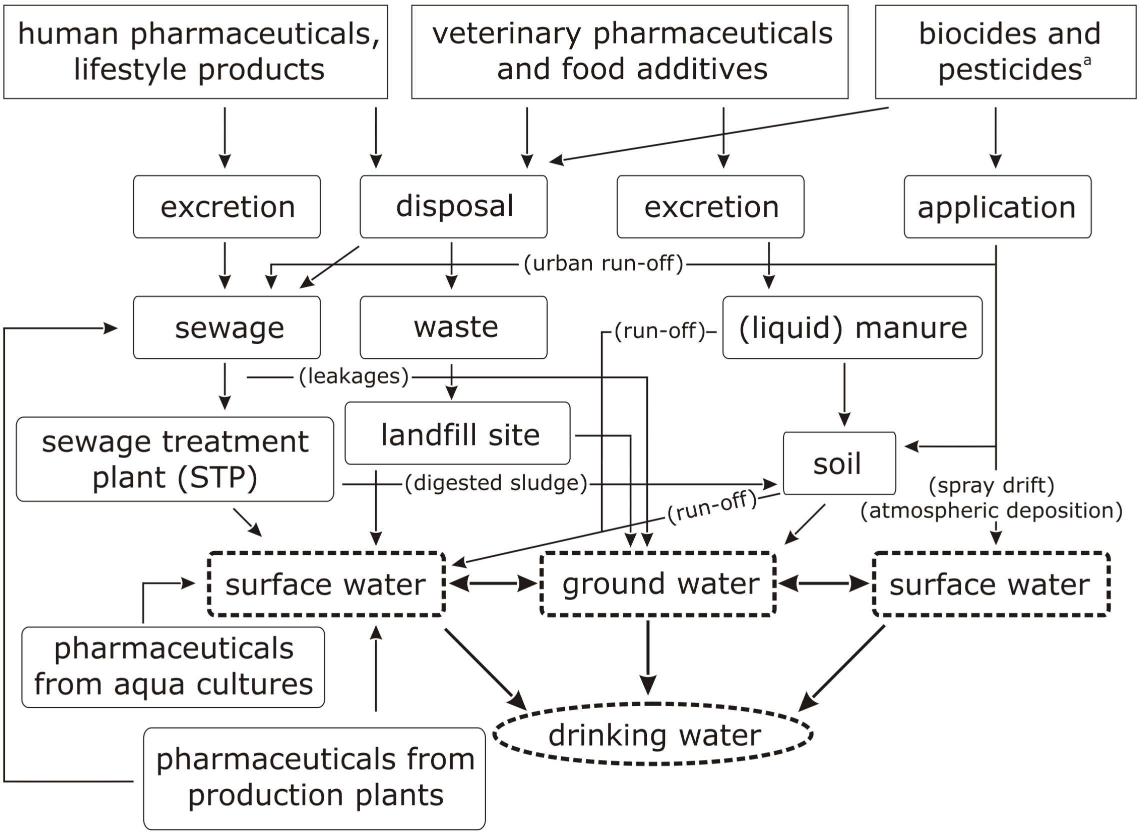


Figure 1.1: Significant entry routes of micro-contaminants into the water cycle (modified after Ternes, 1998; including further information from Bound and Voulvoulis, 2005; Heberer, 2002; Reichenberger *et al.*, 2007; Wittmer *et al.*, 2011); [a]definition of pesticides and biocides according to the European Commission (1998): pesticides are defined as compounds used for plant protection; biocides are defined as compounds used for all non-plant protection purposes.

Due to the continuously growing world population, a generally increasing consumption of these compounds must be assumed. Furthermore, it is widely accepted that the use of the vast majority of pharmaceutically active substances is correlated to the number of elderly people in the society (Kümmerer, 2010). Against the backdrop of demographic changes the usage of these compounds for the characterization of aquifer and catchment properties (heterogeneity, organic carbon distribution, catchment boundaries, land use), groundwater chemistry (redox zones, pH changes), groundwater hydraulics (source apportionment, pathways, transit and residence times, mixing processes) and hence inevitably an evaluation of the anthropogenic impact on water quality and vulnerability of water resources is a highly promising field of application. Especially when one bears in mind that, in contrast to other known chemical indicators for wastewater (e.g. Cl^- and Na^+) and agricultural areas (NO_3^-), there is virtually no natural (i.e. geogenic) background of these compounds. Therefore, the detection of organic micro-contaminants in the aqueous environment is tantamount to the ultimate proof of an anthropogenic impact on water quality.

There were already promising studies, which underlined the high potential of using micro-contaminants as indicators for process optimization, source delineation, and even the quantification of a contamination (e.g. Buerge *et al.*, 2006 and 2009; Dickenson *et al.*, 2009). However, considering the large number of pharmaceuticals, lifestyle products, biocides, and pesticides on the market and the almost unlimited number of known and unknown environmental transformation products of these compounds, the identification of the most promising substances and associated indicator functionalities is an ongoing process. There is still a deep gap between the existing knowledge and the required comprehensive understanding with known dependencies from process interactions and/or boundary conditions in order to associate unambiguous indicator functionalities to these compounds.

1.2 Current stand in water quality evaluation regarding source delineation

The selection of analytes for standard water monitoring usually corresponds to the regulations associated to the responsible water quality guideline. However, in addition to risk and acceptance-based quality parameters, which are not subject of this thesis, the analysis of non-regulated compounds, a careful evaluation of results far beyond the scope of pure limit-value-based considerations, and a deeper understanding of bulk parameters possess a high potential for the assessment of water quality and influencing factors.

The pattern of rare earth elements and yttrium (REY) in water is redox-sensitive. Therefore, redox-induced anomalies offer the potential to be used as indication for certain redox conditions (Bau and Alexander, 2009; Goyne *et al.*, 2010; Kulaksiz and Bau, 2011). Furthermore, the positive anomaly of gadolinium (Gd; from contrast media for magnetic resonance imaging, MRI) can be used as a wastewater indicator in surface water and groundwater (Elbaz-Poulichet *et al.*, 2002; Massmann *et al.*, 2004; Möller *et al.*, 2000; Strauch *et al.*, 2008). In some areas the anthropogenic inputs are even so significant that the Gd anomaly can no longer be used as a clear natural geochemical indicator (Bau and Dulski, 1996). Regarding the delineation of distant sources the significant impact of a single industrial plant on the Rhine River was still detectable 400 km downstream of the plant by using the signal of anthropogenic lanthanum (La; Kulaksiz and Bau, 2011). Furthermore, the authors noted that geogenic background concentrations of La and Gd have to be determined before widespread anthropogenic contamination will have made it impossible.

Elevated boron (B) concentrations in surface water and groundwater can be associated with wastewater inputs (Butterwick *et al.*, 1989; Wolf *et al.*, 2004; Schreiber and Mitch, 2006). Regarding elevated geogenic B concentrations isotope ratio measurements are necessary to distinguish between anthropogenic impact and geogenic background (Gäbler and Bahr, 1999). However, due to technical and environmental developments the strong decreasing European trend of using B-bearing compounds already resulted in decreasing B concentrations in wastewater treatment plant effluents and surface waters (Neal *et al.*, 2010). Therefore, the use of B as water quality indicator is possibly no long-term option for the future.

Turbidity is a well-known and very important water quality indicator, which can be used in many different applications (WHO, 2011a). Also in karst systems turbidity can be associated with (pathogenic) bacteria (Heinz *et al.*, 2009). However, Pronk *et al.* (2007) observed that increasing turbidity is not necessarily connected to elevated cell counts of microorganisms. By using particle size distribution (PSD) they made a distinction between particles from the inside of the aquifer (autochthonous) and from the outside (allochthonous). The latter coincided with *E. coli* levels and the total organic carbon (TOC) concentration. Elevated TOC concentrations can be also associated with microbial contamination. However, in a different study increasing bacterial contamination was also observed at low turbidity and TOC levels (Heinz *et al.*, 2009), which demonstrated the limits of their indicator quality.

The major ions Na^+, Cl^-, and K^+ can also be associated to wastewater impact (Eiswirth and Hötzl, 1997; Heinz *et al.*, 2009; Gasser *et al.*, 2010; Massmann *et al.*, 2004). However, their applicability as wastewater indicators is strictly limited by their natural occurrence in the environment and sources others than wastewater (mining activities, street salt, etc.). Their exclusive application could possibly be recommended if very high fractions of wastewater and/or very low geogenic background concentrations are present. But their significance and resolution for the detection of wastewater after high dilution seem doubtful.

Conclusively, a main limitation of the introduced methods is the presence of (geogenic/autochthonous) background concentrations of the indicators and highly fluctuating background concentrations heavily decrease the significance of additional inputs and thus the sensitivity of the method. Therefore, it is very difficult to assign unambiguous indicator functionalities to these parameters.

1.3 New indicators for water quality assessment and source delineation

With very few exceptions (e.g. caffeine) the geogenic background of micro-contaminants is nonexistent. These compounds are suggested to be source specific and thus offer the potential to be employed as indicators for source delineation in monitoring groundwater and surface water quality, its decline or improvement. This especially applies to selected pharmaceuticals, pesticides, and stimulants (Buerge *et al.*, 2006; Godfrey *et al.*, 2007; Townsend and Young, 2000).

Detrimental environmental effects of pharmaceutical residues are suspected (Hernando *et al.*, 2006; Kümmerer, 2009). However, regarding the toxicological relevance of pharmaceuticals in potable water, adverse health effects are usually not expected at the typically encountered ng L^{-1} to low µg L^{-1} concentrations (Bruce *et al.*, 2010; WHO, 2011b). Consequently, and in contrast to pesticide residuals, these compounds are not regulated and routine monitoring of pharmaceuticals in drinking water is currently not recommended (WHO, 2011b). Among other compounds several pesticides were included into the list of priority substances of the EU Water Framework Directive (European Commission, 2000), but up to now no pharmaceuticals were included (on January 31, 2012 the inclusion of three pharmaceuticals was proposed; European Commission, 2012). However, even if monitoring of these compounds is not deemed necessary at the official level, there is very good reason for monitoring programs focusing on pharmaceuticals and micro-contaminants in general. Examples of how micro-contaminants were used as indicators for water quality independently of limit values or toxicity aspects highlight the potential benefit, which can be drawn from these organic substances:

- The anticonvulsant drug carbamazepine was identified to be much better suited for the calculation of wastewater contamination in nearby drinking water wells than the more "traditional" wastewater tracer Cl^- (Gasser *et al.*, 2010).

- Carbamazepine and primidone (both anticonvulsants) were used to distinguish between different contamination sources and ages (Müller *et al.*, 2012).
- Groundwater pollution by leaking sewers was detected by elevated concentrations of amidotrizoic acid and iothalamic acid (iodinated contrast media) whereas Gd analysis failed to detect the contamination (Wolf *et al.*, 2004 and 2006). Major ions were not used due to the various non-wastewater related parameters affecting their concentrations.
- The concentration of the artificial sweetener acesulfame in surface water and groundwater was found to correlate with the wastewater burden of the respective water bodies (Buerge *et al.*, 2009).
- Caffeine was found to be a suitable wastewater indicator to detect and even quantify the fraction of untreated wastewater discharged to the receiving surface waters (Buerge *et al.*, 2006). Moreover, the concentration of caffeine in different water types was found moderately correlated with fecal coliform bacteria (Sauvé *et al*., 2012).
- The redox sensitivity of the degradation of phenazone (analgesic drug) during artificial recharge was demonstrated (Massmann *et al.*, 2006), which implies that the compound may be used as a valuable redox indicator.
- It was proposed that the desethylatrazine-to-atrazine ratio (pesticide metabolite and parent compound) could be used to distinguish point source from non-point source contamination of an aquifer (Adams and Thurman, 1991).
- Stream-aquifer interaction was verified by using atrazine and its metabolites (Townsend and Young, 2000).
- Regarding biocide and pesticide dynamics in surface waters the biocide mecoprop was used as a representative compound for urban areas (Wittmer *et al.*, 2010).
- Several micro-contaminants of various origins (phenytoin, DEET, meprobamate, and iopromide) were identified as suitable indicators to assess optimized oxidation conditions for wastewater ozonation (Dickenson *et al.*, 2009).

Biological and chemical transformations as well as sorption processes significantly affect micro-contaminant profiles. Specific anthropogenic compounds can only be used as promising and widespread mobile reactive indicators if the boundary conditions leading to biotic and abiotic transformation or sorption are known.

1.4 Laboratory experiments on the fate and transport of micro-contaminants

For many compounds a transformation is observed in field and laboratory studies, but details about the transformation pathway and the products are often unknown (e.g. Baumgarten *et al.*, 2011; Grünheid *et al.*, 2005; Heberer *et al.*, 2008; Putschew and Jekel, 2006; Radke *et al.*, 2009). The biological transformation of specific compounds depends on the availability of suitable electron acceptors and donors, auxiliary substrates, interactions with the sediment material such as sorption or incorporation in organic matrices, and further boundary conditions (Tiehm and Schmidt, 2007). Whereas so far most studies focused on biodegradation of pollutants such as hydrocarbons or chlorinated solvents (Schmidt and Tiehm, 2008; Tiehm *et al.*, 2008), detailed data on the fate and transport of the emerging anthropogenic compounds in aquifers are still limited (Heberer *et al.*, 2008; Grünheid *et al.*, 2005; Massmann *et al.*, 2006; Schulz *et al.*, 2008).

Organic substances may also undergo sorption onto the aquifer material, whereby the type of aquifer material strongly affects the strength of sorption. Many particularly non-polar organics are sorbed on the organic fraction (f_{OC}) of the solid material (Grathwohl, 1990; Razzaque and Grathwohl, 2008). Other organic compounds are also specifically sorbed to clay minerals (Stangroom *et al.*, 2000). Furthermore, sorption depends on a number of boundary conditions (e.g. concentration of natural organic matter (NOM), due to complex formation, or pH in case of organics, which are weak acids or bases; Amiri *et al.*, 2004 and 2005; Schaffer *et al.*, 2012). Because of the high complexity and problems of heterogeneities in the field, laboratory experiments at more defined conditions are useful to understand the processes influencing transport and fate of trace organic compounds. Well-established methods to investigate the transport and degradation behavior of organic trace compounds at defined conditions are particularly batch and column experiments.

Among others, the following experimental approaches were used in former studies:

- Batch experiments / microcosm systems; amongst others variation of: consideration or avoidance of biodegradation, influence and type of sediment, redox and electron acceptor conditions (Balcke *et al.*, 2004; Barbieri *et al.*, 2011 and 2012; Mohatt *et al.*, 2011; Radke *et al.*, 2009; Schmidt and Tiehm, 2008).
- Laboratory column experiments of organic compound and colloid transport in the underground passage, variation: use of disturbed / undisturbed filling of natural sediments, with or without consideration of biodegradation, redox conditions, pH-variation, long-term experiments (Amiri *et al.*, 2004 and 2005; Baumgarten *et al.*, 2011; Bi *et al.*, 2006; Flemming *et al.*, 2007; Grünheid *et al.*, 2005; Jekel and Grünheid, 2006; Leon-Morales *et al.*, 2007; Schaffer *et al.*, 2012; Scheytt *et al.*, 2004; Tiehm and Schmidt, 2007; Weissmahr *et al.*, 1999; Worch *et al.*, 2002).

Different authors mainly investigated the influence of one specific parameter like redox conditions, pH, and NOM or sediment properties on a certain process using column and batch experiments (Massmann *et al.*, 2008; Rahman and Worch, 2005; Delle Site, 2001). In the majority of investigations individual compounds or only a few compounds were used and concentrations of these compounds were usually considerably higher in comparison to field conditions. Biofilms clearly influenced the transport of colloids in the subsurface (Flemming *et al.*, 2007; Leon-Morales *et al.*, 2007). The current state of the art does not allow the comprehensive application of trace organic compounds as indicators on the broader scale. To this end, data on the behavior of these chemicals under conditions relevant for the field scale (e.g. concentrations, boundary conditions) are needed. To underline the relevance of laboratory transformation studies the identified transformation products (TPs) should ideally be detected in environmental samples. This is an important quality measure to distinguish significant results from laboratory artifacts.

1.5 Scope, objectives, and further outline of the thesis

Due to the large number of pharmaceuticals, lifestyle products, biocides, and pesticides on the market and the almost unlimited number of known and unknown environmental transformation products of these compounds, a conclusive evaluation of the applicability of all compounds as water quality indicators is virtually impossible for one single scientific study. However, the presented work is the successful and consequent continuation of ongoing research activities on the evaluation of selected micro-contaminants as water quality indicators, their occurrence and fate in the environment, and their redox-dependent transformation. In the beginning, the development of a sensitive and selective analytical method for the determination of a broad spectrum of micro-contaminants was the main focus. This method was the central tool for all consecutive monitoring-based studies on water quality indicators in surface water and groundwater. Furthermore, a series of redox-specific batch experiments on the degradation of an extensively used and frequently detected antibiotic were conducted. A more detailed outline is presented in the following.

Chapter 2 describes the development and performance of a multi-residue analytical method based on high-performance liquid chromatographic separation and electrospray ionization with tandem mass spectrometric detection (HPLC-MS/MS) for the simultaneous analysis of 46 basic, neutral, and acidic compounds covering a wide range of polarity (log K_{OW} <0–5.9). The selection of analytes was the result of an extensive literature survey on the occurrence and fate of micro-contaminants during wastewater treatment and in the environment. Compounds with known and unknown indicator functionalities were combined in one analytical method. Furthermore, substances with limited data on their occurrence and fate in the environment (e.g. pantoprazole and cetirizine) were also included.

Chapter 3 presents an extensive river monitoring (Leine River, Germany) with focus on the correlation of micro-contaminants with potassium (K^+) and its temporal and spatial variation. A data set of almost 200 samples was analyzed. Since urine is a significant source of K^+ in wastewater treatment plants (WWTP), the basis of this investigation was the hypothesized correlation of (quasi-) persistent pharmaceuticals with K^+. The approach introduced in this study is very useful for the source delineation of contaminants and an excellent addition to studies proving indicator quality on the basis of wastewater burden (the wastewater fraction of a water body). Furthermore, the approach may be used to derive input functions of the correlating micro-contaminants for bank filtration processes. This could significantly improve the evaluation of filtrate water quality at bank filtration sites.

In **Chapter 4** the well-known indicator functionality of caffeine for the sensitive detection of untreated wastewater in surface water was successfully adapted to a karst system. Because of the low residence time of the water and the associated low attenuation potential, karst springs are highly vulnerable to contaminations. Therefore, the development of sensitive detection methods for wastewater contamination is highly important. The presented method is a significant contribution to risk assessment and management tools for karst aquifers used as drinking water resources.

In **Chapter 5** a microcosm study on the transformation of the antibiotic sulfamethoxazole (SMX) under denitrifying conditions is presented. In the scientific community there is an ongoing controversial discussion on the degradation of this compound in the environment and the presented study is an excellent contribution. A selective reaction pathway with the under denitrifying conditions produced nitrogen species nitrogen oxide (NO) and nitrite (NO_2^-) was suspected and the evaluation of two hypothesized TPs was confirmed in the microcosms. The TPs were also detected in environmental samples demonstrating the high relevance of the study regarding the occurrence and fate of SMX in the environment. Furthermore, TPs, which are specific for certain redox conditions, could be potentially used as redox indicators (reactive transport).

Chapter 6 summarizes the conclusions regarding the focus of the thesis and provides suggestions for following research.

In the **Appendix A** additional information regarding Chapter 5 is presented.

In the **Appendix B** journal articles, conference abstracts, and miscellaneous publications authored or co-authored by me and directly related to the presented work are listed.

Please note that as a result of the cumulative nature of this thesis, references are provided at the end of every chapter.

1.6 References

Adams, C.D., Thurman, E.M., 1991. Formation and transport of deethylatrazine in the soil and vadose zone. Journal of Environmental Quality 20 (3), 540–547.

Amiri, F., Rahman, M.M., Börnick, H., Worch, E., 2004. Sorption behaviour of phenols on natural sandy aquifer material during flow through column experiments: the effect of pH. Acta Hydrochimica et Hydrobiologica 32 (3), 214–224.

Amiri, F., Börnick, H., Worch, E., 2005. Sorption of phenols onto sandy aquifer material: the effect of dissolved organic matter (DOM). Water Research 39 (5), 933–941.

Balcke, G.U., Turunen, L.S., Geyer, R., Wenderoth, D.F., Schlosser, D., 2004. Chlorobenzene biodegradation under consecutive aerobic-anaerobic conditions. FEMS Microbiology Ecology 49 (1), 109–120.

Barbieri, M., Carrera, J., Sanchez-Vila, X., Ayora, C., Cama, J., Köck-Schulmeyer, M., López de Alda, M., Barceló, D., Tobella Brunet, J., Hernández García, M., 2011. Microcosm experiments to control anaerobic redox conditions when studying the fate of organic micropollutants in aquifer material. Journal of Contaminant Hydrology 126 (3–4), 330–345.

Barbieri, M., Carrera, J., Ayora, C., Sanchez-Vila, X., Licha, T., Nödler, K., Osorio, V., Pérez, S., Köck-Schulmeyer, M., López de Alda, Barceló, D., 2012. Formation of diclofenac and sulfamethoxazole reversible transformation products in aquifer material under denitrifying conditions: batch experiments, Science of the Total Environment 426, 256–263.

Bau, M., Dulski, P., 1996. Anthropogenic origin of positive gadolinium anomalies in river waters. Earth and Planetary Science Letters 143 (1–4), 245–255.

Bau, M., Alexander, B.W., 2009. Distribution of high field strength elements (Y, Zr, REE, Hf, Ta, Th, U) in adjacent magnetite and chert bands and in reference standards FeR-3 and FeR-4 from the Tamagami iron-formation, Canada, and the redox level of the Neoarchean ocean. Precambrian Research 174 (3–4), 337–346.

Baumgarten, B., Jährig, J., Reemtsma, T., Jekel, M., 2011. Long term laboratory column experiments to simulate bank filtration: factors controlling removal of sulfamethoxazole. Water Research 45 (1), 211–220.

Bergmann, A., Fohrmann, R., Hembrock-Heger, A., 2008. Bewertung der Umweltrelevanz von Arzneistoffen. Umweltwissenschaften und Schadstoff-Forschung 20 (3), 197–208.

Bi, E., Schmidt, T.C., Haderlein, S.B., 2006. Sorption of heterocyclic organic compounds to reference soils: column studies for process identification. Environmental Science and Technology 40 (19), 5962–5970.

Bound, J.P., Voulvoulis, N., 2005. Household disposal of pharmaceuticals as a pathway for aquatic contamination in the United Kingdom. Environmental Health Perspectives 113 (12), 1705–1711.

Bruce, G.M., Pleus, R.C., Snyder, S.A., 2010. Toxicological relevance of pharmaceuticals in drinking water. Environmental Science and Technology 44 (14), 5619–5626.

Buerge, I.J., Buser, H.-R., Kahle, M., Müller, M.D., Poiger, T., 2009. Ubiquitous occurrence of the artificial sweetener acesulfame in the aquatic environment: An ideal chemical marker of domestic wastewater in groundwater. Environmental Science and Technology 43 (12), 4381–4385.

Buerge, I.J., Poigner, T., Müller, M.,D., Buser, H.-R., 2006. Combined sewer overflow to surface waters detected by the anthropogenic marker caffeine. Environmental Science and Technology 40 (13), 4096–4102.

Butterwick, L., de Oude, N., Raymond, K., 1989. Safety assessment of boron in aquatic and terrestrial environments. Ecotoxicology and Environmental Safety 17 (3), 339–371.

Delle Site, A., 2001. Factors affecting sorption of organic compounds in natural sorbent/ water systems and sorption coefficients for selected pollutants. Journal of Physical and Chemical Reference Data 30 (1), 187–439.

Dickenson, E.R.V., Drewes, J.E., Sedlak, D.L., Wert, E.C., Snyder, S.A., 2009. Applying surrogates and indicators to assess removal efficiency of trace organic chemicals during chemical oxidation of wastewaters. Environmental Science and Technology 43 (16), 6242–6247.

Eiswirth, M., Hötzl, H. 1997. The impact of leaking sewers on urban groundwater, in: Chilton, J, *et al.* (Eds.), Groundwater in the urban environment. Vol. 1: Problems, processes and management. Balkema, Rotterdam, pp. 399–404.

Elbaz-Poulichet, F., Seidel, J.-L., Othoniel, C., 2002. Occurrence of an anthropogenic gadolinium anomaly in river and coastal waters of southern France. Water research 36 (4), 1102–1105.

European Commission, 1998. Directive 98/8/EC of the European Parliament and of the Council of 16 February 1998 concerning the placing of biocidal products on the market. http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:31998L0008:EN:NOT - Accessed 13 April 2012.

European Commission, 2000. Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 - Establishing a Framework for Community Action in the Field of Water Policy. Official Journal 22 December 2000 L 327/1. Brussels: European Commission.

European Commission, 2012. COM/2011/0876 - Proposal for a directive of the European Parliament and of the Council amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy. http://www.ipex.eu/IPEXL-WEB/dossier/files/download/082dbcc534ad5cc6013533875c5a05f2.do - Accessed 15 April 2012.

Flemming, H.-C., Strathmann, M., Leon-Morales. C.F., 2007. Biofilms and their role in sediment dynamics and pollutant mobility. In: Westrich, B., Förstner U (eds.) Sediment dynamics and pollutant mobility in rivers, Springer-Verlag, Berlin Heidelberg New York, 344–357.

Gasser, G., Rona, M., Voloshenko, A., Shelkov, R., Tal, N., Pankratov, I., Elhanany, S., Lev, O., 2010. Quantitative evaluation of tracers for quantification of wastewater contamination of potable water sources. Environmental Science and Technology 44 (10), 3919–3925.

Gäbler, H.-E., Bahr, A., 1999. Boron isotope ratio measurements with a double-focusing magnetic sector ICP mass spectrometer for tracing anthropogenic input into surface and ground water. Chemical Geology 156 (1–4), 323–330.

Glassmeyer, S.T., Furlong, E.T., Kolpin, D.W., Cahill, J.D., Zaugg, S.D., Werner, S.L., Meyer, M.T., Kryak, D.D., 2005. Transport of chemical and microbial compounds from known wastewater discharges: Potential for use as indicators of human fecal contamination. Environmental Science and Technology 39 (14), 5157–5169.

Godfrey, E., Woessner, W.W., Benotti, M.J., 2007. Pharmaceuticals in on-site sewage effluent and ground water, Western Montana. Ground Water 45 (3), 263–271.

Goyne, K.W., Brantley, S.L., Chorover, J., 2010. Rare earth element release from phosphate minerals in the presence of organic acids. Chemical Geology 278 (1–2), 1–14.

Grathwohl, P., 1990. Influence of organic matter from soils and sediments from various origins on the sorption of some chlorinated aliphatic hydrocarbons: implications on KOC correlations. Environmental Science and Technology 24 (11), 1687–1693.

Grünheid, S., Amy, G., Jekel, M., 2005. Removal of bulk dissolved organic carbon (DOC) and trace organic compounds by bank filtration and artificial recharge. Water Research 39 (14), 3219–3228.

Halling-Sørensen, B., Nors Nielsen, S., Lanzky, P.F., Ingerslev, F., Holten Lützhøft, H.C., Jørgensen, S.E., 1998. Occurrence, fate and effects of pharmaceutical substances in the environment – a review. Chemosphere 36 (2), 357–393.

Heberer, T., 2002. Occurrence, fate, and removal of pharmaceutical residues in the aquatic environment: a review of recent research data. Toxicology Letters 131 (1–2), 5–17.

Heberer, T., Massmann, G., Fanck, B., Taute, T., Dünnbier, U., 2008. Behaviour and redox sensitivity of antimicrobial residues during bank filtration. Chemosphere 73 (4), 451–460.

Heinz, B., Birk, S., Liedl, R., Geyer, T., Straub, K.L., Andresen, J., Bester, K., Kappler, A., 2009. Water quality deterioration at a karst spring (Gallusquelle, Germany) due to combined sewer overflow: evidence of bacterial and micro-pollutant contamination. Environmental Geology 57 (4), 797–808.

Hernando, M.D., Mezcua, M., Fernández-Alba, A.R., Barceló, D., 2006. Environmental risk assessment of pharmaceutical residues in wastewater effluent, surface waters and effluents. Talanta 69 (2 SPEC. ISS.), 334–342.

Isobe, K.O., Tarao, M., Zakaria, M.P., Chiem, N.H., Minh, L.Y., Takada, H., 2002. Quantitative application of fecal sterols using gas chromatography – mass spectrometry to investigate fecal pollution in tropical waters: western Malaysia and Mekong Delta, Vietnam. Environmental Science and Technology 36 (21), 4497–4507.

Isobe, K.O., Tarao, M., Chiem, N.H., Minh, L.Y., Takada, H., 2004. Effect of environmental factors on the relationship between concentrations of coprostanol and fecal indicator bacteria in tropical (Mekong Delta) and temperate (Tokyo) freshwaters. Applied and Environmental Microbiology 70 (2), 814–821.

Jekel, M., Grünheid, S., 2006. Bank filtration and groundwater recharge for treatment of polluted surface waters. In: Recent progress in slow sand and alternative biofiltration processes. IWA Publishing: London, 519–529.

Koch, R., 1893. Die Cholera in Deutschland während des Winters 1892–1893. Medical Microbiology and Immunology 15 (1), 89–165.

Kolpin, D.W., Furlong, E.T., Meyer, M.T., Thurman, E.M., Zaugg, S.D., Barber, L.B., Buxton, H.T., 2002. Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999–2000: a national reconnaissance. Environmental Science and Technology 36 (6), 1202–1211.

Kulaksiz, S., Bau, M., 2011. Rare earth elements in the Rhine River, Germany: First case of anthropogenic lanthanum as a dissolved microcontaminant in the hydrosphere. Environmental International 37 (5), 973–979.

Kümmerer, K., 2009. Antibiotics in the aquatic environment – a review – part II. Chemosphere 75 (4), 435–441.

Kümmerer, K., 2010. Pharmaceuticals in the environment. Annual Review of Environment and Resources 35, 57–75.

Leon-Morales, C.F., Strathmann, M., Flemming, H.-C., 2007. Influence of biofilms on the movement of colloids in porous media. Implications for colloid facilitated transport in subsurface environments. Water Research 41 (10), 2059–2068.

Massmann, G., Knappe, A., Richter, D., Pekdeger, A., 2004. Investigating the influence of treated sewage on groundwater and surface water using wastewater indicators in Berlin, Germany. Acta Hydrochimica et Hydrobiologica 32 (4–5), 336–350.

Massmann, G., Greskowiak, J., Dünnbier, U., Zuehlke, S., Knappe, A., Pekdeger, A., 2006. The impact of variable temperatures on the redox conditions and the behaviour of pharmaceutical residues during artificial recharge. Journal of Hydrology 328 (1–2), 141–156.

Massmann, G., Dünnbier, U., Heberer, T., Taute, T., 2008. Behaviour and redox sensitivity of pharmaceutical residues during bank filtration – Investigation of residues of phenazone-type analgesics. Chemosphere 71 (8), 1476–1485.

Mohatt, J.L., Hu, L., Finneran, K.T., Strathmann, T.J., 2011. Microbially mediated abiotic transformation of the antimicrobial agent sulfamethoxazole under iron-reducing soil conditions. Environmental Science and Technology 45 (11), 4793–4801.

Möller, P., Dulski, P., Bau, M., Knappe, A., Pekdeger, A., Sommer-von Jarmerstedt, C., 2000. Anthropogenic gadolinium as a conservative tracer in hydrology. Journal of Geochemical Exploration 69–70, 409–414.

Müller, B., Scheytt, T., Asbrand, M., Mross de Casas, A., 2012. Pharmaceuticals as indictors (sic!) of sewage-influenced groundwater. Hydrogeology Journal 20 (6), 1117–1129.

Neal, C., Williams, R.J., Bowes, M.J., Harrass, M.C., Neal, M., Rowland, P., Wickham, H., Thacker, S., Harman, S., Vincent, C., Jarvie, H.P., 2010. Decreasing boron concentrations in UK rivers: Insights into reductions in detergent formulations since the 1990s and within-catchment storage issues. Science of the Total Environment 408 (6), 1374–1385.

Pronk, M., Goldscheider, N., Zopfi, J., 2007. Particle-size distribution as indicator for fecal bacteria contamination of drinking water from karst springs. Environmental Science and Technology 41 (24), 8400–8405.

Putschew, A., Jekel, M., 2006. Iodinated X-ray contrast media. In: Reemtsma, T., Jekel M. (eds.): Organic Pollutants in the Water Cycle. Wiley-VCH: Weinheim, 87–98.

Radke, M., Lauwigi, C., Heinkele, G., Mürdter, T.E., Letzel, M., 2009. Fate of the antibiotic sulfamethoxazole and its two major human metabolites in a water sediment test. Environmental Science and Technology 43 (9), 3135–3141.

Rahman, M.M., Worch, E., 2005. Nonequilibrium sorption of ionisable organic chemicals onto geosorbents: the impact of pH on the intraparticle mass transfer coefficient. Chemosphere 61 (10), 1419–1426.

Razzaque, M., Grathwohl, P., 2008. Predicting organic carbon-water partitioning of hydrophobic organic chemicals in soils and sediments based on water solubility. Water Research 42 (14), 3775–3780.

Reichenberger, S., Bach, M., Skitschak, A., Frede, H.-G., 2007. Mitigation strategies to reduce pesticide inputs into ground- and surface water and their effectiveness; A review. Science of the total environment 384 (1–3), 1–35.

Sauvé, S., Aboulfadl, K., Dorner, S., Payment, P., Deschamps, G., Prévost, M., 2012. Fecal coliforms, caffeine and carbamazepine in stormwater collection systems in a large urban area. Chemosphere 86 (2), 118–123.

Savichtcheva, O., Okabe, S. 2006. Alternative indicators of fecal pollution: Relations with pathogens and conventional indicators, current methodologies for direct pathogen monitoring and future application perspectives. Water research 40 (13), 2463–2476.

Schaffer, M., Boxberger, N., Börnick, H., Licha, T., Worch, E. 2012. Sorption influenced transport of ionizable pharmaceuticals onto a natural sandy aquifer sediment at different pH. Chemosphere 87 (5), 513–520.

Scheytt, T., Mersmann, P., Leidig, M., Pekdeger, A., Heberer, T., 2004. Transport of pharmaceutically active compounds in saturated laboratory columns. Ground Water 42 (5), 767–773.

Schmidt, K.R., Tiehm, A., 2008. Natural attenuation of chloroethenes: Identification of sequential reductive/oxidative biodegradation by microcosm studies. Water Science and Technology 58 (5), 1137–1145.

Schreiber, I.M., Mitch, W.A., 2006. Occurrence and fate of nitrosamines and nitrosamine precursors in wastewater-impacted surface waters using boron as a conservative tracer. Environmental Science and Technology 40 (10), 3203–3210.

Schulz, M., Löffler, D., Wagner, M., Ternes, T., 2008. Transformation of the X-ray Contrast Medium Iopromide in Soil and Biological Wastewater Treatment. Environmental Science and Technology 42 (19), 7207–7217.

Schwarzenbach, R.P., Escher, B.I., Fenner, K., Hofstetter, T.B., Johnson, C.A., von Gunten, U., Wehrli, B., 2006. The challenge of micropollutants in aquatic systems. Science 313 (5790), 1072–1077.

Stangroom, S.J., Lester, J.N., Collins, C.D., 2000. Abiotic behaviour of organic micropollutants in soils and the aquatic environment. A review: I. partitioning. Environmental Technology 21 (8), 845–863.

Strauch, G., Möder, M., Wennrich, R., Osenbrück, K., Gläser, H.-R., Schladitz, T., Müller, C., Schirmer, K., Reinstorf, F., Schirmer, M., 2008. Indicators for Assessing Anthropogenic Impact on Urban Surface and Groundwater. Journal of Soils and Sediments 8 (1), 23–33.

Tallon, P., Magajna, B., Lofranco, C., Leung, K.T. 2005. Microbial indicators of faecal contamination in water: A current perspective. Water, Air, and Soil Pollution 166 (1–4), 139–166.

Ternes, T.A., 1998. Occurrence of drugs in German sewage treatment plants and rivers. Water Research 32 (11), 3245–3260.

Ternes, T., 2007. The occurrence of micopollutants (sic!) in the aquatic environment: A new challenge for water management. Water Science and Technology 55 (12), 327–332.

Tiehm, A., Schmidt, K.R., 2007. Methods to evaluate biodegradation at contaminated sites. In: Knödel, K., Lange, G., Voigt, H.-J. (eds.): Environmental Geology, Handbook of Field Methods and Case Studies Springer, Berlin, Heidelberg, pp. 876–920.

Tiehm, A., Schmidt, K.R., Pfeifer, B., Heidinger, M., Ertl, S., 2008. Growth kinetics and stable carbon isotope fractionation during aerobic degradation of cis-1,2-dichloroethene and vinyl chloride. Water Research 42 (10–11), 2431–2438.

Townsend, M.A., Young, D.P., 2000. Atrazine and its Metabolites as Indicators of Stream-aquifer Interaction in Kansas, USA. International Journal of Environmental Ananytical Chemistry 78 (1), 9–23.

Weissmahr, K.W., Hildenbrand, M., Schwarzenbach, R.P., Haderlein, S.B., 1999. Laboratory and field scale evaluation of geochemical controls on groundwater transport of nitroaromatic ammunitions residues. Environmental Science and Technology 33 (15), 2593–2600.

WHO, 2011a. Guidelines for drinking-water quality - 4th ed. WHO Press, World Health Organization, 1211 Geneva 27.

WHO, 2011b. Pharmaceuticals in Drinking-water. WHO Press, World Health Organization, 1211 Geneva 27.

Wittmer, I.K., Bader, H.-P., Scheidegger, R., Singer, H., Lück, A., Hanke, I., Carlsson, C., Stamm, C., 2010. Significance of urban and agricultural land use for biocide and pesticide dynamics in surface waters. Water Research 44 (9), 2850–2862.

Wittmer, I.K., Scheidegger, R., Bader, H.-P., Singer, H., Stamm, C., 2011. Loss rates of urban biocides can exceed those of agricultural pesticides. Science of the Total Environment 409 (5), 920–932.

Worch, E., Grischek, T., Börnick, H., Eppinger, P., 2002. Laboratory tests for simulating attenuation processes of aromatic amines in riverbank filtration. Journal of Hydrology 266 (3–4), 259–268.

Wolf, L., Eiswirth, M., Hötzl, H., 2006. Assessing sewer-groundwater interaction at the city scale based on individual sewer defects and marker species distributions. Environmental Geology 49 (6), 849–857.

Wolf, L., Held, I., Eiswirth, M., Hötzl, H., 2004. Impact of leaky sewers on groundwater quality. Acta Hydrochimica et Hydrobiologica 32 (4–5), 361–373.

Chapter 2

2 Development of a multi-residue analytical method, based on liquid chromatography-tandem mass spectrometry, for the simultaneous determination of 46 micro-contaminants in aqueous samples

Karsten Nödler[1], Tobias Licha[1], Kai Bester[2], Martin Sauter[1]

Citation: Nödler, K., Licha, T., Bester, K., Sauter, M., 2010. Development of a multi-residue analytical method, based on liquid chromatography-tandem mass spectrometry, for the simultaneous determination of 46 micro-contaminants in aqueous samples. Journal of Chromatography A 1217 (42), 6511–6521.

http://dx.doi.org/10.1016/j.chroma.2010.08.048

[1] Department Applied Geology, Geoscience Center of the University of Göttingen, Goldschmidtstr. 3, 37077 Göttingen, Germany

[2] National Environmental Research Institute, Aarhus University, Frederiksborgsvej 399, 4000 Roskilde, Denmark

Abstract

A multi-residue analytical method based on high-performance liquid chromatographic separation, electrospray ionization with tandem mass spectrometric detection (HPLC-MS/MS) was developed for the simultaneous analysis of 46 basic, neutral, and acidic compounds covering a wide range of polarity (log K_{OW} <0–5.9). The compound list included selected iodinated contrast media, analgesics, anti-inflammatories, stimulants, beta-blockers, antibiotics, lipid regulators, anti-histamines, psychiatric drugs, herbicides, corrosion inhibitors, and the gastric acid regulator pantoprazole. The main feature of the presented method was a simultaneous solid phase extraction (SPE) of all analytes followed by simultaneous separation and detection by HPLC-MS/MS with electrospray ionization in both positive and negative polarization within the same chromatogram. Optimization of electrospray drying gas temperature resulted in using a temperature gradient on the ion source. Six different polymeric sorbents for SPE were compared with respect to recoveries, taking into account the specific surface of each sorbent. Method quantitation limits (MQL) in surface and seawater ranged from 1.2 ng L^{-1} to 28 ng L^{-1}, in wastewater from 5.0 ng L^{-1} to 160 ng L^{-1}, respectively. In order to demonstrate the applicability of the method, river water, treated wastewater, and seawater were analyzed.

2.1 Introduction

During the last decades, the field of instrumental analytical sciences has progressed rapidly. This advancement has laid the foundation for the analysis of numerous organic compounds in trace concentrations (ng L^{-1} range), acknowledging the widespread occurrence of organics with anthropogenic origin in all parts of the water cycle (Kolpin *et al.*, 2002; Schwarzenbach *et al.*, 2006; Ternes, 2007). Several multi-residue analytical methods using high performance liquid chromatography (HPLC) with mass spectrometric detection and different ionization techniques have been discussed in the literature (e.g. Busetti *et al.*, 2009; Gómez *et al.*, 2006; Gros *et al.*, 2006; Marín *et al.*, 2009; Petrović *et al.*, 2005; Rodil *et al.*, 2009; Wick *et al.*, 2010). With respect to electrospray ionization, some compounds are preferably ionized in negative (ESI−), while others give better signals in positive mode (ESI+). To support better ionization often different eluents are used for ESI+ and ESI−, respectively. Thus different chromatographic runs have to be performed for chemically different groups of compounds (Gros *et al.*, 2006). Additionally, fast switching between negative and positive mode has not been possible with older instruments in a stable way and up to now there are only a few studies reported in the literature using this feature (Gao *et al.*, 2010; Tutunji *et al.*, 2009; Yang *et al.*, 2010). A critical issue of connecting liquid chromatography with electrospray ionization is that acidic compounds can be best chromatographed in the undissociated form (eluent pH < pKa), while they give best ESI(−) signals in their dissociated form (eluent pH > pKa) (Ardrey, 2003). This illustrates the importance and difficulty of carefully identifying a suitable HPLC column and a generic eluent composition for multi-residue analyses.

For this study, a multi-residue analytical method was chosen over a single group approach in order to reduce cost and time while simultaneously obtaining information on the occurrence and fate of a broad spectrum of xenobiotic compounds. With respect to simultaneous preconcentration, combinations of various pharmaceuticals such as analgesics, anti-inflammatories, beta-blockers, lipid-regulators, antibiotics, psychiatric drugs, anti-histamines and caffeine are common (Gros *et al.*, 2006; Kim *et al.*, 2007; Pedrouzo *et al.*, 2007; Petrović *et al.*, 2005; Vanderford *et al.*, 2003) as well as the combination of herbicides and pharmaceuticals (Rodil *et al.*, 2009). Methods for single group analysis of iodinated contrast media (ICM) are well established (Pérez and Barceló, 2007), but their incorporation into multi-residue methods is rare and with respect to

ICM currently applicable for iopromide only (Kim *et al.*, 2007; Vanderford *et al.*, 2003; Yoon *et al.*, 2010). However, there is a huge difference in concentration e.g., iopromide occurs with thousands of ng L^{-1} on the one hand and the sedative diazepam with only a few ng L^{-1} in the same samples (Yoon *et al.*, 2010). This range of concentrations is highly demanding considering the linear range of the method and cross talk in the mass spectrometer. To the authors knowledge no multi-residue analytical method, which incorporates all of the compounds discussed here, has been developed to this date.

The objective of the presented work was to develop a multi-residue analytical method including simultaneous sample pre-treatment, simultaneous separation and detection of 46 basic, neutral and acidic analytes such as selected ICM, analgesics, anti-inflammatories, stimulants, beta-blockers, antibiotics, lipid regulators, anti-histamines, psychiatric drugs, herbicides, corrosion inhibitors and the gastric acid regulator pantoprazole. Pantoprazole has recently become an over-the-counter drug in the European Union and thus must be regarded as an emerging contaminant in the near future.

2.2 Experimental

2.2.1 Chemicals and materials

Methanol (LC/MS grade) was purchased from Fisher Scientific (Schwerte, Germany), ethyl acetate, acetonitrile, ammonium acetate, and formic acid (all analytical grade) were purchased from VWR (Darmstadt, Germany). Ultrapure water was obtained from a combined water purification system consisting of Elix 5 (Progard 1 silver cartridge) and Milli-Q Gradient A10 (Quantum Ex Ultrapure Organex + Q-Gard 1 cartridge), both from Millipore (Schwalbach, Germany).

Reference standards of erythromycin A, diclofenac, paraxanthine, theobromine, theophylline, 1-methylxanthine, 3-methylxanthine, metoprolol, roxithromycin, sulfamethoxazole, bezafibrate, clofibric acid, carbamazepine, citalopram, fluoxetine, sertraline, tolyltriazole, pantoprazole, iohexol, primidone, and 1H-benzotriazole were purchased from Sigma-Aldrich (Steinheim, Germany). Ibuprofen and caffeine were from Fisher Scientific (Schwerte, Germany), naproxen, paracetamol (acetaminophen), phenazone, atenolol, trimethoprim, and diazepam from Fagron (Barsbüttel, Germany). Atrazine, desethylatrazine, desisopropylatrazine, diuron, isoproturon, mecoprop, and metazachlor were purchased from Dr. Ehrenstorfer (Augsburg, Germany), gemfibrozil from Salutas (Barleben, Germany), tetrazepam from Welding Pharma (Hamburg, Germany), and benzoylecgonine from LGC Promochem (Wesel, Germany). Cetirizine and loratadine were from KSK-Pharma (Berghausen, Germany), iopamidol and iopromide from Bayer (Leverkusen, Germany), and iomeprol from Bracco Imaging (Konstanz, Germany). Sotalol and clarithromycin were obtained from pharmaceutical preparations for intravenous injection, respectively, from Carinopharm, Gronau (Leine), Germany (Carino Sotalol i.v. 40 mg) and Abbott, Wiesbaden, Germany (Klacid® i.v. 500 mg). A summary of all analytes is presented in Table 2.1.

Table 2.1: Analytes and their application/origin.

Application or origin	Compound	Application or origin	Compound
Analgesics/ anti-inflammatories	Diclofenac	Lipid regulators	Bezafibrate
	Ibuprofen		Clofibric acid
	Naproxen		Gemfibrozil
	Paracetamol	Antihistamines	Cetirizine
	Phenazone		Loratadine
Stimulants/ caffeine metabolites	Caffeine	Anticonvulsants/sedatives	Carbamazepine
	Paraxanthine		Diazepam
	Theobromine		Primidone
	Theophylline		Tetrazepam
	1-Methylxanthine	Antidepressants	Citalopram
	3-Methylxanthine		Fluoxetine
Antihypertensive agents	Atenolol		Sertraline
	Metoprolol	Herbicides/herbicide metabolites	Atrazine
	Sotalol		Desethylatrazine
Iodinated contrast media	Iohexol		Desisopropylatrazine
	Iomeprol		Diuron
	Iopamidol		Isoproturon
	Iopromide		Mecoprop
Antibiotics	Clarithromycin		Metazachlor
	Erythromycin	Corrosion inhibitors	1H-Benzotriazole
	Roxithromycin		Tolyltriazole
	Sulfamethoxazole	Cocaine metabolite	Benzoylecgonine
	Trimethoprim	Gastric acid regulator	Pantoprazole

To avoid internal reactions, individual standard solutions were prepared. Unless otherwise stated, individual standard solutions with concentrations up to 10 mg mL^{-1} were prepared in methanol. Cetirizine was dissolved in acetonitrile in order to prevent esterification with methanol. This reaction occurred rapidly when the compound was dissolved in methanol (< 1 week). Caffeine, paraxanthine, theobromine, theophylline and the mono-methylxanthines were dissolved jointly in warm water (60 °C, 100 µg mL^{-1} each) and diluted to 10 µg mL^{-1} in methanol. Individual standard solutions of iopamidol, iopromide, iohexol and iomeprol were prepared in methanol containing 20% water (v/v). Multi-standard solutions for calibration were obtained by mixing individual standard solutions and gradual dilution with acetonitrile (to prevent cetirizine esterification). All individual reference standard solutions and multi-standard solutions were stored at −18 °C.

Paraxanthine-D_6, atenolol-D_7, erythromycin-N-methyl-^{13}C-D_3, ibuprofen-D_3, and lansoprazole were purchased from Sigma Aldrich (Steinheim, Germany). Desmethoxyiopromide, sulfamethoxazole-$^{13}C_6$, carbamazepine-D_{10}, diazepam-D_5, and fluoxetine-D_6 were from LGC Promochem (Wesel, Germany). Isoproturon-D_6, atrazine-D_5, and mecoprop-D_3 were from Dr. Ehrenstorfer (Augsburg, Germany) while loratadine-D_4 was purchased from Biomol (Hamburg, Germany). The internal standard (E)-9-[O-(2-methyloxime)]-erythromycin (in the following called ery-methyloxime) was synthesized according to Schlüsener *et al.* (2003). However, instead of purification on silica gel the crude product containing significant amounts of erythromycin and anhydro-erythromycin has been washed ten times with acetonitrile. The identity and purity of the final product was verified by HPLC-MS/MS and neither erythromycin nor its anhydro-derivative were detected after washing. The purified macrolide internal standard showed two resolved peaks in HPLC-MS/MS with a peak area ratio of 1:9 probably caused by isomeric effects as erythromycin (purity ≥95%) from Fagron (Barsbüttel, Germany) was used as reactant in the synthesis. The more intensive peak was used as the internal standard and the resolution of both was used as a qualitative indicator for matrix-induced deterioration of separation efficiency for basic compounds.

An internal standard (IS) mix with concentrations of 40 ng μL^{-1} desmethoxyiopromide, 30 ng μL^{-1} fluoxetine-D_6, 20 ng μL^{-1} of lansoprazole, erythromycin-N-methyl-^{13}C-D_3, paraxanthine-D_6, ibuprofen-D_3, and 10 ng μL^{-1} of the other respective internal standard compounds was prepared in acetonitrile.

During the method development the following disposable cartridges (6 mL) for solid-phase extraction (SPE) were used: OASIS HLB 500 mg from Waters, Eschborn, Germany; Bond Elut Plexa 200 mg, AbsElut Nexus 200 mg and Bond Elut PPL 500 mg all from Varian (Darmstadt, Germany); Strata X 500 mg from Phenomenex (Aschaffenburg, Germany), and Isolute ENV+ 500 mg from Separtis (Grenzach-Wyhlen, Germany). The intention was to identify the most suitable sorbent for extracting the selected analytes. Environmental samples can contain humic and fulvic acids. In comparison to acidified samples, co-extraction of these matrix compounds may be significantly lower at neutral pH (Pichon *et al.*, 1996). Regarding that and the final method, which should be applicable to environmental analysis, all experiments on solid phase extraction were performed at pH values buffered by dihydrogen phosphate to 7 ± 0.2. Additionally, some analytes such as erythromycin A are susceptible to chemical degradation due to acidification and/or alkalinization (Hassanzadeh *et al.*, 2007; Paesen *et al.*, 1995).

2.2.2 Surface-normalized comparison of selected SPE-sorbents

A phosphate buffer containing 13.4 g potassium dihydrogen phosphate and 6.22 g disodium hydrogen phosphate dihydrate (both from VWR Darmstadt, Germany) per liter buffer solution was used in a ratio of 10 mL buffer solution per 1000 mL sample (pH 7 ± 0.2).

Six commercially available cartridges were tested. An approach following typical adsorption studies, i.e. technical adsorption on carbon black by using a surface normalized approach in a matrix-free system, was applied (Sontheimer *et al.*, 1985). In this way, a more realistic comparison for the individual sorbent capacities and mechanisms can be obtained. For this purpose, each cartridge was tested with buffered ultrapure water containing 1 µg L^{-1} of iohexol, iomeprol, iopamidol, and iopromide, and 0.5 µg L^{-1} of any other analyte, respectively. A constant ratio of 1 mL solution per m^2 sorbent was applied resulting in a range of 500 mL for 500 mg Isolute ENV+ to 90 mL for 200 mg Bond Elut Plexa. Therefore, a constant analyte to surface ratio of 1 ng m^{-2} ICM and 0.5 ng m^{-2} for all compounds other than ICM was applied to all types of sorbent. For a better illustration, a sample preparation scheme of the experiment is shown in Table 2.2.

Table 2.2: Sample preparation scheme of the surface-normalized SPE experiments.

Sorbent	Specific surface [m^2 g^{-1}]	Surface per cartridge [m^2]	Extracted standard solution [mL]	Rinse water [µL]	Re-dissolution [µL]
OASIS HLB	810	405	405	1620	1000
Bond Elut Plexa	450	90	90	360	500
AbsElut Nexus	575	115	115	460	500
Isolute ENV+	1000	500	500	2000	1000
Strata X	800	400	400	1600	1000
Bond Elut PPL	600	300	300	1200	1000

In order to exclude effects of varying extraction kinetics of the sorbents, a low extraction speed of 3 mL min^{-1} was used. Prior to extraction, the SPE cartridges were conditioned with 4 mL of methanol and rinsed twice with 4 mL of ultrapure water successively. After extraction each cartridge was rinsed with twice the amount of ultrapure water given in Table 2.2, as the amount of rinsing water was also adjusted to the specific surface of the respective sorbent. The rinsed cartridges were dried by drawing air through the cartridges for 30–45 minutes and sequentially eluted with 2×2 mL of methanol and 2×2 mL ethyl acetate. The combined extract was spiked with 5 µL of internal standard by using a 10-µL syringe. Internal standards were applied to compensate any adverse ionization effects. Thus, for calculating the absolute recovery in this experiment, the IS-compensated signal of the analyte was used. The extract was evaporated to dryness at 40 °C with a gentle stream of nitrogen and re-dissolved in aqueous 5 mM ammonium acetate solution containing 4% methanol (see Table 2.2 for exact volume). The extract was then transferred into an autosampler vial and centrifuged (Christ RVC 2-18, purchased from Fisher Scientific Schwerte, Germany) for 30 min before analysis. For this experiment the HPLC-MS/MS was calibrated with diluted multi-standard solutions in aqueous 5 mM ammonium acetate solution containing 4% methanol.

2.2.3 Sampling and sample preparation

Water samples were collected from the river Leine (Göttingen, Germany), the Baltic Sea (Ahlbeck, Usedom) and a municipal wastewater treatment plant. The river sample was taken directly at the riverbank and the seawater sample at a pier, in 200 m distance from the seashore. Salinity of the seawater sample was 6.2‰. The wastewater sample was collected from the effluent of a municipal wastewater treatment plant (WWTP), which was located in a city with a population of around 120,000 inhabitants. The WWTP consisted of a mechanical treatment for the separation of solid material (i.e. a grit, fat separator and a primary settler) followed by activated sludge treatment, including nitrification and denitrification. Additionally, chemical P-removal was performed. Under dry weather discharge conditions, the mean hydraulic residence time was 20–24 h. WWTP effluent and river water were sampled in October 2009, the Baltic Sea in May 2009. 1 liter (clear glass and screw cap) bottles were used for sampling (Fisher Scientific, Schwerte, Germany). Samples were allowed to settle at 4 °C in the dark for 12–24 h. Careful handling prevented resuspension of settled particles and the supernatant was used for analysis instead of a filtered aliquot.

OASIS HLB was selected for sample extraction. Samples were extracted in duplicate. Prior to extraction the sorbent was conditioned with 4 mL of methanol and rinsed twice with 4 mL of ultrapure water. The sample (500 mL for river and seawater and 100 mL for treated wastewater) was spiked with

10 µL of IS by using a 10-µL syringe and with buffer concentrate (10 mL per liter sample, see 2.2.2.). It was extracted with a flow rate (applied by vacuum suction) of 15 mL/min. All sample matrices had pH 7.0 ± 0.2 after buffering.

After extraction, the sorbent was rinsed with 2×1.5 mL of ultrapure water in order to remove the inorganic salt matrix. Afterwards, the sorbent was dried by drawing air through the cartridges under vacuum for 30 min. The analytes were eluted with 2×2 mL of methanol, followed by 2×2 mL of ethyl acetate. The extract was evaporated to dryness at 40 °C with a gentle stream of nitrogen and re-dissolved in 1 mL of aqueous 5 mM ammonium acetate solution, containing 4% methanol. Prior to analysis, the extract was transferred into an autosampler vial and centrifuged for 30 min.

2.2.4 Liquid chromatography tandem mass spectrometry (HPLC-MS/MS)

2.2.4.1 Liquid chromatography

The HPLC system consisted of a Varian ProStar 410 autosampler and a high-pressure gradient system of two Varian ProStar 210 pumps. For chromatographic separation a Polaris C18-Ether column 150 mm×2 mm i.d., 3 µm particle size (Varian, Darmstadt, Germany) was used. The flow was 200 µL min^{-1}. The separation was operated at 30 °C and the injection volume was set to 100 µL. In comparison to studies with similar dimensions concerning HPLC column and flow rate, the applied injection volume of 100 µL was relatively high (e.g. in comparison to 10 µL (Rodil *et al.*, 2009), 25 µL (Busetti *et al.*, 2009). Therefore, the extracts were re-dissolved in a solution of elution strength slightly lower than the starting eluent in order to enable sufficient retention and excellent peak shape of early eluting analytes (e.g. sotalol). A direct dissolution in acidic start gradient was not applied in order to preserve acid instable compounds.

Eluent A was 0.015% formic acid + 5% methanol in ultrapure water, eluent B was methanol. The elution started isocratically for 50 seconds with 100% A, followed by a gradient of 10 seconds to 95% A. This rapid step was due to the minimum flow rate with reliable accuracy of the used pump given by the manufacturer (10 µL min^{-1}), which corresponds to 5% of eluent B at a total flow rate of 200 µL min^{-1}. Accordingly, this sharp gradient must be considered as a switch-on of pump B in order to overcome its range of unreliable accuracy as fast as possible, rather than as part of the eluting gradient. This step was followed by a 39-min linear gradient to 95% B. This was held for 5 minutes followed by a 1-min linear gradient to 100% A, which was maintained for 11 minutes to equilibrate the system.

2.2.4.2 Mass spectrometry (MS/MS)

A Varian 1200 L triple quadrupole with electrospray interface (ESI) was used for detection and quantification. A spray voltage of 5.5 kV in positive mode, −4.5 kV in negative mode, and shield voltages of 0.5 kV and −0.5 kV were used, respectively. Detection was performed in multiple reaction monitoring (MRM) and with the exception of paracetamol, fluoxetine, ibuprofen, iohexol, gemfibrozil, mecoprop, and the internal standards two transitions per analyte were monitored (European Commission, 2002). Compounds were quantified using their most intensive MRM. Minimum dwell times of the individual MRM were 40 ms and 20 ms for quantifier and qualifier, respectively. Fragmentation experiments, optimization of capillary voltage and collision energy were achieved by direct injection of stock solutions with a concentration of 5 µg mL^{-1}. Individual MS/MS parameters of analytes and IS are shown in Table 2.3 and Table 2.4. Argon 5.0 was used as the collision gas with a pressure of 0.27 Pa. Following the manufacturer’s guidelines the pressure of the drying and nebulizing gases were 180×10^3 Pa and 386×10^3 Pa, respectively.

Table 2.3: Log K_{ow} and pKa of analytes, ESI-MS/MS conditions for the analysis of the selected compounds.

Charge at neutral water pH	*[1]Related IS	Compound	*[2]Log Kow	*[2]pKa Acidic	*[3]Basic	Quantifier	*[4]Cap U [V]	*[5]CE [V]	Qualifier	*[4]Cap U [V]	*[5]CE [V]
Neutral	1	1H-Benzotriazole	1.34 ± 0.25	8.4 ± 0.1	1.2 ± 0.3	120 > 65	+ 65	- 11.5	120 > 92	+ 65	- 9.5
	1	3-Methylxanthine	-0.79 ± 0.39	10.5 ± 0.2	1.3 ± 0.7	165 > 122	- 55	+ 19.0	165 > 150	- 55	+ 18.0
	6	Atrazine	2.63 ± 0.21		2.4 ± 0.5	216 > 174	+ 55	- 9.0	216 > 104	+ 55	- 18.0
	7	*[6]Benzoylecgonine	2.72 ± 0.38	3.4 ± 0.4	10.8 ± 0.4	290 > 168	+ 60	- 12.0	290 > 105	+ 60	- 21.0
	1	Caffeine	-0.13 ± 0.37		0.7 ± 0.7	195 > 138	+ 55	- 9.5	195 > 110	+ 55	- 9.0
	7	Carbamazepine	2.67 ± 0.38	13.9 ± 0.2		237 > 194	+ 45	- 11.0	237 > 179	+ 45	- 27.0
	7	*[6]Cetirizine	2.17 ± 0.84	2.9[a]	(1.5 & 8,3)[a]	389 > 201	+ 40	- 14.5	389 > 166	+ 40	- 34.5
	6	Desethylatrazine	1.50 ± 0.25		2.1 ± 0.5	188 > 146	+ 50	- 8.5	188 > 104	+ 50	- 16.0
	6	Desisopropylatrazine	1.16 ± 0.25		2.6 ± 0.5	174 > 104	+ 50	- 13.5	174 > 132	+ 50	- 8.5
	10	Diazepam	2.96 ± 0.55		3.4 ± 0.1	285 > 193	+ 65	- 21.0	285 > 154	+ 65	- 16.5
	12	Diuron	2.78 ± 0.33	13.6 ± 0.7		231 > 186	- 40	+ 15.0	231 > 150	- 40	+ 22.0
	3	Iohexol	-4.16 ± 0.85	11.4 ± 0.5		820 > 127	- 60	+ 11.5	-		
	3	Iomeprol	-3.08 ± 0.86	11.4 ± 0.5		776 > 127	- 50	+ 14.0	778 > 558	+ 60	- 21.5
	3	Iopamidol	-2.09 ± 1	10.9 ± 0.5		776 > 127	- 50	+ 14.0	778 > 558	+ 60	- 21.5
	3	Iopromide	-2.95 ± 0.91	10.6 ± 0.7		790 > 127	- 45	+ 8.0	792 > 573	+ 65	- 14.5
	5	Isoproturon	2.32 ± 0.29		0.9 ± 0.5	207 > 72	+ 45	- 10.0	207 > 165	+ 45	- 7.0
	11	Loratadine	5.94 ± 0.77		4.8 ± 0.2	383 > 337	+ 55	- 15.5	383 > 267	+ 55	- 26.5
	7	Metazachlor	2.11 ± 0.48		1.3 ± 0.5	278 > 134	+ 30	- 17.5	278 > 210	+ 30	- 6.0
	1	Paracetamol	0.34 ± 0.21	9.9 ± 0.1	1.7 ± 0.5	152 > 110	+ 40	- 20.0	-		
	1	Paraxanthine	-0.63 ± 0.59	8.5 ± 0.5	0.2 ± 0.7	181 > 124	+ 60	- 8.0	181 > 96	+ 60	- 10.5
	7	Phenazone	0.27 ± 0.33		0.7 ± 0.7	189 > 146	+ 60	- 10.0	189 > 131	+ 60	- 9.5
	4	Primidone	0.40 ± 0.52	12.3 ± 0.4		219 > 162	+ 35	- 9.0	219 > 91	+ 35	- 21.0
	10	Tetrazepam	3.13 ± 0.65		2.0 ± 0.2	289 > 225	+ 60	- 24.0	289 > 253	+ 60	- 9.5
	1	Theobromine	-0.72 ± 0.55	9.9 ± 0.5	0.6 ± 0.7	181 > 138	+ 55	- 9.5	181 > 110	+ 55	- 13.0
	1	Theophylline	-0.18 ± 0.31	8.6 ± 0.5	1.5 ± 0.7	181 > 124	+ 60	- 8.0	181 > 96	+ 60	- 10.5
	1	Tolyltriazole	1.89[b]	not available		134 > 77	+ 55	- 15.0	134 > 106	+ 55	- 5.0
Anionic	12	Bezafibrate	3.46 ± 0.45	3.3 ± 0.1		360 > 274	- 40	+ 13.0	360 > 154	- 40	+ 21.0
	12	Clofibric acid	2.72 ± 0.27	3.2 ± 0.1		213 > 127	- 25	+ 12.0	213 > 85	- 25	+ 8.5
	12	Diclofenac	4.06 ± 0.41	4.2 ± 0.1		294 > 250	- 30	+ 8.5	296 > 214	+ 30	- 24.0
	12	Gemfibrozil	4.39 ± 0.49	4.8 ± 0.5		249 > 121	- 30	+ 11.0	-		
	12	Ibuprofen	3.72 ± 0.23	4.4 ± 0.1		205 > 161	- 25	+ 5.5	-		
	13	Mecoprop	2.83 ± 0.27	3.2 ± 0.2		213 > 141	- 30	+ 12.5	-		
	12	Naproxen	3.00 ± 0.24	4.8 ± 0.3		231 > 185	+ 40	- 10.0	231 > 170	+ 40	- 21.5
	4	Sulfamethoxazole	0.89 ± 0.42	5.8 ± 0.5	1.4 ± 0.1	254 > 156	+ 35	- 13.5	254 > 108	+ 35	- 17.5
Cationic	2	Atenolol	0.10 ± 0.28		9.2 ± 0.4	267 > 145	+ 55	- 20.0	267 > 190	+ 55	- 11.0
	9	Clarithromycin	3.16 ± 0.78	13.1 ± 0.7	8.2 ± 0.7	748 > 158	+ 60	- 13.5	748 > 590	+ 60	- 6.0
	10	Citalopram	2.51 ± 0.74		9.6 ± 0.3	325 > 109	+ 60	- 17.5	325 > 262	+ 60	- 11.0
	8	Erythromycin	2.83 ± 0.78	13.1 ± 0.7	8.2 ± 0.7	734 > 158	+ 60	- 16.0	734 > 576	+ 60	- 6.0
	14	Fluoxetine	4.09 ± 0.45		10.1 ± 0.1	310 > 148	+ 25	- 6.5	-		
	2	Metoprolol	1.79 ± 0.4		9.2 ± 0.4	268 > 116	+ 55	- 11.0	268 > 191	+ 55	- 10.0
	9	Roxithromycin	2.75[c]		8.8[c]	837 > 158	+ 70	- 17.0	837 > 679	+ 70	- 6.0
	14	Sertraline	4.81 ± 0.41		9.5 ± 0.4	306 > 159	+ 25	- 19.0	306 > 275	+ 25	- 7.5
	2	Sotalol	0.32 ± 0.37	9.6 ± 0.5	9.2 ± 0.4	273 > 255	+ 40	- 7.5	273 > 213	+ 40	- 17.0
Neutral/Cationic	7	Trimethoprim	0.79 ± 0.38		7.2 ± 0.1	291 > 230	+ 65	- 15.0	291 > 123	+ 65	- 15.5
Neutral/Anionic	1	1-Methylxanthine	-0.58 ± 0.59	7.7 ± 0.7	1.2 ± 0.7	165 > 108	- 55	+ 19.0	165 > 80	- 55	+ 25.0
	15	Pantoprazole	1.69 ± 1.12	7.7 ± 0.7	3.6 ± 0.3	384 > 200	+ 25	- 8.5	384 > 138	+ 25	- 21.0

*[1]corresponding to Table 4 - *[2]SciFinder predicted values unless otherwise noted - *[3]'Basic' pKa implies pKa of the conjugate acid - *[4]Capillary voltage - *[5]Collision energy

*[6]Dipolar ion, [a]Hanocq et al. (1989), [b]Giger et al. (2006), [c]Beausse (2004)

Table 2.4: ESI-MS/MS conditions of the internal standards.

Internal standard		MRM	[a]Cap U [V]	[b]CE [V]
1	Paraxanthine-D_6	187 > 127	+ 60	− 9.0
2	Atenolol-D_7	274 > 145	+ 55	− 17.5
3	Desmethoxyiopromide	760 > 127	− 40	+ 8.5
4	Sulfamethoxazole-$^{13}C_6$	260 > 162	+ 35	− 13.5
5	Isoproturon-D_6	213 > 171	+ 50	− 8.0
6	Atrazine-D_5	221 > 179	+ 55	− 10.5
7	Carbamazepine-D_{10}	247 > 204	+ 45	− 13.0
8	Erythromycin-N-methyl-^{13}C-D_3	738 > 162	+ 65	− 16.5
9	Ery-methyloxime	763 > 158	+ 60	− 20.0
10	Diazepam-D_5	290 > 198	+ 65	− 24.5
11	Loratadine-D_4	387 > 269	+ 55	− 26.5
12	Ibuprofen-D_3	208 > 164	− 25	+ 6.0
13	Mecoprop-D_3	218 > 146	− 35	+ 12.0
14	Fluoxetine-D_6	316 > 154	+ 25	− 6.5
15	Lansoprazole	370 > 252	+ 25	− 8.0

[a] Capillary voltage.

[b] Collision energy.

2.2.5 Performance of the multi-residue method

Method quantitation limits (MQL) were calculated throughout the complete method by linear regression of a 10-point calibration incorporating SPE and HPLC-MS/MS analysis (confidence interval 0.95). The highest and lowest concentration levels were extracted in triplicate, all intermediate concentrations in duplicate. Single injection of each extract was applied. For calculating the MQL of surface- and seawater 500 mL buffered aqueous standard solutions were prepared and extracted according to the method described in 2.2.3. The extracts were analyzed according to 2.2.4. With respect to treated wastewater 100 mL were used. For both sample volumes 10 equidistant absolute levels corresponding to a low concentration range were prepared: 5–50 ng for the iodinated contrast media, fluoxetine, sertraline, 1-methylxanthine and 3-methylxanthine and 1.25–12.5 ng for all other compounds.

The linear range of the method was evaluated using artificial samples of 500 mL Milli-Q water spiked with the analytes. In contrast to instrumental linearity studies, SPE was included to account for breakthrough of analytes during extraction. Linearity ranges for 100 mL sample volume were calculated from the 500 mL results as empirical worst-case values, hereby possibly underestimating the real range of linearity for the smaller volume.

2.3 Results and discussion

2.3.1 Surface-normalized comparison of selected SPE-sorbents

In contrast to other comparative recovery studies, the sorbents were tested considering their specific surface areas. The results are presented in Figure 2.1. With respect to the used sorbents, there is no universal correlation of recovery and log K_{OW} of neutral compounds (Figure 2.1 a & b). However, recoveries clearly decreased with increasing basic character of the compounds (Figure 2.1 c & d). Standard deviation (SD) of the triple-extractions was ≤10%, except for erythromycin A on AbsElut Nexus (14%) and diuron on Isolute ENV+ (11%).

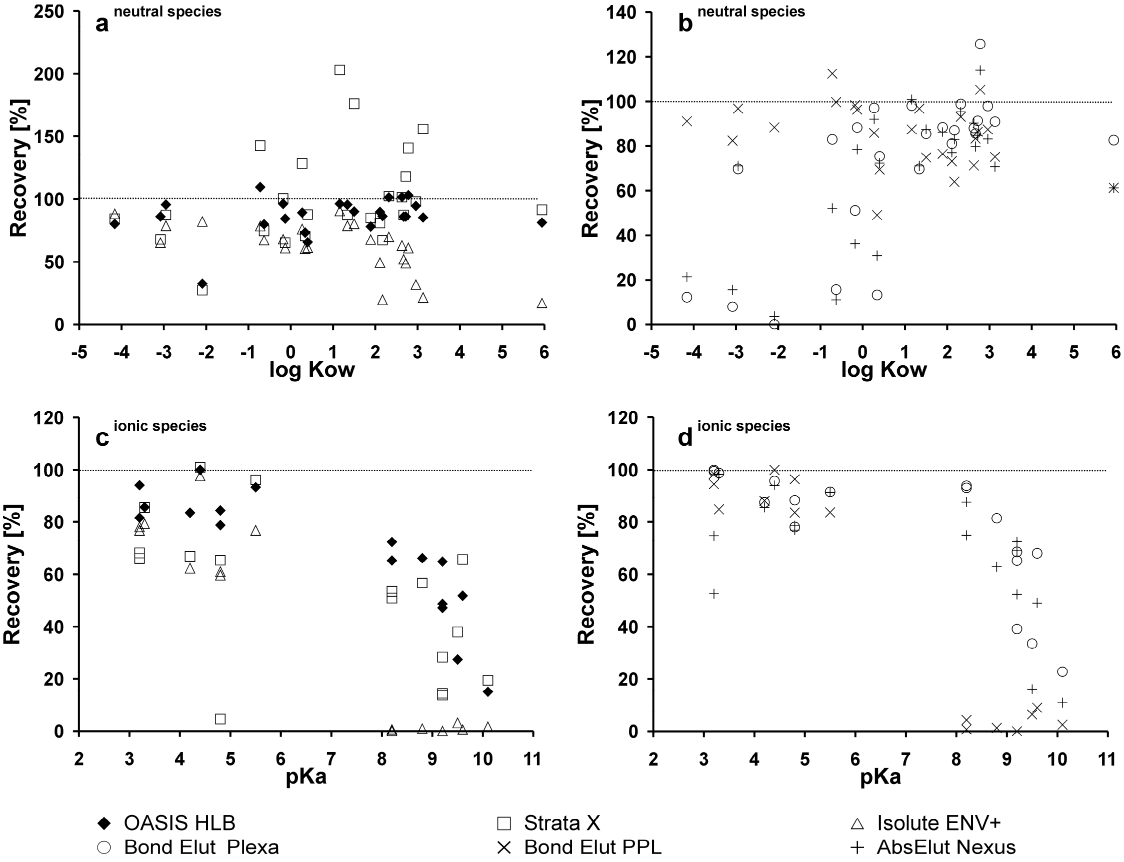


Figure 2.1: Recovery rates (RR) of compounds on different SPE sorbents; correlation of RR, log K_{OW} and pKa. In case of multiple pKa per compound, the one that was relevant at experimental conditions was chosen. Chart a + c illustrate RR of OASIS HLB, Strata X and Isolute ENV+, chart b + d illustrate RR of Bond Elut Plexa, Bond Elut PPL and AbsElut Nexus. High RR (>120%) of Strata X in Chart 'a' are most likely attributed to over-compensation by internal standardization. Standard deviation (SD) of the triple-extractions was ≤10% except for erythromycin A on AbsElut Nexus (14%) and diuron on Isolute ENV+ (11%).

The recoveries of cationic compounds and ICM turned out to be the critical ones, since recoveries of neutral and anionic compounds were mostly in the same range for all sorbents. Out of all analytes, fluoxetine and sertraline were exceptional cases, since both compounds exhibited very poor recoveries for all of the tested sorbents (3–38% and 2–23%, respectively). Apart from these compounds, AbsElut Nexus (acidic basic screen ELUT), a polystyrene-divinylbenzene (PS-DVB) / methyacrylate copolymer, and Bond Elut Plexa exhibited the highest recoveries of cationic compounds with a median of 69%. With Bond Elut Plexa and AbsElut Nexus recoveries of the ICM iopamidol, iomeprol and iohexol were unacceptably low (0–21%). Surprisingly, Bond Elut PPL showed the best recoveries for all of the ICM tested (≥82%). This sorbent was never mentioned in the literature for extraction of ICM (Pérez and Barceló, 2007). Verification of this sorbent for a specialized analytical method for both non-ionic and ionic ICM may be promising since recoveries of all anionic compounds were also high (≥84%). Unfortunately, this sorbent exhibited very low recoveries for beta-blockers, macrolide antibiotics and SSRI (<10%). With the exception of fluoxetine and sertraline, OASIS HLB demonstrated high recoveries of cationic compounds (65% median recovery) and all ICM except iopamidol (33%) were recovered with yields of >80% by this sorbent. For a multi-residue analytical method of the required analytes, OASIS HLB represented the best compromise, since it was the only sorbent with sufficient recoveries of ICM and basic compounds.

During the elution step of Strata X and Isolute ENV+, turbidity of the extracts indicated possible elution/dissolution of sorbent material. Since the turbidity endured in the re-dissolved extracts of both sorbents, analytes and internal standards may be sorbed to these particles and were removed by centrifugation. Additionally, adverse ionization effects in ESI may eventually be caused by soluble cartridge material. Figure 2.1a shows remarkable recoveries of up to 200% for the Strata X. This may be attributed to overcompensation of the effects mentioned above with the internal standard, since the analytical system was calibrated with a diluted stock solution of the reference standards. Thus, the calibration did not represent any matrix effect caused by the sample or sample pre-treatment (Bester *et al.*, 2001; Mallet *et al.*, 2004). This may underestimate the extraction quality of Strata X, since a sorbent-specific matrix would be compensated during a calibration, which includes the extraction process. The partially disappointing recoveries with Isolute ENV+ may also be influenced by a similar effect.

2.3.2 Liquid chromatography tandem mass spectrometry (HPLC-MS/MS)

2.3.2.1 Liquid chromatography

The applied gradient allowed sufficient retention and separation of analytes on a Polaris C18-Ether HPLC column. A chromatogram of a standard solution is presented in Figure 2.2. During the optimization process, formic acid concentrations of eluent A were varied over the concentration range 0.01–0.1%. The influence of formic acid concentration (and thus pH) on response of ibuprofen-D_3 (MRM 208 → 164) is presented in Figure 2.3. It clearly demonstrates a striking signal suppression of ibuprofen-D_3 in presence of more acidic pH values (higher formic acid concentrations). This is most likely attributed to a lower dissociation rate of ibuprofen at higher acid concentrations (Ardrey, 2003). Determination of acidic pharmaceuticals in negative mode (ESI−) is usually performed with organic solvents and ultrapure water, or aqueous ammonium acetate, usually with alkaline pH values (Gros *et al.*, 2006). However, Busetti *et al.* (2009) used a low formic acid concentration of 0.01% for the analysis of naproxen, ibuprofen, clofibric acid and gemfibrozil with negative ESI on a Micromass Quattro Ultima Quadrupole instrument. Yang *et al.* (2010) reported suppressed ionization of phenolic acids in the presence of 0.1% formic acid in one eluent while using negative ionization. On the other hand, ionization of adenosine and coumarins were enhanced in positive polarity due to the acid. When using switching electrospray ionization it is obvious that the gradient composition must represent an appropriate compromise. Therefore, a formic acid concentration of 0.015% was found to be optimal in the multi-residue analytical method described here.

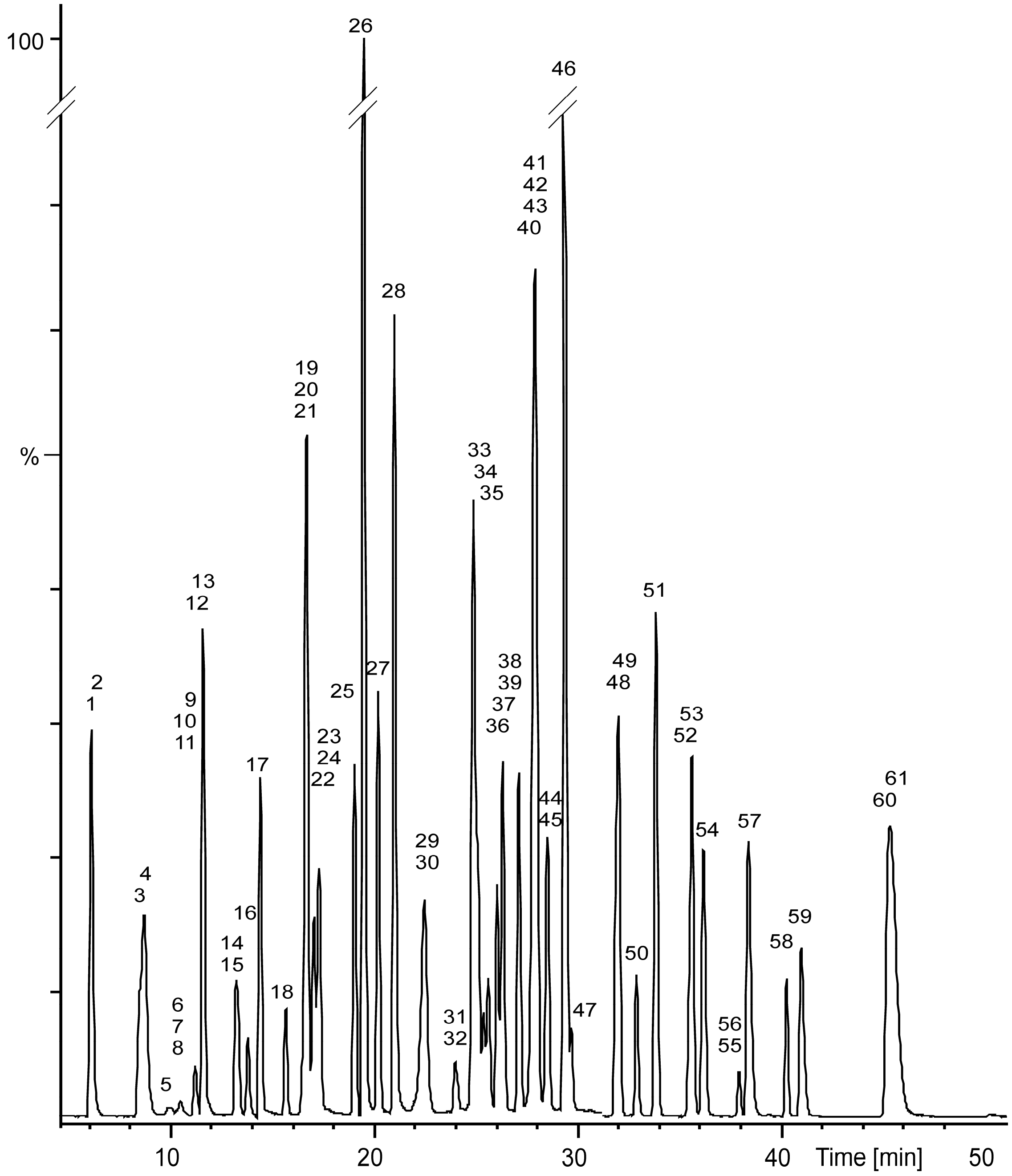


Figure 2.2: Chromatogram (Total Ion Current, TIC) of a standard solution. Analytes and internal standards in order of elution: (1) sotalol, (2) iopamidol, (3) atenolol-D$_7$, (4) atenolol, (5) 3-methylxanthine, (6) iomeprol, (7) 1-methylxanthine, (8) iohexol, (9) desmethoxyiopromide, (10) paracetamol, (11) iopromide, (12) theobromine, (13) trimethoprim, (14) paraxanthine-D$_6$, (15) paraxanthine, (16) theophylline, (17) metoprolol, (18) caffeine, (19) desisopropylatrazine, (20) benzoylecgonine, (21) 1H-benzotriazole, (22) phenazone, (23) sulfamethoxazole-^{13}C$_6$, (24) sulfamethoxazole, (25) primidone, (26) citalopram, (27) desethylatrazine, (28) tolyltriazole, (29) erythromycin-N-methyl-^{13}C-D$_3$, (30) erythromycin, (31) fluoxetine-D$_6$, (32) fluoxetine, (33) pantoprazole, (34) sertraline, (35) clarithromycin, (36) ery-methyloxime, (37) roxithromycin, (38) carbamazepine-D$_{10}$, (39) carbamazepine, (40) lansoprazole, (41) atrazine-D$_5$, (42) metazachlor, (43) atrazine, (44) isoproturon-D$_6$, (45) isoproturon, (46) cetirizine, (47) diuron, (48) diazepam-D$_5$, (49) diazepam, (50) naproxen, (51) tetrazepam, (52) loratadine-D$_4$, (53) loratadine, (54) bezafibrate, (55) ibuprofen-D$_3$, (56) ibuprofen, (57) clofibric acid, (58) gemfibrozil, (59) diclofenac, (60) mecoprop-D$_3$ and (61) mecoprop.

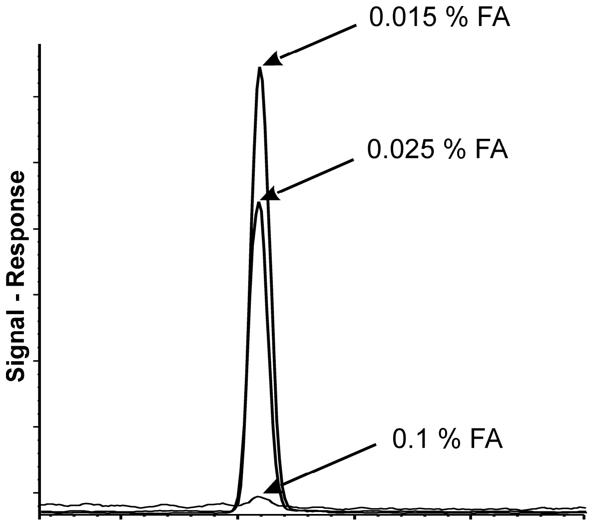


Figure 2.3: Correlation of signal response and formic acid (FA) fraction of eluent A [%], 20 ng ibuprofen-D$_3$ using the reaction of the negatively charged (M−H$^+$)- ion for detection.

2.3.2.2 Mass spectrometry (MS)

Selected results of the optimization experiments of ESI drying gas temperature are shown in Figure 2.4. The optimization of the drying gas temperature was achieved by analyzing a multi-standard solution with 100 ng mL^{-1} for each analyte in 5 mM ammonium acetate and 4% methanol according to 2.4. Signal response (peak area) and relative standard deviation (3 injections per temperature) of each respective quantifier MRM was investigated at drying gas temperatures of 260–360 °C with 20 K increments. The drying gas temperature had significant effects for only a few of the compounds; however, effects were remarkable. Figure 2.4a shows the results for those analytes for which signals increased with temperature (with the highest signal set to 100%). Since drying efficiency is a function of the organic nature of the mobile phase, eluents with high organic fractions may evaporate more easily than eluents with low organic fractions. Therefore, the most promising enhancement due to a high drying gas temperature was expected at gradient conditions, which are tendentially unfavorable for evaporation. Increasing signal responses of iohexol, iopromide and iopamidol, which were eluting with methanol fractions below 20%, confirmed the expectation. However, with respect to the analytes eluting with high methanol fractions (>50%), the excessive improvement of bezafibrate was the only remarkable example.

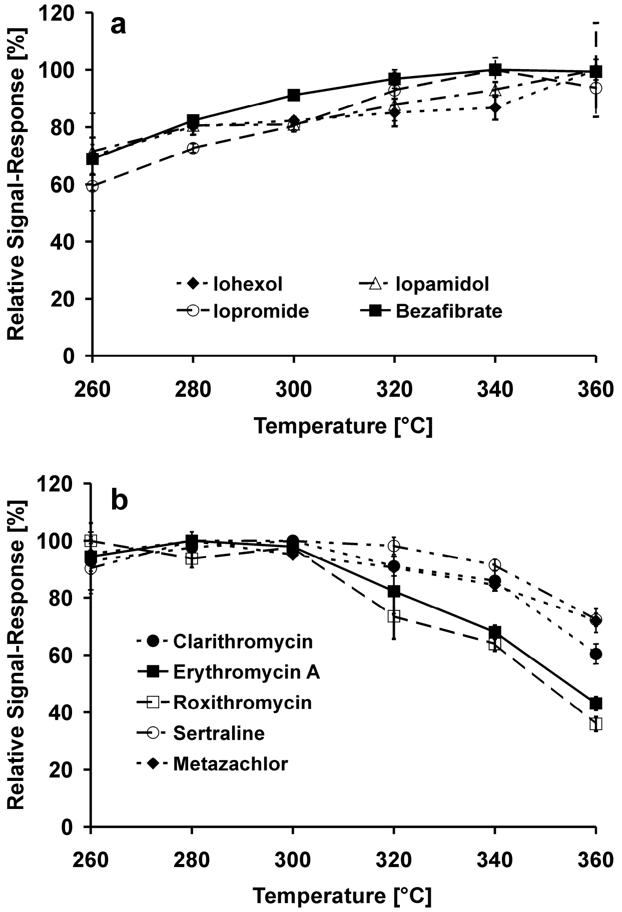


Figure 2.4: Correlation of signal response and drying gas temperature of selected compounds.

With respect to the analytes, drying gas temperature is basically limited by the thermal stability of individual compounds. The substances that were most susceptible to thermal decay were the macrolide antibiotics erythromycin A, clarithromycin and roxithromycin (Figure 2.4b). Direct LC/MS injection experiments with the chosen macrolides at different drying gas temperatures demonstrated the temperature dependent decay of the respective $[M+H]^+$ into its $[M+H-C_8H_{15}O_3]^+$ derivative (elimination of cladinosyl). This equates to the macrolide qualifier reaction presented in this MS/MS method. Signal responses of sertraline and metazachlor were also negatively affected by increased drying gas temperature, but to a lesser extent.

Reproducibility, expressed as the relative standard deviation of each compound with three injections per temperature, also depended on a change in drying gas temperature. The temperature range of 280–340 °C showed RSD <10% for all analytes whereas 260 and 360 °C revealed values of up to 15 and 16%, respectively. Thus, 340 °C was used during the first 19 min of the analytical run to optimize sensitivity of the ICM. 280 °C was used from 19–55 min to prevent thermolabile compounds from thermal degradation and 340 °C from 55–57 min to prepare the system for the following run.

If mentioned at all, the optimization process of electrospray drying gas temperature is infrequently described in the literature and studies with quantified temperature/response relations are an exception (Takino *et al.*, 2000). As demonstrated, signal response of compounds from different chemical classes to varying drying gas temperatures can be highly individual. If supported by the MS instrument, the implementation of a temperature gradient can significantly increase the sensitivity of a given method. This application is primarily relevant for multi-residue analytical methods, as described in this study.

2.3.3 Performance of the developed multi-residue analytical method

Method quantitation limits (MQL) and linear range of the method were determined according to section 2.2.5. They are presented in Table 2.5. In comparison to studies on organic pollutants in seawater the sample pre-concentration factor applied in this study was rather low. Sample volumes of 1–100 liters were used frequently for target and non-target analysis in seawater (Bester and Hühnerfuss, 1993; Bester and Theobald, 2000; Buser *et al.*, 1998; Weigel *et al.*, 2002, 2004). However, MQL of the developed method are adequate for environmental analysis in the low ng L^{-1} concentration range. In contrast to other studies on multi-residue analytical methods (Busetti *et al.*, 2009; Gómez *et al.*, 2006; Rodil *et al.*, 2009), a high number and variety of individual compounds were analyzed here simultaneously, with only one injection, by using a generic gradient and switching electrospray ionization. Furthermore, the linear range and correlation factors presented in Table 2.5 were obtained as a result of the total analytical procedure and do not represent instrument levels only (Busetti *et al.*, 2009; Rodil *et al.*, 2009). This may allow a more realistic assessment of the method's linearity within the scope of an application. However, one drawback of the presented method, as well as for all multi-residue methods in general, is their compromise nature. In comparison with single group analysis, MQL of analytes can be significantly higher as it is exemplary for ICM (Ternes and Hirsch, 2000). Fortunately, if present in environmental samples, the concentrations of these compounds are usually several times higher than the MQL of the developed method (Pérez and Barceló, 2007; Ternes and Hirsch, 2000).

Table 2.5: Performance of the developed multi-residue method.

	500 mL sample volume				100 mL sample volume		
Compound	MQL	Linear range[a]	(R^2)[b]	RSD[c]	MQL	Linear range[d]	RSD[c]
	[ng L^{-1}]	[ng L^{-1}]		[%]	[ng L^{-1}]	[ng L^{-1}]	[%]
1H-Benzotriazole	4.7	2.5 – 1400	0.9947	6.1	18	12.5 – 7000	5.2
1-Methylxanthine	21	10 – 1000	0.9934	4.1	130	50 – 5000	7.5
3-Methylxanthine	28	10 – 1000	0.9903	5.6	135	50 – 5000	7.8
Atenolol	3.5	2.5 – 2000	0.9983	4.9	12	12.5 – 10000	3.1
Atrazine	1.4	2.5 – 2000	0.9969	1.9	8.9	12.5 – 10000	2.4
Benzoylecgonine	2.3	2.5 – 1000	0.9942	3.2	8.8	12.5 – 5000	2.4
Bezafibrate	3.5	2.5 – 900	0.9868	4.9	7.3	12.5 – 4500	2.0
Caffeine	4.3	2.5 – 2000	0.9937	6.1	39	12.5 – 10000	12
Carbamazepine	2.2	2.5 – 1600	0.9900	3.1	8.8	12.5 – 8000	2.4
Cetirizine	2.2	2.5 – 750	0.9901	2.9	12	12.5 – 3750	3.2
Citalopram	3.2	2.5 – 2000	0.9978	4.5	35	12.5 – 10000	8.5
Clarithromycin	7.5	2.5 – 2000	0.9959	11	18	12.5 – 10000	5.0
Clofibric acid	3.4	2.5 – 1000	0.9964	4.7	15	12.5 – 5000	4.1
Desethylatrazine	1.7	2.5 – 2000	0.9987	2.3	8.7	12.5 – 10000	2.4
Desisopropylatrazine	5.6	2.5 – 1400	0.9949	8.1	15	12.5 – 7000	4.2
Diazepam	1.4	2.5 – 2000	0.9963	1.9	11	12.5 – 10000	3.0
Diclofenac	2.0	2.5 – 1000	0.9850	2.7	15	12.5 – 5000	4.1
Diuron	3.3	2.5 – 1000	0.9890	4.6	14	12.5 – 5000	3.8
Erythromycin	7.5	2.5 – 2000	0.9928	6.2	29	12.5 – 10000	8.4
Fluoxetine	16	10 – 2000	0.9984	5.5	82	50 – 10000	5.9
Gemfibrozil	2.0	2.5 – 1000	0.9861	2.7	17	12.5 – 5000	4.5
Ibuprofen	3.6	2.5 – 2000	0.9920	5.0	19	12.5 – 10000	5.4
Iohexol	21	12.5 – 4000	0.9913	6.0	160	60 – 20000	9.3
Iomeprol	19	12.5 – 4000	0.9970	5.1	145	60 – 20000	8.4
Iopamidol	19	12.5 – 4000	0.9945	5.2	135	60 – 20000	7.9
Iopromide	19	12.5 – 4000	0.9949	5.2	105	60 – 20000	6.0
Isoproturon	3.0	2.5 – 2000	0.9981	4.2	19	12.5 – 10000	5.1
Loratadine	2.7	2.5 – 1400	0.9905	3.8	11	12.5 – 7000	2.8
Mecoprop	1.2	2.5 – 2000	0.9972	1.5	5.0	12.5 – 10000	1.4
Metazachlor	1.8	2.5 – 1000	0.9938	2.5	12	12.5 – 5000	3.1
Metoprolol	4.1	2.5 – 2000	0.9959	5.9	23	12.5 – 10000	6.3
Naproxen	4.8	2.5 – 900	0.9950	6.9	24	12.5 – 4500	6.9
Pantoprazole	4.8	2.5 – 1600	0.9977	6.8	36	12.5 – 8000	11
Paracetamol	3.7	2.5 – 2000	0.9905	5.1	26	12.5 – 10000	7.5
Paraxanthine	3.2	2.5 – 2000	0.9954	4.4	13	12.5 – 10000	3.5
Phenazone	2.0	2.5 – 1000	0.9896	2.7	18	12.5 – 5000	4.9
Primidone	2.7	2.5 – 2000	0.9977	3.7	14	12.5 – 10000	3.7
Roxithromycin	9.5	2.5 – 1400	0.9954	2.0	15	12.5 – 7000	4.2
Sertraline	16	10 – 1200	0.9981	3.3	115	50 – 6000	17
Sotalol	4.8	2.5 – 1800	0.9950	6.9	32	12.5 – 9000	9.4
Sulfamethoxazole	2.6	2.5 – 2000	0.9988	3.5	14	12.5 – 10000	3.8
Tetrazepam	2.5	2.5 – 2000	0.9955	3.4	15	12.5 – 10000	4.2
Theobromine	5.1	2.5 – 750	0.9971	7.4	24	12.5 – 3750	6.9
Theophylline	3.4	2.5 – 2000	0.9912	4.7	18	12.5 – 10000	5.0
Tolyltriazole	4.9	2.5 – 1800	0.9931	7.0	35	12.5 – 9000	10
Trimethoprim	2.5	2.5 – 2000	0.9890	3.4	15	12.5 – 10000	4.2

[a] Experimental values; 15 point calibration: extraction of 6.25–2000 ng ICM; 5–1000 ng fluoxetine, sertraline and mono-methylxanthines; 1.25–1000 ng for all other compounds; concentration range with R^2 ≥0,9850 was defined as linear range.

[b] corresponds to linear range of 500 mL sample volume.

[c] coefficient of the variation of the procedure, calculated from MQL determination (linear regression, n = 22).

[d] calculated values from 500 mL sample volume.

The identified linear ranges of all analytes are generally in accordance with concentrations of micro-contaminants frequently detected in environmental samples (Giger *et al.*, 2006; Hirsch *et al.*, 1999; Kiss and Fries, 2009; Kosonen and Kronberg, 2009; Maurer *et al.*, 2007; Pérez and Barceló, 2007; Ternes, 1998; Ternes and Hirsch, 2000; Voutsa *et al.*, 2006; Zuccato *et al.*, 2000). Coefficients of the variation of the procedure are, apart from a few exceptions, less than 10%, indicating a good precision of the total method.

2.3.4 Matrix effects

Undesirable matrix effects such as signal suppression and enhancement are often the concern of LC-MS/MS studies. The goal of the presented study was at this stage to study the possibilities of a single gradient method. However, signal suppression was observed for almost all analytes in extracts from environmental samples.

Mechanisms of matrix effects, as well as strategies to overcome them, were discussed in the literature (Antignac *et al.*, 2005; Bester, 2008; Cappiello, 2008; Matiszewski *et al.*, 2003). As stated, ESI is more susceptible than i.e. LC-MS interfaces, which are based on heated nebulization such as atmospheric pressure chemical ionization (APCI) and, very recently, electron impact (EI). However, limited distribution of EI and a higher sensitivity for many frequently detected compounds in environmental samples justify the frequent use of ESI in multi-residue analytical methods (Petrović *et al.*, 2005; Wick *et al.*, 2010).

A simple strategy to reduce matrix effects in SPE-based methods requires rinsing the loaded SPE cartridge with water, or low concentrated organic acids containing little portions of organic solvents (Kosonen and Kronberg, 2009). In the presented method, rinsing the sorbent twice with 1.5 mL of ultrapure water showed no significant influence on recovery of analytes, but was very effective in removal of inorganic salt matrix, as verified by ion chromatography. Particularly for the analysis of seawater matrix, this step is recommendable, since otherwise the formation of sodium adducts would result in reduced sensitivity of selected compounds (Bester, 2008). Furthermore, compared to extraction at acidic pH values, extraction at neutral pH minimizes potential co-extraction of typical organic components of environmental samples like humic and fulvic acids (Pichon *et al.*, 1996).

The application of isotope-labeled internal standards was identified as a very powerful tool to compensate adverse matrix effects with ESI for various aqueous matrices (Busetti *et al.*, 2009; Marín *et al.*, 2009; Rodil *et al.*, 2009; Wick *et al.*, 2010). However, for a multi-residue analysis with a wide range of chemically diverse compounds as presented here, the availability of suitable surrogates and their considerable costs make a suitable selection of internal standards with excellent performance for all matrices quite challenging. In the presented analytical method a high number of 15 IS were used as a strategy to overcome matrix effects, which represented a good compromise between precision and surrogate availability.

2.3.5 Application to environmental samples

To demonstrate the applicability of the method, real samples with different matrices were analyzed in duplicates. For that purpose, three different water types were used, from low organic concentration in seawater samples (commonly 0.1 mg L^{-1} total organic carbon, TOC) to a highly complex organic matrix in treated effluent with ca. 10 mg L^{-1} TOC. Additionally, the samples differed with respect to their ionic strength (from 0.01 mol L^{-1} to 0.5 mol L^{-1}). The results are presented in Table 2.6. Expanded uncertainties were calculated according to Konieczka & Namieśnik (2010). They are comparable between samples over wide concentration ranges, i.e. 1H-benzotriazole between 29 ng L^{-1} and 3846 ng L^{-1}. Furthermore, analytes in the sub ng L^{-1} as well as analytes in the µg L^{-1} concentration range were successfully quantified in the same sample.

36 of the calibrated 46 analytes were detected in at least one sample. As expected, the lowest concentrations of micro-contaminants were detected in seawater. The WWTP effluent exhibited the highest individual concentrations and the river presented the highest variety of compounds. 1H-benzotriazole, tolyltriazole, carbamazepine, cetirizine, clarithromycin, sulfamethoxazole, iomeprol and iopromide were detected in all three samples.

The river water was particularly interesting because the sampling location was about 2 km downstream of the sampled WWTP and both samples were taken at approximately the same time. It is remarkable that significant amounts of paracetamol, caffeine and its degradation products were detected in the river but not in the treated effluent. Micro-contaminants are suggested to be source specific and thus offer the potential to be employed as indicators for source delineation in monitoring ground- and surface water quality, its decline or improvement. This especially applies to selected pharmaceuticals, pesticides and stimulants (Buerge *et al.*, 2006; Godfrey *et al.*, 2007; Townsend and Young, 2000). Since caffeine is generally readily biodegradable in WWTPs, it has been used as an indicator for the input of raw sewage into surface waters (Buerge *et al.*, 2006). Elimination rates of paracetamol in WWTPs are also very high (up to 99%) (Gómez *et al.*, 2007). Therefore, there is an indication of inflow of raw sewage into the river. The same applies to the analyzed seawater sampling location since caffeine, paraxanthine and theobromine were detected.

Table 2.6: Concentrations of analytes in three different sample matrices expressed as mean result of duplicate extraction ± expanded uncertainty[a] in ng L^{-1}.

Compound	River sample	Seawater sample	Treated effluent sample
1H-Benzotriazole	670 ± 41	29 ± 3.3	3846 ± 277
1-Methylxanthine	61 ± 14	nd[c]	–
3-Methylxanthine	–[b]	nd	–
Atenolol	66 ± 3.6	–	245 ± 16
Atrazine	2 ± 0.8	–	–
Benzoylecgonine	–	–	–
Bezafibrate	29 ± 2.5	–	168 ± 11
Caffeine	101 ± 8.7	58 ± 5.4	–
Carbamazepine	265 ± 10	26 ± 1.6	1429 ± 46
Cetirizine	20 ± 2.0	4 ± 1.3	105 ± 8.2
Citalopram	54 ± 4.7	–	431 ± 41
Clarithromycin	77 ± 12	14 ± 9.4	520 ± 35
Clofibric acid	21 ± 2.4	–	103 ± 9.3
Desethylatrazine	6 ± 1.0	–	–
Desisopropylatrazine	7 ± 3.4	–	–
Diazepam	10 ± 0.9	–	27 ± 6.1
Diclofenac	156 ± 6.0	–	1492 ± 89
Diuron	–	–	–
Erythromycin	22 ± 2.7	–	173 ± 23
Fluoxetine	–	nd	–
Gemfibrozil	–	–	–
Ibuprofen	–	–	–
Iohexol	1214 ± 88	–	5574 ± 718
Iomeprol	1258 ± 106	40 ± 11	6272 ± 572
Iopamidol	785 ± 100	–	5569 ± 619
Iopromide	268 ± 30	25 ± 11	2670 ± 208
Isoproturon	43 ± 2.0	–	35 ± 11
Loratadine	–	4 ± 4.0	–
Mecoprop	26 ± 1.2	–	159 ± 5.2
Metazachlor	–	–	–
Metoprolol	337 ± 22	–	2513 ± 161
Naproxen	–	–	–
Pantoprazole	13 ± 2.8	nd	149 ± 23
Paracetamol	1992 ± 198	–	–
Paraxanthine	36 ± 3.3	22 ± 2.4	–
Phenazone	27 ± 1.7	–	56 ± 23
Primidone	60 ± 4.3	–	216 ± 20
Roxithromycin	16 ± 6.4	–	78 ± 11
Sertraline	–	nd	–
Sotalol	195 ± 9.0	–	1314 ± 130
Sulfamethoxazole	93 ± 4.4	7 ± 1.5	509 ± 35
Tetrazepam	–	–	–
Theobromine	–	23 ± 4.2	–
Theophylline	21 ± 3.0	–	–
Tolyltriazole	741 ± 45	37 ± 3.6	4803 ± 404
Trimethoprim	95 ± 4.2	–	681 ± 28

[a] expanded uncertainties calculated according to Konieczka & Namieśnik (2010).

[b] not detected.

[c] not determined.

Concentrations of iomeprol, iopamidol and iopromide are in the typical range of these compounds in German river water and WWTP effluents (Ternes and Hirsch, 2000). Considering concentrations of several µg L^{-1} ICM in WWTP effluents, detection of ICM in seawater was not surprising. Concentration ranges of antibiotics and other compounds such as atenolol, metoprolol, diclofenac and phenazone were also found within the typical range of these compounds in river water and WWTP effluents (Hirsch *et al.*, 1999; Maurer *et al.*, 2007; Ternes, 1998; Zucchato *et al.*, 2000).

In Germany, the herbicide atrazine has been banned for almost two decades now. However, this compound as well as its metabolites, desethylatrazine and desisopropylatrazine, were detected in the river sample. The occurrence of desethylatrazine cannot be attributed to former usage of atrazine with absolute certainty, since this compound is also a metabolite of the formerly used herbicide propazine (Thurman *et al.*, 1994). However, depending on local herbicide patterns, a desethylatrazine/atrazine-ratio (DAR) > 1 can indicate a breakdown of atrazine in soil and groundwater (Townsend and Young, 2000). In the presented case, the DAR is ~3. Therefore, if attributed to atrazine, a recent application of the herbicide is unlikely and a non-point source of these compounds is likely. This outcome was expected; with the developed method it is possible to interpret this ratio in the very low concentration range.

Kosonen & Kronberg (2009) revealed that high concentrations of anti-histamines in WWTP wastewaters correlate with an outbreak of allergic reactions, due to a high abundance of plant pollen in the air in April and May. Furthermore, they identified cetirizine as a relatively stable compound in the aquatic environment, which can be detected far from the discharge point. This can explain its detection in seawater, since sampling took place in late spring. Cetirizine concentrations in WWTP effluent and river water are comparable to those of the Finnish study.

The widely used corrosion inhibitors 1H-benzotriazole and tolyltriazole were detected in all matrices at expected concentrations. They have frequently been detected in surface waters and WWTP effluents (Giger *et al.*, 2006; Kiss and Fries, 2009; Voutsa *et al.*, 2006). Due to typical WWTP effluent concentrations, combined with the mobility and persistence of these compounds, a widespread distribution in marine environments has to be predicted.

2.4 Conclusions

Sensitive detection of 46 compounds, belonging to different chemical classes, was successfully applied with only one injection. In the presented multi-residue analytical method, the use of switching electrospray ionization and SPE requires compromises in terms of a generic gradient and sorbent material, respectively. However, experimental MQL and linear ranges of analytes are in accordance with typical environmental concentrations and analytes in the sub ng L^{-1} together with analytes in the µg L^{-1} concentration range can be quantified in the same sample. If supported by the instrument, the application of a drying gas temperature gradient is certainly worth testing. Depending on the analyzed compound classes, implementation of such a gradient can significantly improve the sensitivity of the method. This is primarily interesting for multi-residue analytical methods as described in this study. The simplified purification step of the IS ery-methyloxime may increase its attraction, as alternative to expensive isotope-labeled compounds. In contrast to other comparative studies, six SPE sorbents were tested considering their specific surface areas. As the specific surfaces of commercially available sorbents differ, the approach applied here allows a more realistic comparison of different sorbents. With respect to TOC and ionic strength, three distinct water-types were analyzed in order to demonstrate the applicability of the method. Expanded uncertainties were comparable between samples over wide concentration ranges. Analyte concentrations were found within the typical range of the respective sample matrices.

2.5 Acknowledgments

This study was funded by the European Commission (contract number 518118-1, GABARDINE, "Groundwater Artificial Recharge Based on Alternative Sources of Water: Advanced Integrated Technologies and Management"), and by the German Federal Ministry of Education and Research (promotional reference No. 02WM0802, SMART, "Sustainable and Integral Management of Available Water Resources Using Innovative Technologies").

KSK-Pharma (Berghausen, Germany) supported this research with cetirizine and loratadine, Bayer (Leverkusen, Germany) with iopamidol and iopromide and Bracco Imaging (Konstanz, Germany) with iomeprol. We thank Ulf Diederichsen and Olaf Senge for their support and ideas during the synthesis and purification of (E)-9-[O-(2-methyloxime)]-erythromycin.

2.6 References

Antignac, J.-P., de Wasch, K., Monteau, F., De Brabander, H., Andre, F., Le Bizec, B. 2005. The ion suppression phenomenon in liquid chromatography–mass spectrometry and its consequences in the field of residue analysis. Analytica Chimica Acta 529 (1–2), 129–136.

Ardrey, B., 2003. Liquid chromatography – mass spectrometry: an introduction. John Wiley & Sons Ltd, The Atrium, Southern Gate, Chichester, West Sussex PO19 8SQ, England.

Beausse, J., 2004. Selected drugs in solid matrices: A review of environmental occurrence, determination and properties of principal substances, Energy Research Centre of the

Netherlands. http://www.ecn.nl/docs/society/horizontal/hor_desk_26_pharmaceuticals.pdf - Accessed 27 September 2011.

Bester, K., 2008. Quantification with HPLC-MS/MS for environmental issues: quality assurance and quality assessment. Analytical and Bioanalytical Chemistry 391 (1), 15–20.

Bester, K., Bordin, G., Rodriguez, A., Schimmel, H., Pauwels, J., VanVyncht, G., 2001. How to overcome matrix effects in the determination of pesticides in fruit by HPLC-ESI-MS-MS. Fresenius Journal of Analytical Chemistry 371 (4), 550–555.

Bester, K., Hühnerfuss, H., 1993. Triazines in the Baltic and North Sea. Marine Pollution Bulletin 26 (8), 423–427.

Bester, K., Theobald, N., 2000. Results of non target screening of lipophilic organic pollutants in the German Bight V: Xanthen-9-one. Water Research 34 (8), 2277–2282.

Buerge, I.J., Poiger, T., Müller, M.D., Buser, H.-R., 2006. Combined sewer overflows to surface waters detected by the anthropogenic marker caffeine. Environmental Science and Technology 40 (13), 4096–4102.

Buser, H.-R., Poiger, T., Müller, M.D., 1998. Occurrence and fate of the pharmaceutical drug diclofenac in surface waters: Rapid photodegradation in a lake. Environmental Science and Technology 32 (22), 3449–3456.

Busetti, F., Linge, K.L., Heitz, A., 2009. Analysis of pharmaceuticals in indirect potable reuse systems using solid-phase extraction and liquid chromatography-tandem mass spectrometry. Journal of Chromatography A 1216 (31), 5807–5818.

Cappiello, A., Famiglini, G., Palma, P., Pierini, E., Termopoli, V., Trufelli, H., 2008. Overcoming matrix effects in liquid chromatography-mass spectrometry. Analytical Chemistry 80 (23), 9343–9348.

European Commision (2002). Commision Decision 2002/657/EC implementing Council Directive 96/23/EC concerning performance of analytical methods and the interpretation of results. Official Journal of the European Community L221/8.

Gao, F., Zhang, M., Cui, X., Wang, Z., Sun, Y., Gu, J., 2010. Simultaneous quantitation of hydrochlorothiazide and metoprolol in human plasma by liquid chromatography-tandem mass spectrometry. Journal of Pharmaceutical and Biomedical Analysis 52 (1), 149–154.

Giger, W., Schaffner, C., Kohler, H.-P.E., 2006. Benzotriazole and tolyltriazole as aquatic contaminants. 1. Input and occurrence in rivers and lakes. Environmental Science and Technology 40 (23), 7186–7192.

Godfrey, E., Woessner, W.W., Benotti, M.J., 2007. Pharmaceuticals in on-site sewage effluent and ground water, Western Montana. Ground Water 45 (3), 263–271.

Gómez, M.J., Martínez Bueno, M.J., Lacorte, S., Fernández-Alba, A.R., Agüera, A., 2007. Pilot survey monitoring pharmaceuticals and related compounds in a sewage treatment plant located on the Mediterranean coast. Chemosphere 66 (6), 993–1002.

Gómez, M.J., Petrović, M., Fernández-Alba, A.R., Barceló, D., 2006. Determination of pharmaceuticals of various therapeutic classes by solid-phase extraction and liquid chromatography–tandem mass spectrometry analysis in hospital effluent wastewaters. Journal of Chromatography A 1114 (2), 224–233.

Gros, M., Petrović, M., Barceló, D., 2006. Multi-residue analytical methods using LC-tandem MS for the determination of pharmaceuticals in environmental and wastewater samples: a review. Analytical and Bioanalytical Chemistry 386 (4), 941–952.

Hanocq, M., Croisier, P., van Damme, M., Aelvoet, C., 1989. Macro ionization constants of hydroxyzine, cetirizine and an analog. Analytical Letters 22 (1), 117–140.

Hassanzadeh, A., Barber, J., Morris, G.A., Gorry, P.A., 2007. Mechanism for the degradation of erythromycin A and erythromycin A 2'-ethyl succinate in acidic aqueous solution. Journal of Physical Chemistry A 111 (40), 10098–10104.

Hirsch, R., Ternes, T., Haberer, K., Kratz, K.-L., 1999. Occurrence of antibiotics in the aquatic environment. Science of the Total Environment 225 (1–2), 109–118.

Kim, S.D., Cho, J., Kim, I.S., Vanderford, B.J., Snyder, S.A., 2007. Occurrence and removal of pharmaceuticals and endocrine disruptors in South Korean surface, drinking, and waste waters. Water Research 41 (5), 1013–1021.

Kiss, A., Fries, E., 2009. Occurrence of benzotriazole in the rivers Main, Hengstbach, and Hegbach (Germany). Environmental Science and Pollution Research 16 (6), 702–710.

Kolpin, D.W., Furlong, E.T., Meyer, M.T., Thurman, E.M., Zaugg, S.D., Barber, L.B., Buxton, H.T., 2002. Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999–2000: A national reconnaissance. Environmental Science and Technology 36 (6), 1202–1211.

Konieczka, P., Namieśnik, J., 2010. Estimating uncertainty in analytical procedures based on chromatographic techniques. Journal of Chromatography A 1217 (6), 882–891.

Kosonen, J., Kronberg, L., 2009. The occurrence of antihistamines in sewage waters and in recipient rivers. Environmental Science and Pollution Research 16 (5), 555–564.

Mallet, C.R., Lu, Z., Mazzeo, J.R., 2004. A study of ion suppression effects in electrospray ionization from mobile phase additives and solid-phase extracts. Rapid Communications in Mass Spectrometry 18 (1), 49–58.

Marín, J.M., Gracia-Lor, E., Sancho, J.V., López, F.J., Hérnandez, F., 2009. Application of ultra-high-pressure liquid chromatography–tandem mass spectrometry to the determination of multi-class pesticides in environmental and wastewater samples. Study of matrix effects. Journal of Chromatography A 1216 (9), 1410–1420.

Matuszewski, B.K., Constanzer, M.L., Chavez-Eng, C.M., 2003. Strategies for the assessment of matrix effect in quantitative bioanalytical methods based on HPLC-MS/MS. Analytical Chemistry 75 (13), 3019–3030.

Maurer, M., Escher, B.I., Richle, P., Schaffner, C., Alder, A.C., 2007. Elimination of β-blockers in sewage tretment plants. Water Research 41 (7), 1614–1622.

Paesen, J., Khan, K., Roets, E., 1995. Study of the stability of erythromycin in neutral and alkaline solutions by liquid chromatography on poly(styrene-divinylbenzene). J. Hoogmartens, International Journal of Pharmaceutics 113 (2), 215–222.

Pedrouzo, M., Reverté, S., Borrull, F., Pocurull, E., Marcé, R.M., 2007. Pharmaceutical determination in surface and wastewaters using high-performance liquid chromatography-(electrospray)-mass spectrometry. Journal of Separation Science 30 (3), 297–303.

Pérez, S., Barceló, D., 2007. Fate and occurrence of X-ray contrast media in the environment. Analytical and Bioanalytical Chemistry 387 (5), 1235–1246.

Petrović, M., Hernando, M.D., Díaz-Cruz, M.S., Barceló, D., 2005. Liquid chromatography-tandem mass spectrometry for the analysis of pharmaceutical residues in environmental samples: a review. Journal of Chromatography A 1067 (1–2), 1–14.

Pichon, V., Cau Dit Coumes, C., Chen, L., Guenu, S., Hennion, M.-C., 1996. Simple removal of humic and fulvic acid interferences using polymeric sorbents for the simultaneous solid-phase extraction of polar acidic, neutral and basic pesticides. Journal of Chromatography A 737 (1), 25–33.

Rodil, R., Quintana, J.B., López-Mahía, P., Muniategui-Lorenzo, S., Prada-Rodríguez, D., 2009. Multi-residue analytical method for the determination of emerging pollutants in water by solid-phase extraction and liquid chromatography–tandem mass spectrometry. Journal of Chromatography A 1216 (14), 2958–2969.

Schlüsener, M.P., Bester, K., Spiteller, M., 2003. Determination of antibiotics such as macrolides, ionophores and tiamulin in liquid manure by HPLC-MS/MS. Analytical and Bioanalytical Chemistry 375 (7), 942–947.

Schwarzenbach, R.P., Escher, B.I., Fenner, K., Hofstetter, T.B., Johnson, C.A., von Gunten, U., Wehrli, B., 2006. The challenge of micropollutants in aquatic systems. Science 313 (5790), 1072–1077.

Sontheimer, H., Frick, B., Fettig, J., Hörner, G., Hubele, C., Zimmer, G., 1985. Adsorptionsverfahren zur Wasserreinigung, Karlsruhe: DVGW-Forschungsstelle am Engler-Bunte-Institut der Universität Karlsruhe.

Takino, M., Daishima, S., Yamaguchi, K., 2000. Determination of nonylphenol ethoxylate oligomers by liquid chromatography-electrospray mass spectrometry in river water and non-ionic surfactants. Journal of Chromatography A 904 (1), 65–72.

Ternes, T., 2007. The occurrence of micopollutants (sic!) in the aquatic environment: A new challenge for water management. Water Science and Technology 55 (12), 327–332.

Ternes, T.A., 1998. Occurrence of drugs in German sewage treatment plants and rivers. Water Research 32 (11), 3245–3260.

Ternes, T.A., Hirsch, R., 2000. Occurrence and behavior of X-ray contrast media in sewage facilities and the aquatic environment. Environmental Science and Technology 34 (13), 2741–2748.

Thurman, E.M., Meyer, M.T., Mills, M.S., Zimmerman, L.R., Perry, C.A., 1994. Formation and transport of deethylatrazine and deisopropylatrazine in surface water. Environmental Science and Technology 28 (13), 2267–2277.

Townsend, M.A., Young, D.P., 2000. Atrazine and its Metabolites as Indicators of Stream-aquifer Interaction in Kansas, USA. International Journal of Environmental Ananytical Chemistry 78 (1), 9–23.

Tutunji, M.F., Ibrahim, H.M., Khabbas, M.H., Tutunji, L.F., 2009. Simultaneous determination of bisoprolol and hydrochlorothiazide in human plasma by HPLC coupled with tandem mass spectrometry. Journal of Chromatography B 877 (16–17), 1689–1697.

Vanderford, B.J., Pearson, R.A., Rexing, D.J., Snyder, S.A., 2003. Analysis of endocrine disruptors, pharmaceuticals, and personal care products in water using liquid chromatography/tandem mass spectrometry. Analytical Chemistry 75 (22), 6265–6274.

Voutsa, D., Hartmann, P., Schaffner, C., Giger, W., 2006. Benzotriazoles, alkylphenols and bisphenol A in municipal wastewaters and in the Glatt River, Switzerland. Environmental Science and Pollution Research 13 (5), 333–341.

Weigel, S., Berger, U., Jensen, E., Kallenborn, R., Thoresen, H., Hühnerfuss, H., 2004. Determination of selected pharmaceuticals and caffeine in sewage and seawater from Tromsø/Norway with emphasis on ibuprofen and its metabolites. Chemosphere 56 (6), 583–592.

Weigel, S., Kuhlmann, J., Hühnerfuss, H., 2002. Drugs and personal care products as ubiquitous pollutants: occurrence and distribution of clofibric acid, caffeine and DEET in the North Sea. Science of the Total Environment 295 (1–3), 131–141.

Wick, A., Fink, G., Ternes, T.A., 2010. Comparison of electrospray ionization and atmospheric pressure chemical ionization for multi-residue analysis of biocides, UV-filters and benzothiazoles in aqueous matrices and activated sludge by liquid chromatography-tandem mass spectrometry. Journal of Chromatography A 1217 (14), 2088–2103.

Yang, W., Feng, C., Kong, D., Shi, X., Zheng, X., Cui, Y., Liu, M., Zhang, L., Wang, Q., 2010. Simultaneous determination of 15 components in Radix Glehniae by high performance liquid chromatography—electrospray ionization tandem mass spectrometry. Food Chemistry 120 (3), 886–894.

Yoon, Y., Ryu, J., Oh, J., Choi, B.-G., Snyder, S.A., 2010. Occurrence of endocrine disrupting compounds, pharmaceuticals, and personal care products in the Han River (Seoul, South Korea). Science of the Total Environment 408 (3), 636–643.

Zuccato, E., Calamari, D., Natangelo, M., Fanelli, R., 2000. Presence of therapeutic drugs in the environment. Lancet 355 (9217), 1789–1790.

Chapter 3

3 A case study on the correlation of micro-contaminants and potassium in the Leine River (Germany)

Karsten Nödler[1], Tobias Licha[1], Steffi Fischer[1], Bianca Wagner[1], Martin Sauter[1]

Citation: Nödler, K., Licha, T., Fischer, S., Wagner, B., Sauter, M., 2011. A case study on the correlation of micro-contaminants and potassium in the Leine River (Germany). Applied Geochemistry 26 (12), 2172–2180.

http://dx.doi.org/10.1016/j.apgeochem.2011.08.001

[1] Department Applied Geology, Geoscience Center of the University of Göttingen, Goldschmidtstr. 3, 37077 Göttingen, Germany

Abstract

River monitoring with focus on the correlation of 41 micro-contaminants with potassium (K^+) and its temporal and spatial variation was carried out at the Leine River (Germany). A positive correlation of concentrations of wastewater-related micro-contaminants and K^+ is to be expected in receiving waters of wastewater treatment plant (WWTP) effluent since urine is a significant source of K^+ in WWTP effluent. This correlation was found for compounds, which meet the following criteria: 1) WWTP effluent is the dominating source of the compound 2) Variability of its mass flux in the WWTP is negligible and 3) The compound is persistent in WWTPs and in the environment. The excellent positive correlation of carbamazepine with K^+ resulted in the fitting of a universal linear equation to the summer and winter data. For almost all other correlating compounds (1H-benzotriazole, citalopram, diclofenac, metoprolol, sotalol, sulfamethoxazole and tolyltriazole), slopes of the line fittings were steeper in winter (x-axis: K^+, y-axis: respective micro-contaminant). This has been attributed to a presumed lower degree of degradation and attenuation within WWTPs and in the environment due to low temperatures as well as an increase in consumption of these compounds in the winter months. As part of this research, a sampling event along the entire flow path of the Leine River (~280 km) was conducted to identify compounds demonstrating stable ratios of various respective micro-contaminants with K^+. Among other compounds, carbamazepine, sulfamethoxazole, and tolyltriazole demonstrated the best correlations with $R^2 > 0.89$. K^+-equivalents of the individual micro-contaminants depended on land use and population structure of the investigated river section.

3.1 Introduction

During the last two decades micro-contaminants such as pharmaceuticals, pesticides, and their respective metabolites have been detected in virtually all parts of the aquatic environment, including surface waters (Ternes, 1998; Schwarzenbach *et al.*, 2006; Ternes, 2007). For this reason, these compounds are currently receiving more and more attention regarding their sources, occurrence, fate, and effects in the environment (Ternes, 1998; Kolpin *et al.*, 2002; Schwarzenbach *et al.*, 2006; Wolf *et al.*, 2006; Ternes, 2007; Katz and Griffin, 2008; Sacher *et al.*, 2008; Daneshvar, 2010a; Daneshvar, 2010b; Wittmer *et al.*, 2010). A large variety of compounds cannot be completely eliminated by conventional wastewater treatment technology and are introduced into the aquatic environment (Reemtsma *et al.*, 2006; Maurer *et al.*, 2007; Choi *et al.*, 2008). Due to their high persistency in wastewater treatment plants (WWTP) and in the aqueous environment, micro-contaminants such as the antiepileptic carbamazepine were identified as suitable compounds for the quantification of wastewater in lakes and groundwater (Clara *et al.*, 2004; Kahle *et al.*, 2009; Gasser *et al.*, 2010).

Regarding wastewater, human urine is an important source of potassium (K^+) (Jönsson *et al.*, 1997). This was further demonstrated by a study from Eiswirth and Hötzl (1997) who linked elevated concentrations of K^+ in groundwater to leaking subsurface sewers. For the same reason, and as K^+ is not eliminated in a WWTP, effluents from municipal WWTPs may be significant K^+-sources for the respective effluent-receiving surface waters (i.e. rivers). Therefore, in rivers with WWTP effluents as significant K^+-sources, K^+-concentration should correlate with WWTP persistent micro-contaminant concentrations. Thus, it is less relevant if micro-contaminants originate from human excrements but more important that these micro-contaminants discharge at significant concentrations to a municipal WWTP together with human excrements. In addition, concentrations of compounds from sources other than WWTP effluents such as agricultural herbicides and substances, which are readily biodegradable in WWTPs and environment, should not correlate with K^+.

The focus of the study presented here is to investigate the correlation quality of K^+ with a large number of micro-contaminants present in the Leine River basin (Thuringia and Lower Saxony, Germany). Sampling events were carried out with a focus on temporal and spatial variability of these correlations. Major anions, cations, and 41 micro-contaminants covering a broad spectrum of different applications were analyzed in more than 200 samples.

3.2 Materials and methods

3.2.1 Sampling

3.2.1.1 Correlation between micro-contaminants and K^+ in the river basin

One sampling event was carried out in June 2009, covering nearly the whole length of the river (~280 km). 14 sampling locations were selected (L01–L14), starting at the spring and ending close to the junction with the Aller River north of Hanover (Figure 3.1). Both riverbanks were sampled individually at each location except for L09. In total, 27 samples were taken. With respect to micro-contaminants, 0.5 liter bottles (clear glass and screw cap) were used for sampling. For major ions, samples were taken in 20 mL (HD-PE) bottles.

3.2.1.2 Temporal and seasonal variation of the correlation between micro-contaminants and K^+

Two sampling events were carried out in June/July 2009 and January 2010, covering a river section of 11 km. Three equidistant sampling locations (G1–G3) were selected (Figure 3.1). A sampling frequency of 24 h was applied for 13 and 14 days in summer and winter, respectively. Grab samples were taken at 1.5 meters distance from the riverbank and at each location, with both riverbanks sampled individually. Between G2 and G3 the treated effluent of the municipal WWTP of Göttingen (~120,000 inhabitants, Figure 3.1) was being introduced 4 km upstream of G3. The WWTP and sampling location G2 are located within the city limits of Göttingen. The treated effluent was sampled with the same frequency as the river. 189 samples were taken in total. Sampling procedure was as described in the previous section.

The Göttingen WWTP consists of mechanical treatment for the separation of solid material (i.e. a grit, fat separator, and a primary settler) followed by activated sludge treatment, including nitrification and denitrification. Additionally, chemically facilitated P-removal is performed. Under dry weather discharge conditions, the mean hydraulic residence time is approximately 20–24 h.

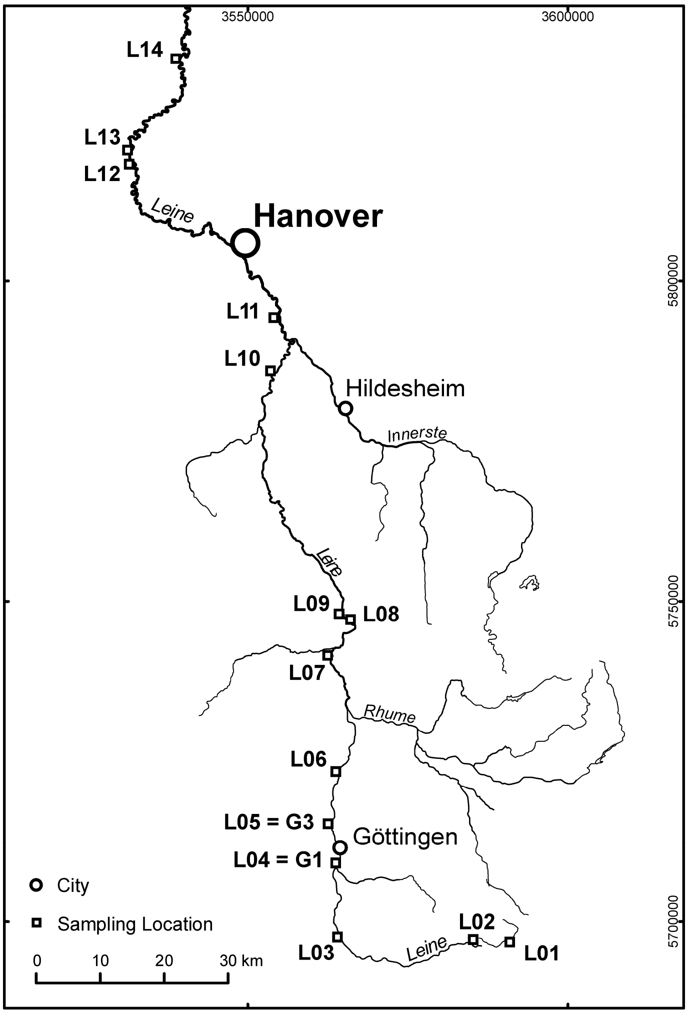


Figure 3.1: Leine River with sampling locations and the most significant confluences and cities. Additional sampling locations specific to the study on temporal and seasonal variations of the correlation between micro-contaminants and K$^+$ (G2 and the respective WWTP) are located within the city limits of Göttingen).

3.2.2 Chemical Analysis

3.2.2.1 Organic micro-contaminants

A multi-residue analytical method based on solid phase extraction (SPE) and high-performance liquid chromatographic separation with tandem mass spectrometric detection (HPLC-MS/MS) was used for the analysis of 41 organic micro-contaminants. Details have been published previously (Nödler *et al.*, 2010). Sample volumes of 500 and 100 mL were used for river water and treated effluent, respectively. All analytes and their applications are shown in Table 3.1. Method quantitation limits (MQL) ranged from 1.2 to 19 ng L^{-1} in river water and from 5.0 to 160 ng L^{-1} in treated effluent.

3.2.2.2 Ion chromatography

Prior to ion chromatography (IC), samples were filtered (Whatman Anotop 10 IC, 0.2 µm). For the determination of cations (Na^+, K^+, Mg^{2+}, Ca^{2+}), the analytical system consisted of a Dionex DX-500 with conductometric detection, a Dionex IonPac CS16 (3x250 mm) column and a 35 mM methanesulfonic acid eluent (isocratic separation at 44 °C). For the determination of anions (Cl^-, NO_3^-, SO_4^{2-}), a Dionex DX-320 with conductometric detection, a Dionex AS11-HC (2×250 mm) column, a 23 mM KOH eluent (isocratic separation at 30 °C), and a flow rate of 0.38 mL min^{-1} were used.

Table 3.1: Analytes and their applications.

Application	Compound	Application	Compound
Analgesics/anti-inflammatories	Diclofenac	Lipid regulators	Bezafibrate
	Ibuprofen		Clofibric acid
	Naproxen		Gemfibrozil
	Paracetamol	Antiallergics	Cetirizine
	Phenazone		Loratadine
Stimulants/caffeine metabolites	Caffeine	Anticonvulsants/sedatives	Carbamazepine
	Paraxanthine		Diazepam
	Theobromine		Primidone
	Theophylline		Tetrazepam
Antihypertensives	Atenolol	Antidepressant	Citalopram
	Metoprolol	Herbicides/herbicide metabolites	Atrazine
	Sotalol		Desethylatrazine
Iodinated contrast media	Iohexol		Desisopropylatrazine
	Iomeprol		Diuron
	Iopamidol		Isoproturon
	Iopromide		Mecoprop
Antibiotics	Clarithromycin		Metazachlor
	Erythromycin	Corrosion inhibitors	1H-benzotriazole
	Roxithromycin		Tolyltriazole
	Sulfamethoxazole	Cocaine metabolite	Benzoylecgonine
	Trimethoprim		

3.3 Results and discussion

3.3.1 Temporal and seasonal variation of the correlation between micro-contaminants and K^+

The summer and winter sampling events covering a river section of 11 km were performed in order to identify compounds with significant K^+-correlation with respect to the seasons. Due to snowmelt and rain events, significant amounts of road salt were introduced into the river during the second week of the winter sampling event. A significant correlation of K^+ with Na^+ and Cl^-, which was not pronounced at this river section under normal flow conditions, proved that this data must be excluded from the correlation experiments. The K^+-concentration in summer ranged from 3.3–4.3 mg L^{-1} in the river water upstream of the first major WWTP (Göttingen) and the range downstream of the same WWTP was 5.2–7.4 mg L^{-1}. In winter, the results were 2.7–3.3 mg L^{-1} and 4.5–5.2 mg L^{-1}, respectively and exclusive of the snowmelt event. Concentrations of K^+ in the Göttingen WWTP effluent ranged from 29–36 mg L^{-1} and 24–29 mg L^{-1} in summer and winter, respectively.

For increased significance and robustness of the outcome of this research effort, only the micro-contaminants which had detection frequencies >50% in this section of the Leine River (sum of locations G1–G3) in at least one season were subject to analysis with respect to the correlation with K^+. All the correlations of micro-contaminant and K^+, which obtained squared correlation coefficients (R^2) of > 0.8 are shown in Table 3.2. Table 3.3 presents the minimum, maximum, and median concentrations of the respective compounds and their detection frequencies during the summer event and the first week of the winter sampling event, respectively.

Table 3.2: Slope (m), intercept (b), and squared correlation coefficient (R^2) of selected compounds (y-axis) and K^+-concentration (x-axis) as monitored on a selected section of the Leine River (11 km, Figure 3.1: G1–G3). Summer and winter data of compounds, which demonstrated R^2 > 0.8 and a detection frequency >50% in at least one season.

	Summer			Winter		
Compound	m[a]	b[b]	R^2	m	b	R^2
1H-benzotriazole	133	−401	0.863	307	−840	0.972
Carbamazepine	65	−148	0.950	65	−157	0.988
Cetirizine	15	−53	0.970	-[c]		
Citalopram	11	−35	0.830	22	−63	0.899
Diclofenac	40	−134	0.934	42	−110	0.961
Metoprolol	72	−240	0.933	102	−255	0.937
Primidone	215	−836	0.586[d]	11	−27	0.972
Sotalol	60	−211	0.964	70	−201	0.974
Sulfamethoxazole	20	−47	0.650	33	−106	0.868
Tolyltriazole	113	−279	0.930	190	−471	0.970

[a] m in (ng L^{-1}) (mg L^{-1})$^{-1}$.

[b] b in ng L^{-1}.

[c] Detection frequency of cetirizine in winter <50%.

[d] Bad correlation possibly due to disposal of a primidone-containing preparation (see discussion).

Table 3.3: The minimum, maximum, and median concentrations and the detection frequencies of micro-contaminants correlating with K^+-concentration ($R^2 > 0.8$ and detection frequency >50% in at least one season) as monitored on a selected section of the Leine River (11 km, Figure 3.1: G1–G3). Data of the summer sampling event (S) and of the first week of the winter sampling event (W). Two sampling locations upstream and one sampling location downstream the Göttingen WWTP.

		Upstream WWTP				Downstream WWTP			
Compound		Min [ng L^{-1}]	Max [ng L^{-1}]	Median [ng L^{-1}]	DF [%][c]	Min [ng L^{-1}]	Max [ng L^{-1}]	Median [ng L^{-1}]	DF [%][d]
1H-benzotriazole	S [a]	34	176	102	100	248	733	408	100
	W [b]	69	132	89	100	480	845	612	100
Carbamazepine	S	64	144	98	100	163	346	261	100
	W	32	50	39	100	129	185	157	100
Cetirizine	S	<2.2	7.0	3.8	98	19	57	43	100
	W	<2.2	<2.2	<2.2	0	4.9	16	6.7	100
Citalopram	S	<3.2	43	4.3	79	20	44	33	100
	W	<3.2	17	5.1	61	32	60	43	100
Diclofenac	S	6.4	47	14	100	69	176	123	100
	W	8.6	23	19	100	69	127	93	100
Metoprolol	S	21	63	32	100	136	338	222	100
	W	32	154	55	100	182	309	228	100
Primidone	S	<2.7	12	7.3	87	43	1460	218	100
	W	2.9	8.9	5.6	100	22	29	24	100
Sotalol	S	5.4	31	16	100	99	220	188	100
	W	8.0	15	11	100	94	181	129	100
Sulfamethoxazole	S	13	36	18	100	53	178	106	100
	W	5.2	60	9.1	100	31	71	40	100
Tolyltriazole	S	77	258	146	100	295	644	413	100
	W	109	167	126	100	419	575	474	100

[a] S: Summer data.

[b] W: Winter data.

[c] Detection frequency (DF) Related to a total number of 52 (S) and 28 (W) samples.

[d] DF related to a total number of 26 (S) and 14 (W) samples.

3.3.1.1 Correlation of micro-contaminants and K^+ during a season

As hypothesized, concentrations of persistent micro-contaminants in the Leine River, which can be directly attributed to WWTP effluent, correlated very well with K^+-concentrations (Table 3.2). However, compounds such as the iodinated contrast media (ICM) did not demonstrate this correlation although they meet the mentioned criteria. ICM concentrations followed strong weekly cycles in the Göttingen WWTP effluent according to the examination times in hospitals and radiological practices. Ternes and Hirsch (2000) recorded similar observations. Obviously, a constant mass flux of the compound in WWTPs is an additional prerequisite for the respective micro-contaminant correlating with K^+.

Kreuzinger (2008) observed very low weekly dynamics of concentrations of carbamazepine and diclofenac in a WWTP. Both compounds are part of a classical chemical therapy of chronic diseases such as neurological disorders (carbamazepine) and rheumatoid arthritis (diclofenac), which require a daily intake of the active ingredient (Espir and Millac, 1970; Brogden *et al.*, 1980). This explains the low variation of these two compounds in the Göttingen WWTP.

As mass flux of urine-related K^+ may also be regarded as being relatively constant, the good correlation of carbamazepine and diclofenac with K^+ can be explained along this line of reasoning.

The same applies to the antihypertensives sotalol and metoprolol. Elimination rates of <40% in different WWTPs were observed by Maurer *et al.* (2007) and Scheurer *et al.* (2010). Atenolol was also expected to show the correlation with K^+. However, its detection frequency in the Leine River did not meet the preconditions, which can be explained by its higher elimination rates in WWTPs and a comparatively lower consumption in Germany (Maurer *et al.*, 2007; Sadezky *et al.*, 2008; Scheurer *et al.*, 2010).

Benzotriazoles such as 1H-benzotriazole (BTri) and tolyltriazole (TTri) do not belong to any class of pharmaceuticals and their concentrations are independent of human excretion. However, they are widely used as anticorrosives in many industrial applications, as well as in deicing/antiicing fluids and in detergents for household dishwashers (Giger *et al.*, 2006; Weiss *et al.*, 2006; Reemtsma *et al.*, 2010). Therefore, these compounds are typically continuously introduced into municipal WWTPs and due to their high persistence they are frequently detected in WWTP effluents, rivers, and even in coastal seawater (Giger *et al.*, 2006; Voutsa *et al.*, 2006; Nödler *et al.*, 2010; Reemtsma *et al.*, 2010). Emissions of BTri are expected to be constant over time if they can be attributed solely to dishwashing activities (Kiss and Fries, 2009). However, in our study concentrations of BTri demonstrated a clear weekly cycle with minimum concentration on Sundays and Mondays in the Göttingen WWTP effluent. Taking into account the mean residence time of wastewater in this WWTP (20–24 h), a significant input from industrial application is strongly indicated. However, compared to the contrast media, the weekly cycle of BTri was less pronounced and a reasonable correlation with K^+-concentration was demonstrated. A weekly cycle of TTri was only slightly apparent.

Among the correlating compounds, the antidepressant citalopram and the antiallergic cetirizine exhibited the lowest median concentrations in all sample matrices (Table 3.3). Median summer concentration of citalopram in the Göttingen WWTP effluent (300 ng L^{-1}) was in accordance with findings of Himmelsbach *et al.* (2006) in several Austrian WWTP effluents. In contrast to cetirizine, citalopram concentrations were highly varied in the Leine River upstream of the Göttingen WWTP, which can explain the comparatively poor R^2 of the fitting linear equation. The antiallergic drug cetirizine is used for treatment of allergic rhinoconjunctivitis ('hay fever') and during allergen exposure, a continuous treatment is preferred instead of 'treatment on demand' (Ciprandi *et al.*, 1997). Kosonen and Kronberg (2009) observed very low elimination and attenuation rates of cetirizine in WWTPs and in the aquatic environment, which is in line with the exceptional R^2 of 0.97 in summer presented in this study. The concentrations of sulfamethoxazole correlated moderately well with K^+-concentrations. Haggard and Bartsch (2009) demonstrated the high persistence of sulfamethoxazole in river water.

A unique case is the relatively low squared correlation coefficient of primidone in summer ($R^2 = 0.586$). This compound is known to be highly persistent during wastewater treatment and bank filtration (Kahle *et al.*, 2009). Furthermore, its scope of application (anticonvulsant) includes a continuous treatment and this compound is expected to show a relatively high positive correlation with K^+, as was found during winter. However, during the summer sampling event, a sudden peak concentration of 15–20 µg L^{-1} was detected in the Göttingen WWTP effluent resulting in a 10-fold higher concentration in the Leine River than usual. Such high concentrations and sudden appearances can hardly be achieved by an appropriate consumption of the compound. Once opened, the liquid formulation of primidone Liskantin® is only stable for the duration of three months. After its use-by date, it is possible that the leftover was disposed off into the sewage system. Accordingly, Bound and Voulvoulis (2005) demonstrated that the direct disposal of unused pharmaceuticals via the sink or toilet might be an underestimated route of pharmaceuticals into the aquatic environment. Of course, this may not solely apply to primidone. However, the total annual consumption of primidone in Germany is at least one order of magnitude lower than for most of the other correlating compounds presented in Table 3.2 (Sadezky *et al.*, 2008; Schwabe and Paffrath, 2009). Therefore, during the summer sampling event the concentration of primidone in the 11 km section of the Leine River was more subject to the occasional disposal of primidone via the toilet or sink than the more frequently used compounds such as carbamazepine or metoprolol, in turn affecting the consistency of the correlation of primidone with K^+.

Caffeine, paraxanthine, theobromine and theophylline were detected in almost 100% of the river samples but at no time in the Göttingen WWTP effluent, indicating the input of untreated sewage into the river (Buerge *et al.*, 2006). Due to the degradable nature of the compounds, a correlation with K^+ was neither expected, nor observed.

Compounds with sources typically other than WWTPs showed no correlation with K^+-concentration. This was observed for herbicides and herbicide metabolites. Upstream of Göttingen, the river basin is dominated by agricultural areas. In catchments of mixed land use, such as this, the input of selected biocidal products into surface waters via urban areas is at least as significant as those from agricultural origin (Wittmer *et al.*, 2010). With the exception of desethylatrazine, a rapid occurrence of herbicide-related compounds in the Leine River was positively correlated with the previously mentioned precipitation and snowmelt event during the winter sampling period. In contrast to atrazine, its metabolite desethylatrazine was detected in all river samples (2.6–8.3 ng L^{-1}). Desethylatrazine concentration in the respective river section was negatively correlated with river discharge, which indicates the dilution of a diffusive input from former applications of atrazine within the catchment (Adams and Thurman, 1991).

3.3.1.2 Correlation of micro-contaminants and K^+ between seasons

The correlation of K^+ with carbamazepine was the only positive correlation with a universal linear equation across the seasons. The striking total correlation of carbamazepine in comparison with tolyltriazole is shown in Figure 3.2. Constant inflow concentrations and almost negligible elimination rates in WWTPs across the seasons can be regarded as the main reasons for this observed time independent correlation (Kreuzinger, 2008; Sacher *et al.*, 2008; Oulton *et al.*, 2010). This reinforces the validity of carbamazepine as a highly promising organic wastewater tracer (Clara *et al.*, 2004; Kahle *et al.*, 2009; Gasser *et al.*, 2010).

Apart from carbamazepine, cetirizine, and primidone, the correlation-slopes (m) of all compounds demonstrated higher values in winter, indicating higher micro-contaminant loads per urine and K^+-unit. As there is no evidence for greater use of antihypertensives during winter, the steeper slopes of sotalol and metoprolol can be attributed to significantly decreased elimination/attenuation at low ambient temperatures (Daneshvar *et al.*, 2010a). The same applies to diclofenac, but to a lesser extent in the presented study (Vieno *et al.*, 2005; Kreuzinger, 2008).

Regarding citalopram, different overlapping effects may be responsible for a steeper slope of the winter K^+-correlation equation. Examples of these overlapping effects are low attenuation at low temperatures and the high likelihood of greater use of antidepressants during the winter (Gardarsdottir *et* *al.*, 2010; Suarez *et al.*, 2010). Suarez *et al.* (2010) demonstrated the influence of temperature on citalopram elimination in WWTPs. However, the observed effect quantified by Suarez *et al.* (2010) was a 4% increase in citalopram elimination. As the effluent concentrations doubled from summer to winter in the present study, a higher consumption of these drugs by the population is likely to be the more significant reason for the large increase of concentration. With respect to sulfamethoxazole the better correlation during winter may be also attributed to a higher and more continuous consumption of the compound.

Cetirizine, which indicated an excellent correlation with K^+-concentration during summer, was detected with a frequency of <50% in winter. As concentrations of antiallergics usually correlate with the outbreak of allergic symptoms during the pollen season, the low detection frequency in winter can clearly be attributed to its scope of application (Kosonen and Kronberg, 2009).

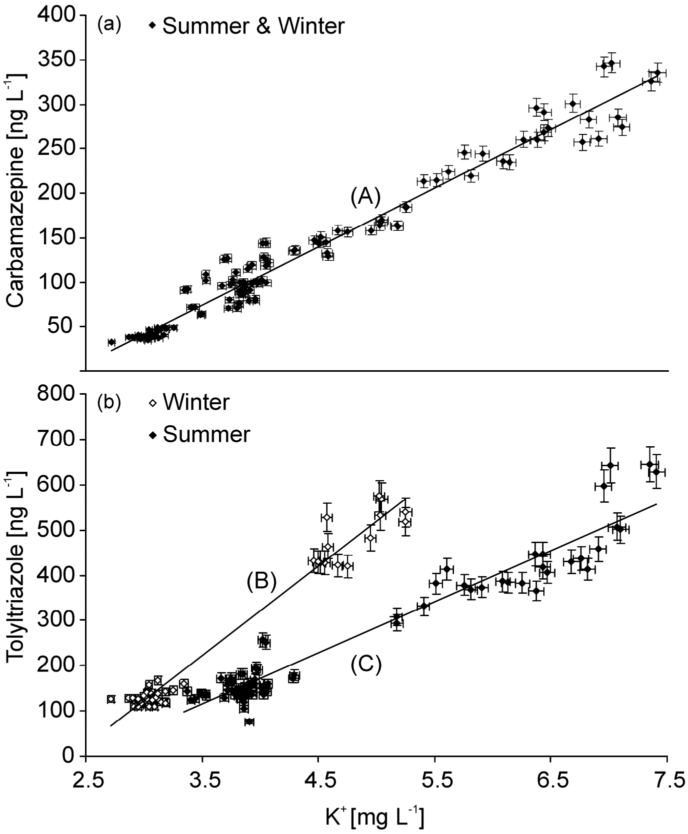


Figure 3.2: Seasonal dependency of the correlation of micro-contaminants with K^+ as monitored on a selected section of the Leine River (11 km, Figure 3.1: G1–G3). Expanded uncertainties of micro-contaminants were calculated according to Konieczka and Namieśnik (2010). Line (A) shows the universal correlation of K^+ with carbamazepine across the seasons ($y=66x-155$, $R^2=0.964$). Lines (B) and (C) show correlations with tolyltriazole in winter and summer, respectively.

Regarding BTri and TTri, the excessive increase of the slope and the decrease of the intercept (b) of the fitting equations for the winter data are remarkable. Benzotriazoles are also used in deicing/antiicing fluids and concentration peaks in surface waters during winter are usually attributed to deicing activity at airports (Giger *et al.*, 2006; Voutsa *et al.*, 2006; Weiss *et al.*, 2006). However, as there are no airports in the catchment, this option as a reason for increasing loads can be excluded. None of the benzotriazoles can be completely removed in WWTPs and removal rates are usually highly variable between different treatment plants (Reemtsma *et al.*, 2010). Technical TTri is a mixture of the 4- and 5-isomer and with the applied analytical method it was not possible to separate both isomers. The 4-TTri is usually much more persistent than the 5-isomer and BTri (Weiss *et al.*, 2006; Reemtsma *et al.*, 2010). Therefore, it can be assumed that in winter reduced biological degradation rates of benzotriazoles mostly affect BTri and 5-TTri. In the presented study, the slopes of BTri and TTri (sum of both isomers) increased by 130% and 68%, respectively, reflecting the negligible influence of temperature on 4-TTri degradation.

3.3.2 Correlation of micro-contaminants and K^+ in the whole river basin

As demonstrated in the previous chapters, a set of compounds has been identified demonstrating a good positive correlation with K^+ along a flow path of the Leine River, which was 11 km and without any tributaries. Another sampling event was also undertaken and was focused on the spatial variation of micro-contaminants and K^+. It was performed in order to identify the compounds, which demonstrate stable ratios of micro-contaminant and K^+ along the whole flow path of the Leine River. In this sampling event, the K^+-concentration varied between 2.6–13.2 mg L^{-1}. The resulting concentration profiles of K^+, carbamazepine, sulfamethoxazole and tolyltriazole are shown in Figure 3.3.

For a better interpretation, it was useful to separate the data into two distinct river sections: river section A (RS.A) from sampling location L01–L10, which covered the section from the river’s spring until the junction with the Innerste River exclusively, and river section B (RS.B) from L11–L14, which covered the section after the junction with the Innerste River almost to the end of the Leine River (Figure 3.1). Correlations of micro-pollutants and K^+ in the respective river sections are shown in Table 3.4. In order to use a significant amount of data, only the micro-contaminants demonstrating a detection frequency >50%

in each river section were analyzed for the correlation. Those obtaining squared correlation coefficients $R^2 > 0.8$ in at least one river section were selected. Table 3.4 also presents the minimum, maximum and median concentrations of the respective compounds and their detection frequencies during the sampling event.

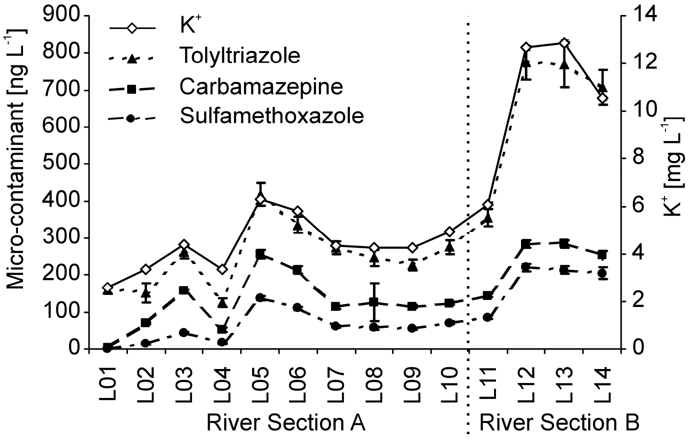


Figure 3.3: Concentrations of K^+, tolyltriazole, carbamazepine, and sulfamethoxazole in the whole Leine River basin. Averaged results of both riverbanks are presented. Expanded uncertainties of micro-contaminants were calculated according to Konieczka and Namieśnik (2010). River sections A and B include sampling locations from L01–L10 and L11–14, respectively. Flow paths of the river sections were 141 and 84 km for A and B, respectively. Maximum concentrations were detected at locations after Göttingen (L05) and Hanover (L12/L13).

As demonstrated, 9 out of 41 micro-contaminants exhibited detection frequencies >50% and a reasonable correlation with K^+ in at least one river section. It was possible to track the ratios of some micro-contaminants and K^+ emitted by the most dominant source in RS.A (WWTP Göttingen) until a more dominant source was involved (WWTPs in Hildesheim and Hanover). This implies that the respective ratios were stable for 90 km, which is noteworthy. However, although a high persistence of the individual compounds is an important prerequisite in order to demonstrate this behavior, it is not possible to conclude that the compounds were not partly degraded along the flow path. Natural waters usually contain K^+ and the inflow of pristine groundwater, brooks, and rivers would have diluted the micro-contaminant concentration to a higher extent than the concentration of K^+. Figure 3.3 clearly indicates several dilution steps of wastewater related compounds. As the correlations still remained valid, the diluting waters have contained a K^+ to micro-contaminant ratio quite similar to the receiving Leine River. The correlation on the long flow path RS.A was best for carbamazepine and sulfamethoxazole. In laboratory scale experiments, Boreen *et al.* (2004) and Matamoros *et al.* (2009)

demonstrated photodegradation half-lives of ≥8 h of these compounds. However, the authors classified the relevance of photodegradation for sulfamethoxazole and carbamazepine in natural waters to be limited by water turbidity, light-attenuation in the upper surface of the water and shading by humic substances. Therefore, a generally high persistence of carbamazepine and sulfamethoxazole in the environment was demonstrated for both compounds (Clara *et al.*, 2004; Haggard and Bartsch, 2009).

Table 3.4: Slope (m), intercept (b), and squared correlation coefficient (R^2) of selected compounds (y-axis) and K^+-concentration (x-axis) in the Leine River. The data set was divided into river section A (L01–L10) and river section B (L11–L14). Data of compounds, which demonstrated $R^2 > 0.8$ in at least one river section and a detection frequency >50% in each river section. The minimum, maximum, and median concentrations and the detection frequencies of micro-contaminants correlating with K^+-concentration in the whole Leine River basin.

	River section A			River section B			Min	Max	Median	DF
Compound	m^a	b^b	R^2	m	b	R^2	[ng L^{-1}]	[ng L^{-1}]	[ng L^{-1}]	[%]^c
Atenolol	13	−45	0.732	3.5	−20	0.958	<3.5	52	13	78
Carbamazepine	63	−150	0.928	21	20	0.982	6.5	285	144	100
Cetirizine	12	−37	0.868	3.4	3.5	0.932	<2.2	47	20	85
Diclofenac	28	−74	0.654	9.8	−34	0.837	<2.0	137	51	93
Mecoprop	−5.1	35	0.378	2.1	−3.2	0.891	<1.2	33	12	93
Metoprolol	59	−140	0.842	24	9.7	0.946	3.5	336	163	100
Sotalol	61	−191	0.792	10	16	0.975	<4.8	223	78	93
Sulfamethoxazole	39	−113	0.956	20	−25	0.926	<2.6	222	61	93
Tolyltriazole	74	−76	0.888	62	−4.9	0.962	118	797	280	100

[a] in (ng L^{-1}) (mg L^{-1})$^{-1}$.

[b] b in ng L^{-1}.

[c] Detection frequency (DF) related to a total number of 27 samples.

Diclofenac, on the other hand, demonstrated a low photodegradation half-life of only < 1 h and photodegradation was identified as its predominant elimination mechanism in lakes (Buser *et al.*, 1998). In the presented study, diclofenac indicated a rather poor correlation in RS.A, which is in perfect accordance with its low half-life.

RS.B begins after the inflow of the Innerste River. The Innerste River is the receiving water body of the city of Hildesheim (~100.000 inhabitants) and the shift of K^+-correlations from RS.A to RS.B can be attributed to the wastewater from Hildesheim. However, as the WWTP-network of Hanover directly discharges its effluents into the Leine River, the wastewater signal and the shift of K^+ correlations were much more evident.

Interestingly, the slopes of all pharmaceutical compounds decreased by at least 50% from RS.A to RS.B. It is unlikely that this could be attributed to significantly better elimination rates in the respective WWTP, as carbamazepine followed the trend of decreasing slopes. Generally, carbamazepine is a highly persistent compound in WWTP (Sadezky *et al.*, 2008). Most likely, the per capita consumption of the respective pharmaceuticals was lower compared to the mostly rural area in RS.A. In order to explain this observation, socioeconomic effects must also be taken into consideration: It is generally accepted, that a considerably increased use of pharmaceuticals is correlated to an increasing number of older people (Kümmerer, 2010). Additionally to a positive migration balance, the area of Hanover has a lower percentage of people older than 55 years. In the area of RS.A the situation was found to be vice versa (Jung *et al.*, 2010). Therefore, as the per capita discharge of K^+ should not be affected, lower K^+-equivalents of pharmaceuticals used in the treatment of age-related chronic diseases such as hypertension were expected and observed in the area of Hanover.

On the other hand, compounds for which the age distribution of populations is only of minor importance, significantly altered slopes were not expected. In fact, the slope of the widely used tolyltriazole only decreased to a minor extent. If attributed to dishwashing activities only, a difference due to a different age distribution would not have been expected. However, as this compound is widely used in different applications, it is just one possible interpretation. Nevertheless, the almost identical curve shapes of K^+ and tolyltriazole concentrations demonstrated in Figure 3.3 are highly impressive. In analogy to the universal correlation of carbamazepine across the seasons, tolyltriazole was the only compound, which demonstrated a universal linear equation throughout the whole river ($y=65x-36$, $R^2 = 0.979$).

In the present study, mecoprop correlated in the urban area RS.B with K^+. This outcome is in accordance with the literature: Beside agricultural applications, mecoprop is widely used as a protective agent for roof material and roof runoff has been identified as a major source of mecoprop into the environment (Bucheli *et al.*, 1998). In a study on the significance of urban and agricultural land use for biocide and pesticide dynamics in waters, Wittmer *et al.* (2010) used mecoprop as a representative compound for urban areas. This implies that the correlation approach with K^+ is useful to identify a WWTP as the dominating source of a contaminant.

3.3.3 Correlation of micro-contaminants and inorganic ions different to K^+

The concentrations of Ca^{2+}, Mg^{2+}, NO_3^- and SO_4^{-2} in the treated effluent of the Göttingen WWTP were equal or even lower than the background concentrations of the ions in the Leine River before the inflow of the WWTP. No correlation of micro-contaminants and these ions was observed in any of the sampling events. Regarding surface waters, domestic wastewater is also a significant source of Na^+ and $Cl^−$ (Massmann *et al.*, 2004). Therefore, a correlation approach with micro-contaminants and these ions may be promising. However, although some compounds demonstrated considerable correlations ($R^2 > 0.8$), the best R^2 were generally obtained with K^+.

Only carbamazepine demonstrated a considerable correlation with Na^+ ($R^2 = 0.91$) during the summer sampling event on the 11 km river section. However, during winter none of the analyzed organic compounds demonstrated a considerable correlation with Na^+ and in contrast to the universal correlation of carbamazepine with K^+ across the seasons, no universal correlation with Na^+ was observed. Regarding a correlation with $Cl^−$ during summer, the R^2 varied from slightly lower to comparable to those with K^+ on the 11 km river section in Göttingen. However, during the winter sampling event only the correlations with metoprolol, carbamazepine and primidone exceeded R^2 of 0.8. Regarding the whole river basin, none of the organic compounds given in Table 3.4 correlated with Na^+ and $Cl^−$.

Boron and anthropogenic gadolinium were identified as conservative wastewater markers (Gäbler and Bahr, 1999; Möller *et al.*, 2000). Initially, a correlation approach of these compounds and organic micro-contaminants was thought to be promising. However, as anthropogenic gadolinium originates from contrast media used for magnetic resonance imaging, a strong weekly cycle in WWTP effluent as demonstrated for the iodinated contrast media is very likely. Therefore, this approach is not practical. A significant proportion of the anthropogenic boron in surface waters originates from bleaching agents in detergents. However, due to technical and environmental developments the strong decreasing European trend of using boron-bearing compounds leads to decreasing boron concentrations in surface waters (Neal *et al.*, 2010). Therefore, a correlation approach with boron might be still promising but, however, not future-oriented. In contrast, the strong benefit of using K^+ for the correlation approach is that the mass flux of urine-borne K^+ in WWTPs is no subject of legal restrictions and, as far as one can discern, technical modifications.

3.3.4 Limitations of the K^+-correlation approach

The approach introduced in this case study is a useful addition to studies proving indicator quality on the basis of wastewater burden (Buerge *et al.*, 2009). However, a prerequisite for the correlation approach with wastewater related K^+ is the exclusion of significant K^+-sources different to WWTPs. Various studies demonstrated the input of soluble and clay-associated K^+ into surface waters by agricultural runoff and peak concentrations of K^+ were observed during storm events (i.e. Walling and Foster, 1975; Sharpley *et al.*, 1988). However, the data sets used for the presented correlation approach were obtained during dry weather periods and potential stormwater related inputs were ruled out with reasonable certainty.

Additional K^+ input due to potash mining or potash industries are serious obstacles for the applicability of the presented correlation approach. However, monitoring of major ions can give indications for an input of wastewaters from potash industries. According to Pöppinghaus *et al.* (1994) $MgCl_2$ and $MgSO_4$ are quantitatively by far the most relevant inorganic wastewater components from potash industry (one order of magnitude higher than K-salts). Therefore, a significant inflow of respective wastewaters can be identified by a strongly increasing Mg^{2+}-concentration relative to the K^+-concentration. This was not observed during the sampling events as Mg^{2+}-concentration increased by only 2.5 mg L^{-1} in the potash industrial area of Hanover. However, an impact cannot be completely excluded but a mining-related K^+-input was obviously not significant for the K^+-balance of the Leine River. In contrast to the Leine River, the Wipper River (Thuringia, Germany) is exemplary for a significant impact of former potash mining. Due to the low average discharge of the Wipper River (< 4 m^3 s^{-1}, one order of magnitude lower than the Leine River), the inflow of drainage water from salt dumps resulted in a severe salinization of the river, including a K^+-concentration of 118 mg L^{-1} in 1998 (Ziemann *et al.*, 2001). Regarding the Wipper River, the presented correlation approach is not feasible because of the insignificance of municipal wastewater with respect to the K^+-balance of the whole river. Summarizing these limitations, outside of storm water events, the approach is supposed to be applicable to any river where WWTPs are a significant K^+-source.

Due to river-specific land uses and background signals of K^+, the linear fits of K^+ and selected micro-contaminant concentrations presented in this study are supposed to be characteristic for the individual river and most likely not directly applicable to other rivers. This was demonstrated by different equations for different river sections of the Leine River.

Untreated wastewater is another possible source of wastewater related compounds different to WWTPs. In untreated wastewater the readily degradable compounds caffeine, paraxanthine, ibuprofen and paracetamol are present at high µg L^{-1} concentrations (Gómez *et al.*, 2007; Choi *et al.*, 2008). Therefore, an inflow of raw sewage into surface waters can be sensitively detected by significantly elevated concentrations of these compounds even after high dilution in the recipient water body. At several sampling locations the comparison of both riverbanks revealed significant concentration differences of i.e. caffeine and paraxanthine depending on the riverbank. Thus, local direct discharges of untreated wastewater were detected. However, regarding the concentrations of the K^+-correlating micro-contaminants at both riverbanks of a certain sampling location, significant differences were exceptional (location L08). Carbamazepine, as an example for a compound correlating with K^+, is usually present at much lower concentrations in raw sewage than caffeine and paracetamol (Gómez *et al.*, 2007; Choi *et al.*, 2008; Kreuzinger, 2008). Thus, small amounts of raw sewage in surface waters do not lead to significantly increased concentrations. The same applies to K^+. Therefore, a significant input of the correlating micro-contaminants into the Leine River via direct discharges of raw sewage was unlikely in the observation period

3.4 Conclusions

An extensive monitoring program on the temporal and spatial correlation quality between 41 micro-contaminants and K^+ in the Leine River clearly demonstrated, that the concentrations of several compounds correlated excellently with K^+-concentration. To the authors' knowledge, no such study has been reported to date. The following preconditions for a micro-contaminant to correlate with K^+ in rivers were derived from this study:

- WWTP effluent is the dominating source of the compound;
- variability of its mass flux in WWTPs is negligible;
- the compound is persistent in WWTPs and in the environment.

On the other hand, an observed correlation of K^+ and a compound of unknown origin and environmental behavior can be used for source-tracking purposes, since the compound obviously fulfills the criteria stated above. Furthermore, there is evidence that the ratios are specific with respect to land use and population structure.

Carbamazepine was the only compound demonstrating a universal linear correlation with K^+ across the seasons. Additionally, the compounds correlated very well in respective river sections regarding the whole river basin. This is a further indication that carbamazepine is a highly valuable quantitative wastewater indicator. A correlation with K^+ indicates that the concentration of the respective micro-contaminant depends only on river discharge. Following this assumption, the prediction of micro-contaminant concentrations at certain locations could be substantially simplified. Furthermore, regarding bank filtration and the interaction of surface water and groundwater in general, it may be possible to derive input functions of the correlating micro-contaminants.

Though the results and the proposed applications are promising, further studies are required to validate the presented approach in different river systems and to identify any limitation. In rivers containing, for example, high natural or anthropogenic K^+-concentrations, the approach may not be feasible. The presented study identified the input of road salt into the river as a limitation.

3.5 Acknowledgments

The presented study was funded by the European Commission (contract number 518118-1, GABARDINE, "Groundwater Artificial Recharge Based on Alternative Sources of Water: Advanced Integrated Technologies and Management"), and by the German Federal Ministry of Education and Research (promotional references No. 02WM0802 and 02WM1081, SMART, "Sustainable and Integral Management of Available Water Resources Using Innovative Technologies").

3.6 References

Adams, C.D., Thurman, E.M., 1991. Formation and transport of deethylatrazine in the soil and vadose zone. Journal of Environmental Quality 20 (3), 540–547.

Boreen, A.L., Arnold, W.A., McNeill, K., 2004. Photochemical fate of sulfa drugs in the aquatic environment: Sulfa drugs containing five-membered heterocyclic groups. Environmental Science and Technology 38 (14), 3933–3940.

Bound, J.P., Voulvoulis, N., 2005. Household disposal of pharmaceuticals as a pathway for aquatic contamination in the United Kingdom. Environmental Health Perspectives 113 (12), 1705–1711.

Brogden, R.N., Heel, R.C., Pakes, G.E., Speight, T.M., Avery, G.S., 1980. Diclofenac sodium: A review of its pharmacological properties and therapeutic use in rheumatic diseases and pain of varying origin. Drugs 20 (1), 24–48.

Bucheli, T.D., Müller, S.R., Voegelin, A., Schwarzenbach, R.P., 1998. Bituminous roof sealing membranes as major sources of the herbicide (R,S)-mecoprop in roof runoff waters: Potential contamination of groundwater and surface waters. Environmental Science and Technology 32 (22), 3465–3471.

Buerge, I.J., Buser, H.-R., Kahle, M., Müller, M.D., Poiger, T., 2009. Ubiquitous occurrence of the artificial sweetener acesulfame in the aquatic environment: An ideal chemical marker of domestic wastewater in groundwater. Environmental Science and Technology 43 (12), 4381–4385.

Buerge, I.J., Poiger, T., Müller, M.D., Buser, H.-R., 2006. Combined sewer overflows to surface waters detected by the anthropogenic marker caffeine. Environmental Science and Technology 40 (13), 4096–4102.

Buser, H.-R., Poiger, T., Müller, M.D., 1998. Occurrence and fate of the pharmaceutical drug diclofenac in surface waters: Rapid photodegradation in a lake. Environmental Science and Technology 32 (22), 3449–3456.

Choi, K., Kim, Y., Park, J., Park, C.K., Kim, M., Kim, H.S., Kim, P., 2008. Seasonal variations of several pharmaceutical residues in surface water and sewage treatment plants of Han River, Korea. Science of the Total Environment 405 (1–3), 120–128.

Ciprandi, G., Passalacqua, G., Mincarini, M., Ricca, V., Canonica, G.W., 1997. Continuous versus on demand treatment with cetirizine for allergic rhinitis. Annals of Allergy, Asthma, and Immunology 79 (6), 507–511.

Clara, M., Strenn, B., Kreuzinger, N., 2004. Carbamazepine as a possible anthropogenic marker in the aquatic environment: Investigations on the behaviour of carbamazepine in wastewater treatment and during groundwater infiltration. Water Research 38 (4), 947–954.

Daneshvar, A., Svanfelt, J., Kronberg, L., Prévost, M., Weyhenmeyer, G.A., 2010a. Seasonal variations in the occurrence and fate of basic and neutral pharmaceuticals in a Swedish river-lake system. Chemosphere 80 (3), 301–309.

Daneshvar, A., Svanfelt, J., Kronberg, L., Weyhenmeyer, G.A., 2010b. Winter accumulation of acidic pharmaceuticals in a Swedish river. Environmental Science and Pollution Research 17 (4), 908–916.

Eiswirth, M., Hötzl, H. 1997. The impact of leaking sewers on urban groundwater, in: Chilton, J, *et al.* (Eds.), Groundwater in the urban environment. Vol. 1: Problems, processes and management. Balkema, Rotterdam, pp. 399–404.

Espir, M.L.E., Millac, P., 1970. Treatment of paroxysmal disorders in multiple sclerosis with carbamazepine (Tegretol). Journal of Neurology, Neurosurgery, and Psychiatry 33 (4), 528–531.

Gäbler, H.-E., Bahr, A., 1999. Boron isotope ratio measurements with a double-focusing magnetic sector ICP mass spectrometer for tracing anthropogenic input into surface and ground water. Chemical Geology 156 (1–4), 323–330.

Gardarsdottir, H., Egberts, T.C., van Dijk, L., Heerdink, E.R., 2010. Seasonal patterns of initiating antidepressant therapy in general practice in the Netherlands during 2002–2007. Journal of Affective Disorders 122 (3), 208–212.

Gasser, G., Rona, M., Voloshenko, A., Shelkov, R., Tal, N., Pankratov, I., Elhanany, S., Lev, O., 2010. Quantitative evaluation of tracers for quantification of wastewater contamination of potable water sources. Environmental Science and Technology 44 (10), 3919–3925.

Giger, W., Schaffner, C., Kohler, H.-P.E., 2006. Benzotriazole and tolyltriazole as aquatic contaminants. 1. Input and occurrence in rivers and lakes. Environmental Science and Technology 40 (23), 7186–7192.

Gómez, M.J., Martínez Bueno, M.J., Lacorte, S., Fernández-Alba, A.R., Agüera, A., 2007. Pilot survey monitoring pharmaceuticals and related compounds in a sewage treatment plant located on the Mediterranean coast. Chemosphere 66 (6), 993–1002.

Haggard, B.E., Bartsch, L.D., 2009. Net changes in antibiotic concentrations downstream from an effluent discharge. Journal of Environmental Quality 38 (1), 343–352.

Himmelsbach, M., Buchberger, W., Klampfl, C.W., 2006. Determination of antidepressants in surface and waste water samples by capillary electrophoresis with electrospray ionization mass spectrometric detection after preconcentration using off-line solid-phase extraction. Electrophoresis 27 (5–6), 1220–1226.

Jönsson, H., Stenström, T.-A., Svensson, J., Sundin, A., 1997. Source separated urine-nutrient and heavy metal content, water saving and faecal contamination. Water Science and Technology 35 (9), 145–152.

Jung, H.-U., Böttcher, F., Hardt, U., Skubowius, A., 2010. Regionalmonitoring Niedersachsen – Regionalreport 2009, Positionierung und Entwicklungstrends ländlicher und städtischer Räume (in German). http://www.ml.niedersachsen.de/download/32678. Accessed 25 July 2011.

Kahle, M., Buerge, I.J., Müller, M.D., Poiger, T., 2009. Hydrophilic anthropogenic markers for quantification of wastewater contamination in ground- and surface waters. Environmental Toxicology and Chemistry 28 (12), 2528–2536.

Katz, B.G., Griffin, D.W., 2008. Using chemicals and microbiological indicators to track the impacts from the land application of treated municipal wastewater and other sources on groundwater quality in a karstic springs basin. Environmental Geology 55 (4), 801–821.

Kiss, A., Fries, E., 2009. Occurrence of benzotriazole in the rivers Main, Hengstbach, and Hegbach (Germany). Environmental Science and Pollution Research 16 (6), 702–710.

Kolpin, D.W., Furlong, E.T., Meyer, M.T., Thurman, E.M., Zaugg, S.D., Barber, L.B., Buxton, H.T., 2002. Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999–2000: A national reconnaissance. Environmental Science and Technology 36 (6), 1202–1211.

Konieczka, P., Namieśnik, J., 2010. Estimating uncertainty in analytical procedures based on chromatographic techniques. Journal of Chromatography A 1217 (6), 882–891.

Kosonen, J., Kronberg, L., 2009. The occurrence of antihistamines in sewage waters and in recipient rivers. Environmental Science and Pollution Research 16 (5), 555–564.

Kreuzinger, N., 2008. Review on the assessment of the removal efficiency of wastewater treatment plants for selected xenobiotics, in: Hlavinek, P., Bonacci, O., Marsalek, J., Mahrikova, I. (Eds.), Dangerous pollutants (xenobiotics) in urban water cycle (NATO Science for peace and security series C: Environmental security), Springer Netherlands, pp. 227–244.

Kümmerer, K., 2010. Pharmaceuticals in the environment. Annual Review of Environment and Resources 35, 57–75.

Massmann, G., Knappe, A., Richter, D., Pekdeger, A., 2004. Investigating the Influence of Treated Sewage on Groundwater and Surface Water Using Wastewater Indicators in Berlin, Germany. Acta Hydrochimica et Hydrobiologica 32 (4–5), 336–350.

Matamoros, V., Duhec, A., Albaigés, J., Bayona, J.M., 2009. Photodegradation of carbamazepine, ibuprofen, ketoprofen and 17α-ethinylestradiol in fresh and seawater. Water, Air, and Soil Pollution 196 (1–4), 161–168.

Maurer, M., Escher, B.I., Richle, P., Schaffner, C., Alder, A.C., 2007. Elimination of β-blockers in sewage tretment plants. Water Research 41 (7), 1614–1622.

Möller, P., Dulski, P., Bau, M., Knappe, A., Pekdeger, A., Sommer-von Jarmerstedt, C., 2000. Anthropogenic gadolinium as a conservative tracer in hydrology. Journal of Geochemical Exploration 69–70, 409–414.

Neal, C., Williams, R.J., Bowes, M.J., Harrass, M.C., Neal, M., Rowland, P., Wickham, H., Thacker, S., Harman, S., Vincent, C., Jarvie, H.P. (2010). Decreasing boron concentrations in UK rivers: Insights into reductions in detergent formulations since the 1990s and within-catchment storage issues. Science of the Total Environment 408 (6), 1374–1385.

Nödler, K., Licha, T., Bester, K., Sauter, M., 2010. Development of a multi-residue analytical method, based on liquid chromatography-tandem mass spectrometry, for the simultaneous determination of 46 micro-contaminants in aqueous samples. Journal of Chromatography A 1217 (42), 6511–6521.

Oulton, R.L., Kohn, T., Cwiertny, D.M., 2010. Pharmaceuticals and personal care products in effluent matrices: a survey of transformation and removal during wastewater treatment and implications for wastewater management. Journal of Environmental Monitoring 12 (11), 1956–1978.

Pöppinghaus, K., Filla, W., Sensen, S., 1994. Abwassertechnologie, second ed. Springer, Berlin.

Reemtsma, T., Miehe, U., Duennbier, U., Jekel, M., 2010. Polar pollutants in municipal wastewater and the water cycle: Occurrence and removal of benzotriazoles. Water Research 44 (2), 596–604.

Reemtsma, T., Weiss, S., Mueller, J., Petrovic, M., Gonźales, S., Barcelo, D., Ventura, F., Knepper, T.P., 2006. Polar pollutants entry into the water cycle by municipal wastewater: A European perspective. Environmental Science and Technology 40 (17), 5451–5458.

Sacher, F., Ehmann, M., Gabriel, S., Graf, C., Brauch, H.-J., 2008. Pharmaceutical residues in the river Rhine – results of a one-decade monitoring programme. Journal of Environmental Monitoring 10 (5), 664–670.

Sadezky, A., Löffler, D., Ternes, T., 2008. KNAPPE (Knowledge and need assessment on pharmaceutical products in environmental waters) deliverable number D1.2 Report: "Proposal of an environmental indicator and classification system of pharmaceutical product residues for environmental management". http://www.knappe-eu.org/fichiers/44-D1.2%20environmental%20indicator%20final%20version.pdf. Accessed 25 July 2011.

Scheurer, M., Ramil, M., Metcalfe, C.D., Groh, S., Ternes, T.A., 2010. The challenge of analyzing beta-blocker drugs in sludge and wastewater. Analytical and Bioanalytical Chemistry 396 (2), 845–856.

Schwabe, U., Paffrath, D., 2009. Arzneiverordnungsreport 2009, Springer Medizin Verlag, Heidelberg.

Schwarzenbach, R.P., Escher, B.I., Fenner, K., Hofstetter, T.B., Johnson, C.A., von Gunten, U., Wehrli, B., 2006. The challenge of micropollutants in aquatic systems. Science 313 (5790), 1072–1077.

Sharpley, A.N., Smith, S.J., Ahuja, L.R., 1988. Soluble potassium transport in agricultural runoff waters. Agricultural Water Management 15 (1), 37–46.

Suarez, S., Lema, J.M., Omil, F., 2010. Removal of pharmaceutical and personal care products (PPCPs) under nitrifying and denitrifying conditions. Water Research 44 (10), 3214–3224.

Ternes, T., 2007. The occurrence of micopollutants (sic!) in the aquatic environment: A new challenge for water management. Water Science and Technology 55 (12), 327–332.

Ternes, T.A., 1998. Occurrence of drugs in German sewage treatment plants and rivers. Water Research 32 (11), 3245–3260.

Ternes, T.A., Hirsch, R., 2000. Occurrence and behavior of X-ray contrast media in sewage facilities and the aquatic environment. Environmental Science and Technology 34 (13), 2741–2748.

Vieno, N.M., Tuhkanen, T., Kronberg, L., 2005. Seasonal variation in the occurrence of pharmaceuticals in effluents from a sewage treatment plant and in the recipient water. Environmental Science and Technology 39 (21), 8220–8226.

Voutsa, D., Hartmann, P., Schaffner, C., Giger, W., 2006. Benzotriazoles, alkylphenols and bisphenol A in municipal wastewaters and in the Glatt River, Switzerland. Environmental Science and Pollution Research 13 (5), 333–341.

Walling, D.E., Foster, I.D.L., 1975. Variations in the natural chemical concentration of river water during flood flows, and the lag effect : some further comments. Journal of Hydrology 26 (3–4), 237–244.

Weiss, S., Jakobs, J., Reemtsma, T., 2006. Discharge of three benzotriazole corrosion inhibitors with municipal wastewater and improvements by membrane bioreactor treatment and ozonation. Environmental Science and Technology 40 (23), 7193–7199.

Wittmer, I.K., Bader, H.-P., Scheidegger, R., Singer, H., Lück, A., Hanke, I., Carlsson, C., Stamm, C., 2010. Significance of urban and agricultural land use for biocide and pesticide dynamics in surface waters. Water Research 44 (9), 2850–2862.

Wolf, L., Eiswirth, M., Hötzl, H., 2006. Assessing sewer-groundwater interaction at the city scale based on individual sewer defects and marker species distributions. Environmental Geology 49 (6), 849–857.

Ziemann, H., Kies, L., Schulz, C.-J., 2001. Desalinization of running waters: III. Changes in the structure of diatom assemblages caused by a decreasing salt load and changing ion spectra in the river Wipper (Thuringia, Germany). Limnologica 31 (4), 257–280.

Chapter 4

4 Caffeine as an indicator for the quantification of untreated wastewater in karst systems

Olav Hillebrand[1], Karsten Nödler[1], Tobias Licha[1], Martin Sauter[1], Tobias Geyer[1]

Citation: Hillebrand, O., Nödler, K., Licha, T., Sauter, M., Geyer, T., 2012. Caffeine as an indicator for the quantification of untreated wastewater in karst systems. Water Research 46 (2), 395–402.

http://dx.doi.org/10.1016/j.watres.2011.11.003

[1]Department Applied Geology, Geoscience Center of the University of Göttingen, Goldschmidtstr. 3, 37077 Göttingen, Germany

Abstract

Contamination from untreated wastewater leakage and related bacterial contamination poses a threat to drinking water quality. However, a quantification of the magnitude of leakage is difficult. The objective of this work is to provide a highly sensitive methodology for the estimation of the mass of untreated wastewater entering karst aquifers with rapid recharge. For this purpose a balance approach is adapted. It is based on the mass flow of caffeine in spring water, the load of caffeine in untreated wastewater and the daily water consumption per person in a spring catchment area. Caffeine is a source-specific indicator for wastewater, consumed and discharged in quantities allowing detection in a karst spring. The methodology was applied to estimate the amount of leaking and infiltrating wastewater to a well-investigated karst aquifer on a daily basis. The calculated mean volume of untreated wastewater entering the aquifer was found to be 2.2 ± 0.5 m^3 d^{-1} (undiluted wastewater). It corresponds to approximately 0.4% of the total amount of wastewater within the spring catchment.

4.1 Introduction

Karst aquifers represent important drinking water resources supplying up to 25% of the world's population with fresh water (Ford and Williams, 2007). Flow and transport in these types of aquifers can be very rapid due to the presence of highly permeable flow paths, e.g. karst conduits in the subsurface (Geyer *et al.*, 2007). Because of thin soil coverage with a low field capacity and highly permeable local infiltration pathways, groundwater recharge generally occurs very rapidly after precipitation events. Consequently, karst springs are highly vulnerable and show strong variations in spring discharge and water quality (Heinz *et al.*, 2009).

Point-source input of wastewater has been shown to be a serious threat to groundwater quality, as it is related to contamination with fecal bacteria (Heinz *et al.*, 2009; Hrudey *et al.*, 2003). Despite the short survival time of coliform bacteria in the environment (McFeters and Stuart, 1972), rapidly transported, untreated wastewater can pose a substantial health risk. Contamination of karst groundwater from point sources can be triggered when single massive inputs of wastewater reach the aquifer in a short time by e.g. overflow of retention basins or failure of wastewater lagoons (Heinz *et al.*, 2009; Heinz *et al.*, 2006; Memon and Azmeh, 2001). Furthermore, leaky sewers or the overflow from undersized sewer networks may be a threat to groundwater quality (Eiswirth and Hötzl, 1997; Rutsch *et al.*, 2006; Seiler *et al.*, 1999; Gasser *et al.*, 2010).

To assess the magnitude of anthropogenic impact on water sources, indicators can be employed. An ideal indicator for anthropogenic source water has to be source-specific and released in sufficient quantities to allow detection after dilution in the environment (Takada *et al.*, 1997). The detection of the anthropogenic impact on water bodies using different indicators has been studied before, proposing and using conservative wastewater-specific indicators, such as carbamazepine (Bahlmann *et al.*, 2009; Gasser *et al.*, 2010; Takada *et al.*, 1997), human-specific antibiotics (Glassmeyer *et al.*, 2005) or artificial sweeteners (Buerge *et al.*, 2009). The lack of specificity of conservative indicators for treated or untreated wastewater is a drawback of those anthropogenic indicators, i.e. a distinction between these two types of wastewater is not possible. To assess the magnitude of pollution solely from untreated wastewater, a specific indicator for untreated wastewater is required. Apart from the traditionally used microbial indicators, which have been discussed controversially (Boehm *et al.*, 2002; Heinz *et al.*, 2009; Ogunseitan, 1996), caffeine was found to be a suitable wastewater indicator that allows even the quantification of wastewater burdens in surface waters (Buerge *et al.*, 2006). It is found in several beverages (Siegener and Chen, 2002) and pharmaceuticals. These are the only sources for caffeine in Germany. Seiler *et al.* (1999) stated, that the main source of caffeine is likely not to be the excreted part of consumed caffeine, but the disposal of unconsumed caffeine-containing beverages, by disposing them down the sink or from rinsing coffee cups. Caffeine is wastewater-specific (Buerge *et al.*, 2003), mobile (Gardinali and Zhao, 2002), shows excellent elimination rates during wastewater processing (Buerge *et al.*, 2006), is degradable in the environment (e.g. Hakil *et al.*, 1998) and has a high detection frequency (this study). Typical loads of caffeine in untreated wastewater are in the range of 16 mg d^{-1} person^{-1} (Buerge *et al.*, 2003). Caffeine is metabolized in the human body mainly to paraxanthine, which is neither found in plants nor food (Schmidt and Schoyerer, 1966; Stavric, 1988; Lelo *et al.*, 1986). Another primary metabolite is theobromine. It is not only a metabolite but is also found in beverages, such as tea or cocoa (Srdjenovic *et al.*, 2008). Both metabolites occur in wastewater, but they can as well be produced by microorganisms. However, since 80% of the ingested caffeine is metabolized to paraxanthine in humans (Lelo *et al.*, 1986), its correlation with caffeine can qualitatively be used to support the specificity of caffeine for wastewater.

The objective of this work is to transfer the methodological approach of wastewater quantification with caffeine in surface waters (Buerge *et al.*, 2006) to groundwater karst systems. For this purpose a karst spring was sampled during a period of several months with a high temporal resolution. Karst springs are often connected to extended conduit networks and drain large catchment areas. The sampling of a single karst spring therefore allows an integral characterization of a catchment area. Thus, wastewater indicators in springs could play an important role for the identification and quantification of untreated wastewater entering a groundwater system.

We hypothesize that caffeine degradation rates and the residence time distribution of water in karst systems allow the indication of rapidly transported, untreated wastewater. Treated wastewater or untreated wastewater that is not transported directly to the spring over highly conductive flow paths, i.e. with higher residence times, is not expected to affect the indication or the quantification.

4.2 Materials and methods

4.2.1 Field work

A long-term sampling campaign was conducted with increased event-based sampling frequency.

4.2.1.1 Study area

The investigated spring (Gallusquelle) is situated in Southwest Germany (Figure 4.1). The spring has an average annual discharge of 0.5 $m^3 s^{-1}$ draining a catchment area of 45 km^2. The total population in the catchment is ca. 4000 inhabitants. Untreated wastewater can reach the karst aquifer by overflows from a retention basin at approximately 9 km distance to the spring or from sewer leakage. The retention basin belongs to a regional combined wastewater drainage and treatment system in the area. The wastewater from the Gallusquelle catchment is treated in wastewater treatment plants outside of the catchment area.

4.2.1.2 Sampling campaign

Due to the high variability of spring discharge and water quality (Geyer *et al.*, 2007) a highly time resolved sampling campaign with 157 water samples was undertaken. Spring water was sampled between March, 3rd and May, 24th 2010. The temporal resolution of sampling was strongly increased during spring discharge events (maximum sampling resolution: 8 samples per day). During recession periods of spring discharge the sampling interval was reduced to one sample per day. The samples were collected in glass bottles, stored at 4 °C and preconcentrated within 24 h by solid phase extraction (SPE). A continuous monitoring system with hourly data for electrical conductivity (ref. T of 20 °C), turbidity and water levels was installed at the spring. Measured water levels were translated to spring discharge applying a rating curve. Hourly rainfall data, air temperature and depth of snow were obtained from weather stations (German Meteorological Service, DWD) located close to the catchment area.

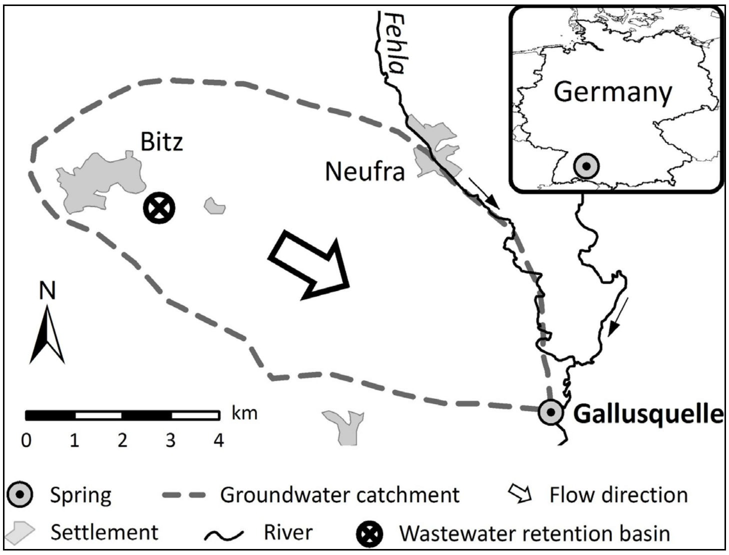


Figure 4.1: The area of investigation. All possible sources for caffeine, settlements and the wastewater retention basin (WWRB), are located in a well defined area (modified after Geyer *et al.*, 2007).

4.2.2 Laboratory analyses

4.2.2.1 Analysis of carbamazepine and methylxanthines

An analytical method based on SPE and high-performance liquid chromatographic separation with tandem mass spectrometric detection (HPLC/MS-MS) was used for the analysis of selected methylxanthines (caffeine and its metabolites paraxanthine, theobromine, theophylline, 1-methylxanthine and 3-methylxanthine), and the anticonvulsant carbamazepine. Details have been published previously (Nödler *et al.*, 2010). In brief, a 500 mL sample volume was buffered at neutral pH (phosphate buffer) and extracted by SPE (500 mg OASIS HLB, Waters, Eschborn, Germany). Prior to extraction, 200 ng of paraxanthine-D_6 and 100 ng of carbamazepine-D_{10} were added as internal standards for the quantification of xanthines and carbamazepine, respectively. After extraction the sorbent was rinsed with ultrapure water and dried. The cartridge was wrapped in aluminum foil and kept frozen (−18 °C) until analysis. The analytes were eluted with methanol and ethyl acetate, consecutively. The solvents were evaporated and the dry residue was re-dissolved in an aqueous 5 mM ammonium acetate solution, containing 4% methanol. In contrast to the volume described by Nödler *et al.* (2010), only 0.8 mL were used to re-dissolve the analytes, resulting in a higher enrichment factor and thus lower method detection and quantification limits.

4.2.2.2 Chemicals

Methanol (LC/MS grade) and caffeine were purchased from Fisher Scientific (Schwerte, Germany), ethyl acetate and ammonium acetate (all analytical grade) were purchased from VWR (Darmstadt, Germany). Carbamazepine, paraxanthine, paraxanthine-D_6, theobromine, theophylline, 1-methylxanthine, and 3-methylxanthine were obtained from Sigma Aldrich (Steinheim, Germany). Carbamazepine-D_{10} was purchased from Promochem (Wesel, Germany). Potassium dihydrogen phosphate and disodium hydrogen phosphate dihydrate were obtained from VWR (Darmstadt, Germany).

4.3 Results and discussion

4.3.1 Occurrence and variation of caffeine together with related methylxanthines and carbamazepine

During the sampling campaign highly variable caffeine concentrations of 10.3 ± 6.3 ng L^{-1} (mean ± standard deviation) were found in the spring water. High concentrations were observed during winter and as response to precipitation events. Caffeine was detected in 95 of 157 samples.

During the sampling campaign a snowmelt event and two rainfall events were observed. The snowmelt event (Figure 4.2; t1) resulted in a significant decrease of caffeine concentrations in spring water, while after each rainfall event (Figure 4.2; t4a/b) a positive caffeine signal in spring water was observed. Concentrations of caffeine in spring water before the snowmelt are relatively high. However, after the snowmelt caffeine concentrations were frequently lower than the limit of detection. This may be related to a lower input and/or a higher dilution of caffeine in the aquifer system by large quantities of melt water.

The comparison of caffeine concentrations and turbidity at the Gallusquelle spring demonstrates that the turbidity curve is only of limited use for wastewater detection. The peak concentration of caffeine after the first rainfall occurs slightly delayed to the turbidity peak and the minimum of the electrical conductivity. It indicates that the caffeine enters the karst aquifer with concentrated point recharge, i.e. recharge through highly conductive flow paths, and is rapidly transported through the aquifer system to the spring. The decrease of electrical conductivity is attributed to the mixing of pre-event groundwater and low mineralized event water. The second rainfall does not appear as clear signal in the data of the turbidity. The electrical conductivity develops a plateau instead of a peak, as the rainfall occurred within the recession period of the first rainfall. Apart from the two turbidity peaks produced by the melt event and the two consecutive rainfall events, a third peak (Figure 4.2; t3) appears between the melt event and the precipitation events, when a water pipe, belonging to the local water supply company burst. The introduced water was processed drinking water and therefore not related to a contamination with wastewater.

Two overflow events of the local wastewater retention basin occurred at the end of March and the beginning of April (only 3 days apart), resulting in a single high peak of caffeine (Figure 4.2; t2). Only one sample indicates the recharge of wastewater for these events. Which one of the overflow events resulted in a caffeine peak is not known, since the events occurred chronologically very close to each other. As no second peak developed, it is to assume, that one of the two overflow events did not result in wastewater recharge. As shown by Heinz *et al.* (2009), water from the overflows of the WWRB percolates to the groundwater and reaches the spring within a few days, producing a turbidity peak. It is noteworthy, that the turbidity may be insensitive for small wastewater quantities and did not evolve a peak for any of the two overflow events mentioned above, whereas the caffeine indicated at least one. Apart from the two overflow events all wastewater intrusions are related to sewer leakage.

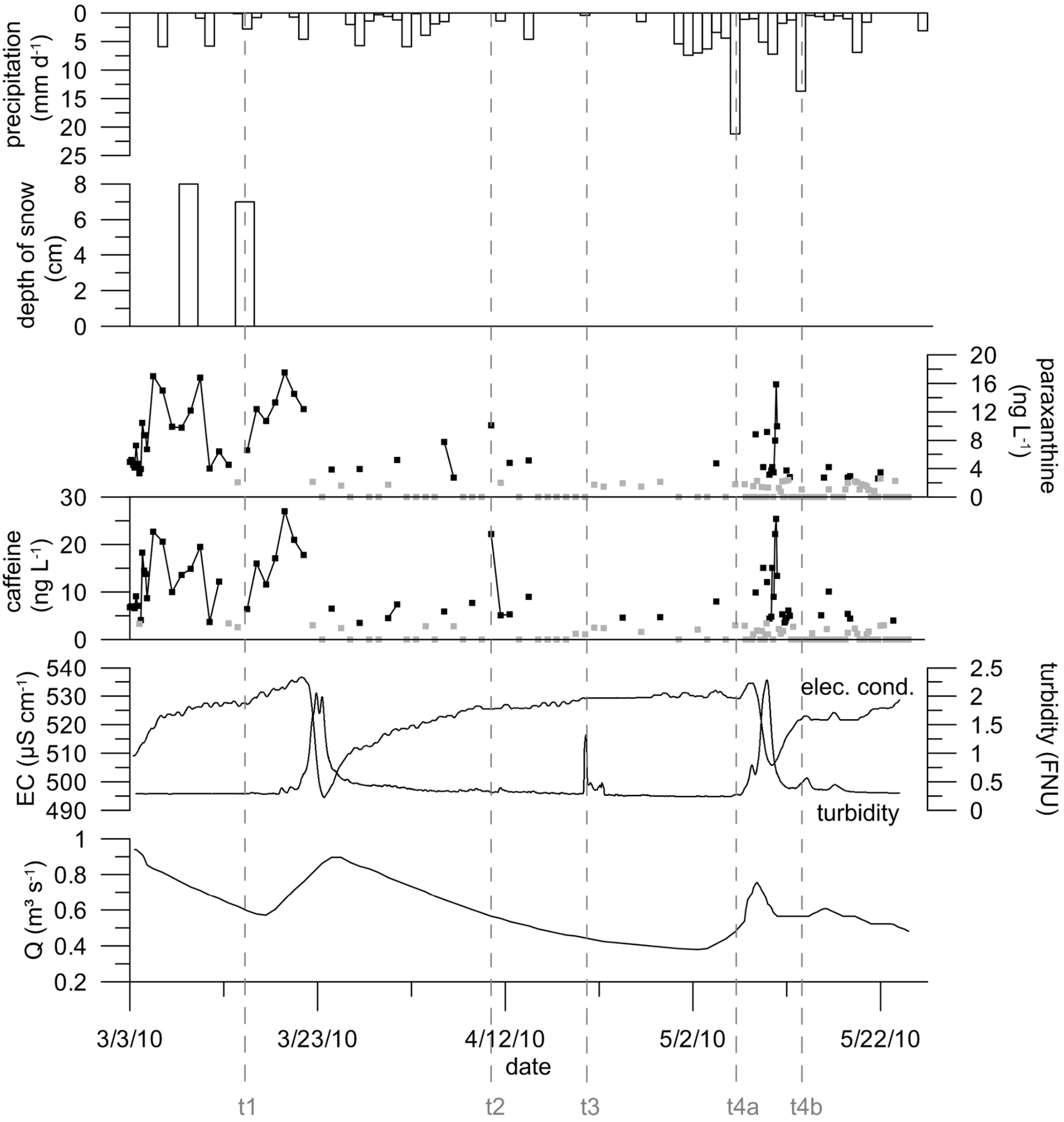


Figure 4.2: Concentrations of caffeine and paraxanthine at the spring. The illustrated daily precipitation amounts are calculated from hourly measurements. Caffeine and paraxanthine concentrations in gray show samples below the methods limit of quantification, concentrations on the abscissa represent samples below method's limit of detection. t1 indicates the commencement of the snowmelt event, t2 the caffeine signal injected by an overflow of the local wastewater retention basin, t3 a burst of a water pipe and t4a/b two rainfall events.

Two other dimethylxanthine compounds, paraxanthine and theobromine, were detected frequently during the sampling campaign and demonstrated reasonable occurrence above their method quantification limits (Table 4.1). The other analyzed methylxanthines appeared only rarely, which is likely related to their higher method detection limits. We tried to verify our results using carbamazepine, as an established marker for wastewater, but it was only detected during spring at very low concentrations that never exceeded the method quantification limit. Applying carbamazepine as indicator for small amounts of wastewater in the investigated highly diluting system is therefore invalid, even if treated wastewater can be excluded as a source. Concentrations of caffeine in untreated domestic wastewater can exceed those of carbamazepine by several orders of magnitude (Miao *et al.*, 2005), emphasizing the superior detectability of caffeine in such systems.

Table 4.1: Concentrations and detection frequencies of caffeine, two metabolites and carbamazepine in spring water for the method limit of quantification. In total 157 samples were taken and analyzed.

	MDL[a] [ng L^{-1}]	MQL[b] [ng L^{-1}]	Min [ng L^{-1}]	Max [ng L^{-1}]	Median [ng L^{-1}]	DF[c] [%]	QF[d] [%]
Caffeine	1.0	3.4	3.5	27.0	7.8	60.5	38.5
Paraxanthine	0.7	2.6	2.6	17.5	5.2	56.7	33.7
Theobromine	1.2	4.1	4.1	29.8	9.4	45.9	36.2
Carbamazepine	0.6	1.8	0	0	0	57.3	0

[a] method detection limit.

[b] method quantification limit.

[c] detection frequency, n=157 samples.

[d] detection frequency above method quantification limit, n=157 samples.

Due to its relatively high detection frequency and its indicator properties for untreated wastewater caffeine can be used as wastewater indicator. Its degradability and hence non-refractory behavior allows even the quantification of untreated domestic wastewater from caffeine concentrations.

4.3.2 Estimation of the amount of domestic wastewater

Since caffeine is wastewater-specific, the measured concentrations of caffeine in spring water allow the estimation of wastewater effectively and directly reaching the karst spring. The specificity of caffeine for domestic wastewater in the spring is verified by the positive correlation with its predominantly human metabolite paraxanthine (Figure 4.3) (Schmidt and Schoyerer, 1966), while caffeine concentrations do not correlate with those of theobromine (data not shown). The exact origin of theobromine in spring water cannot be deduced and a contribution of each possible source (beverages, human metabolism, bacterial degradation) is probable. Groundwater contaminations from wastewater with long residence times do not affect the results because of the restricted lifetime of caffeine and paraxanthine in the groundwater system related to their high degradability.

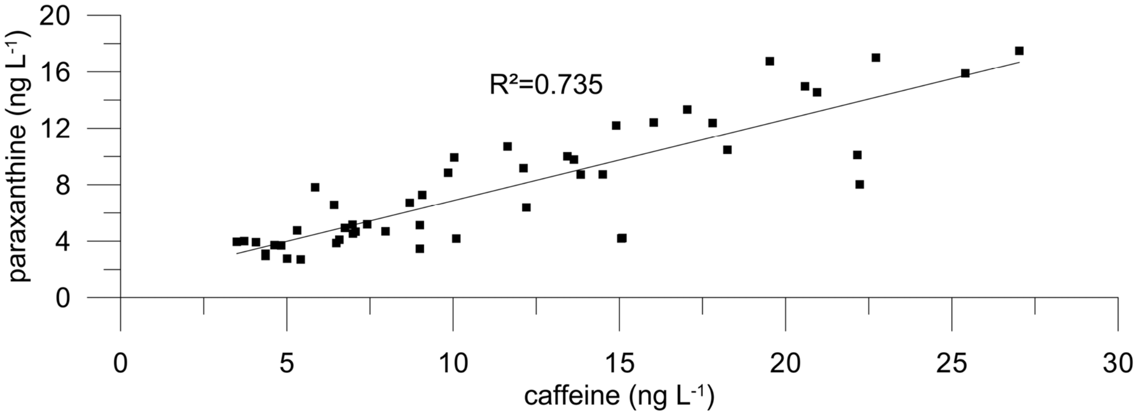


Figure 4.3: Correlation of caffeine with paraxanthine. The correlation demonstrates that caffeine originates from domestic wastewater.

Apart from four farms and two inns all households in the catchment of the Gallusquelle spring are connected to the regional sewer system (personal communication, Mr. Leins, municipality Bitz). Those, not connected to the sewer system dispose their wastewater to septic tanks, which are regularly emptied. Treated wastewater as a source of caffeine can be neglected as the wastewater treatment plants of the region are located outside of the catchment of the spring. Since karst springs are often the point outlet of the catchment, the calculated amounts of wastewater can be interpreted as an integrated signal. The results therefore refer to the whole catchment. The calculation for the amount of untreated wastewater appearing in spring water is as follows:

$$WW = \frac{c}{c_{\max}} \times P \times WC \qquad \text{(Equation 4.1)}$$

with

$$c_{\max} = \frac{P \times I}{Q} \qquad \text{(Equation 4.2)}$$

where WW is the amount of undiluted, rapidly transported and untreated wastewater per day discharging at the spring; P the total population of the catchment of the Gallusquelle is 4000 persons; WC is the daily water consumption per person. It is calculated to be 134 L d^{-1} person^{-1} (yearly data from the sanitary district 'Scher-Lauchert Abwasserverband'). I is the load of caffeine in untreated wastewater. It is stated to be relatively stable at 15.8 ± 3.8 mg d^{-1} person^{-1} (Buerge *et al.*, 2003). This mean value and the standard deviation are used for further calculations as reasonable approximation. c and c_{max} are the actually observed concentrations of caffeine in the spring water and the maximum concentration to be expected for the given population and discharge conditions, if 100% of the wastewater was released and rapidly transported to the spring, respectively. Q is the spring discharge. Only concentrations exceeding the method quantification limit (3.4 ng L^{-1}) are used for the calculations of wastewater volumes. Samples with lower concentrations are set to 0 $m^3\ d^{-1}$ of wastewater. During days with high sampling resolution daily mean values of the recharging wastewater were calculated. This leads to a slide bias of the calculation towards an underestimation of the true amounts of untreated wastewater at the spring. Note that the resulting equation (Equation 4.3) for the amount of untreated wastewater at the spring becomes independent from the number of inhabitants within the catchment.

$$WW = \frac{c \times WC \times Q}{I} \qquad \text{(Equation 4.3)}$$

For the calculation, the relative standard deviation of 6.1% (Nödler *et al.*, 2010) of the analytical method and the relative uncertainty of the caffeine load (24.1%) are taken into account, considering error propagation. The mean amount of domestic wastewater discharging at the spring is calculated to be 2.2 ± 0.5 $m^3\ d^{-1}$ (mean ± standard deviation). Note that this volume refers to normalized wastewater, which is calculated as the product of P and WC. The volume is unaffected by dilution from e.g. precipitation events. The calculated volume corresponds to approximately 0.4% of the total amount of normalized wastewater in the groundwater catchment. The temporal distribution of wastewater load on a daily basis is shown in Figure 4.4. It is evident that the influx of wastewater to the aquifer is highly variable with a maximum estimated volume of untreated wastewater of 14.0 ± 3.5 $m^3\ d^{-1}$, corresponding to 2.6% of the total wastewater. Rutsch *et al.* (2006) stated the leakages in urban environments to be in the order of 1–10%. The calculated volumes in this study therefore represent a relatively low fraction of the total wastewater.

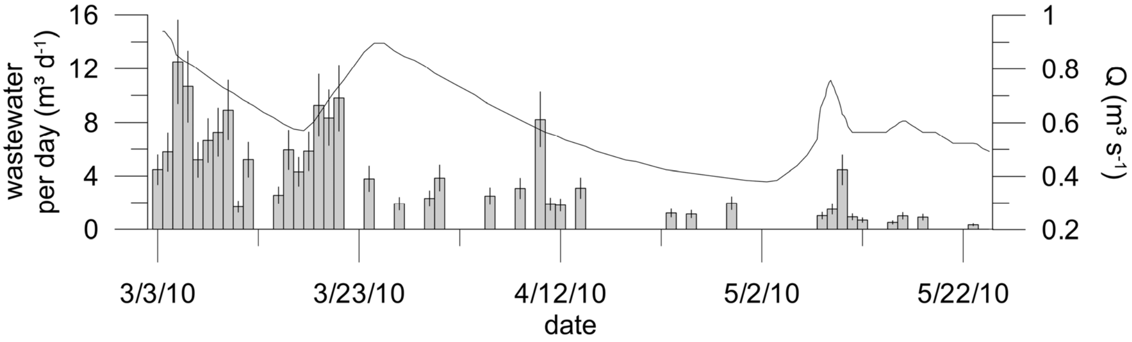


Figure 4.4: Volumes of leaked wastewater. Calculated volumes of wastewater at the spring Gallusquelle with the respective standard deviation.

Heinz *et al.* (2009) stated, that during overflow events on average 23000 m³ of combined wastewater are released. These values cannot be compared to the calculated volumes from this study, as the effective dilution of the wastewater is not known.

Taking into account the method quantification limit of caffeine (c_{mql} = 3.4 ng L^{-1}) and a spring discharge of 0.5 m³ s^{-1}, the minimum daily amount of untreated wastewater (WW_{mql}) which can be quantified is 1.3 ± 0.3 m³ d^{-1} (mean ± standard deviation). The relative amount of wastewater to spring discharge, at which caffeine is still quantifiable, is approximately 2.910^{-5}.

As can be seen from Equation 4.4, the detection limit to quantify wastewater in spring water is inversely proportional to the spring discharge. The highest accuracy for the calculation is given during low flow periods. However, as concluded by Musolff *et al.* (2010) from their findings in an urban area, sewer leakage does not occur evenly distributed over time, but simultaneously to natural recharge, i.e. precipitation events, implying an increase of spring discharge. This means, that the detection of wastewater is, in all cases, impeded by dilution.

$$\alpha = \frac{WW_{mql}}{Q} = \frac{c_{mql} \times WC}{I} \qquad \text{(Equation 4.4)}$$

The above stated result reflects the lower boundary estimation for the amount of untreated domestic wastewater at the spring. The degradation of caffeine in the aquifer during transport is neglected here. This simplification is introduced since only limited information on the effect of the significant dilution and fast transport (i.e. low residence times) on biodegradation exists. However, a more realistic picture may be derived when degradation is considered. For this purpose residence times of wastewater in the aquifer resulting from concentrated recharge events and *in-situ* degradation rates are required.

4.3.3 Mean residence time of caffeine in the aquifer

In a single event of point source recharge of untreated wastewater at a known location and time, the resulting breakthrough curve of caffeine at the spring can be used to identify the residence time of wastewater and thus of caffeine from that recharge event in karst systems.

The residence time of recharging water directly flowing to the spring can be estimated from the time interval between the precipitation event and the occurrence of chemical signals transported by the percolating rainwater at the spring (Hunkeler and Mudry, 2007). The residence time of water in groundwater systems depends on the point of recharge and groundwater flow velocity. In karst systems groundwater flow velocity is related to the spring discharge (Heinz *et al.*, 2009). Since all possible wastewater sources in the investigated area, both small towns and the retention basin (see Figure 4.1) are located in a similar region, estimated residence times of wastewater can be considered to be representative for infiltrating wastewater. Two recharge events from point sources were selected for estimating the mean residence time (Figure 4.2; t4a, t4b). Each of the two rainfall events was of sufficient intensity to result in caffeine peaks at the spring. The mean spring discharge during the two investigated recharge events is similar to the mean spring discharge during the whole sampling campaign. The time of the precipitation events is known at a resolution of 1 hour (German Meteorological Service, DWD). Since the two rainfall events occurred over periods of 10 and 4 hours, respectively, the effective resolution is adjusted appropriately. The respective beginning and the end of the rain events are used as earliest and latest possible points in time inducing the recharge event. The peak concentrations of caffeine in the spring water after rain events are used as the mean point in time for the arrival of caffeine at the spring. The sampling resolution is 3 and 6 hours for the first and second precipitation event, respectively.

The estimated residence time after the first rainfall event is 99 ± 8 h. The second rainfall event yields 130 ± 8 h. The mean spring discharge was 0.64 $m^3\ s^{-1}$ and 0.58 $m^3\ s^{-1}$, respectively. The mean spring discharge during the whole period of investigation is 0.6 $m^3\ s^{-1}$ and therefore similar to the mean spring discharge of the two analyzed precipitation events. As representative residence time for wastewater during the period of investigation the mean of both estimations 115 ± 20 h (mean ± standard deviation) can be assumed. Previous investigations of the residence time, using turbidity, bacteria and uranine as tracer of recharge occurring close to the settlements and from the wastewater retention basin, yielded residence times of approximately 55–238 h depending on the spring discharge (Heinz *et al.*, 2009).

4.3.4 Degradation

Buerge *et al.* (2003) indicated that biodegradation and photodegradation may be relevant degradation processes in surface water bodies. In aquifers the latter can be neglected. However, to improve the estimation on the relative contribution of untreated domestic wastewater in spring discharge over concentrated flow using caffeine as indicator requires a consideration of the *in-situ* biodegradation of caffeine during transport. Estimations on caffeine degradation in the environment are scarce. Swartz *et al.* (2006) estimated the *in-situ* degradation rate in a porous aquifer from a mass balance to be in the order of 0.07–0.014 d^{-1}. This results, for the above stated residence time, in an estimation errors between 6 and 48% for the volume of untreated wastewater at the spring. Since the dilution in karst aquifers is larger than in porous aquifers, a hindered degradation is expected. In contrast to the relatively high degradation rates stated above, Buerge *et al.* (2003) performed incubation experiments with lake water (20 °C, dark), that yielded degradation rates of 0.004–0.006 d^{-1}, while they proposed, that the actual degradation at temperatures below 10 °C is expected to be ≤0.003 d^{-1}. The *in-situ* degradation rate of caffeine in a lake was estimated to be in the range of 0.003–0.007 d^{-1} for different temperatures and depths (Buerge *et al.*, 2006). The temperature of water in the karst aquifer Gallusquelle is almost constant at 8.2 °C. The corresponding half-lives are therefore in the range of 100–240 d. These values refer to a strongly diluted system and are believed to be a better approximation for the *in-situ* degradation rate of caffeine in karst aquifers, than the above mentioned rates of Swartz *et al.* (2006). The estimated residence time of signal-producing wastewater in the conduit system is lower than the half-life of caffeine in the aquifer by more than one order of magnitude. The effect of biodegradation on the estimations of wastewater can therefore be neglected.

Note that the estimated amounts of wastewater should be understood as a lower boundary estimation, since the dilution of the wastewater occurs along the whole flow path and a gradually decreasing degradation of caffeine might occur with reasonable degradation close to the input.

The mean residence time of water in the fissured matrix of karst aquifers (e.g. Maloszewski *et al.*, 2002) is in the order of years to decades and hence high compared to the above stated half-lives in the karst conduit system. Caffeine degradation rates in the environment are therefore high enough not to show refractory behavior and low enough to reach the spring after infiltration. Due to the good degradability in WWTPs and rather stable behavior in the environment, caffeine is an excellent indicator for untreated wastewater in rapid systems, such as karst. As the caffeine concentrations show high variations after recharge events a high sampling rate is required to assess the amount of recharged domestic wastewater. In combined sewer systems during rain events mixing of wastewater with rainwater occurs, which dilutes the input signal. This does not affect the calculated volumes, but it may happen that caffeine concentrations in spring water do not exceed the method quantification limit. Further, constant caffeine loads in untreated wastewater are assumed for the calculations, which may not necessarily be applicable because of seasonal and temporal variations. However, as the exact point in time of the wastewater release is unknown a consideration of short-term variations (e.g. diurnal) in the calculation is not reasonable. However, long-term-trends in caffeine loads may be considered. Deviations of the assumed caffeine load or water consumption result in an inversely proportional and proportional deviation of the calculated wastewater volume respectively. For example, a caffeine load of 20 mg d^{-1} p^{-1} (instead of 15.8 mg d^{-1} p^{-1}, i.e. 21% higher caffeine load) would result in a calculated wastewater volume lower by 21%. Assumptions, deviating from the real situation result in deviations of the wastewater volume appropriately. Literature values can be used in order to approximate the magnitude of contamination.

For the estimation of *in-situ* degradation during transport, a limited source area is required to reliably assess the actual residence time of injected untreated domestic wastewater.

4.4 Conclusions

- Caffeine was detected in a karst spring with a higher frequency than the established wastewater marker carbamazepine, which fails as indicator in karst systems.
- The degradation rates of caffeine and the residence time distribution of water in karst systems allows to infer to untreated and rapidly transported wastewater from caffeine concentrations in spring water.
- A quantification of untreated wastewater at a karst spring is possible, from measured caffeine concentrations, caffeine load in untreated wastewater, water consumption rates and spring discharge. Even literature values can be used to estimate volumes of untreated wastewater, yielding reasonable approximations.
- The degradation of caffeine, which may affect the calculation of wastewater volumes, in karst aquifers is uninvestigated. A tracer test could enhance the estimation, using caffeine and a reference tracer.

4.5 Acknowledgments

We thank the staff of the water supply company Hermentingen / Swabian Alb, especially Mr. Peter Knaus for advice, providing data and field equipment. The German Meteorological Service (DWD, Gewässerdirektion Riedlingen) supplied data on weather parameters, the sanitary-district 'Scher-Lauchert Abwasserverband' supplied data on wastewater volumes. The presented study was funded by the German Federal Ministry of Education and Research (promotional reference No. 02WM1081, SMART, "Sustainable and Integral Management of Available Water Resources Using Innovative Technologies" and promotional reference No. 02WRS1277A, AGRO, "Risikomanagement von Spurenstoffen und Krankheitserregern in ländlichen Karsteinzugsgebieten").

4.6 References

Bahlmann, A., Weller, M.G., Panne, U., Schneider, R.J.,2009. Monitoring carbamazepine in surface and wastewater by an immunoassay based on a monoclonal antibody. Analytical and Bioanalytical Chemistry 395 (6), 1809–1820.

Boehm, A.A., Grant, S.B., Kim, J.,H., Mowbray, S.,L., McGee, C.D., Clark, C.,D., Foley, D.M., Wellman, D.E., 2002. Decadal and shorter period variability of surf zone water quality at Huntington Beach, California. Environmental Science and Technology 36 (18), 3885–3892.

Buerge, I.J., Poigner, T., Müller, M.D., Buser, H.-R., 2003. Caffeine, an anthropogenic marker for wastewater contamination of surface waters. Environmental Science and Technology 37 (4), 691–700.

Buerge, I.J., Poigner, T., Müller, M.,D., Buser, H.-R., 2006. Combined sewer overflow to surface waters detected by the anthropogenic marker caffeine. Environmental Science and Technology 40 (13), 4096–4102.

Buerge, I.J., Buser, H.-R., Kahle, M., Müller, M.D., Poigner, T., 2009. Ubiquitous occurence of the artificial sweetener acesulfame in the aquatic environment: an ideal chemical marker of domestic wastewater in groundwater. Environmental Science and Technology 43 (12), 4381–4385.

Eiswirth, M., Hötzl, H., 1997. The impact of leaking sewers on urban groundwater. In: Groundwater in the urban environment. - Vol. 1, Problems, processes and management; Chilton, J. *et al.* (Eds), A.A. Balkema, Rotterdam, pp. 399–404.

Ford, D., Williams, P., 2007. Karst hydrogeology and geomorphology. Wiley, Chichester.

Gardinali, P.R., Zhao, X., 2002. Trace determination of caffeine in surface water samples by liquid chromatography–atmospheric pressure chemical ionization–mass spectrometry (LC-APCI-MS). Environment International 28 (6), 521–528.

Gasser, G., Rona, M., Voloshenko, A., Shelkov, R., Tal, N., Pankratov, I., Elhanany, S., Lev, O., 2010. Quantitative evaluation of tracers for quantification of wastewater contamination of potable water sources. Environmental Science and Technology 44 (10), 3919–3925.

Geyer, T., Birk, S., Licha, T., Liedl, R., Sauter, M., 2007. Multitracer test approach to characterize reactive transport in karst aquifers. Ground Water 45 (1), 36–45.

Glassmeyer, S.T., Furlong, E.T., Kolpin, D.,W., Cahill, J.D., Zaugg, S.,D., Werner, S.L., Meyer, M.T., Kryak, D.D., 2005. Transport of chemical and microbial compounds from known wastewater discharges: Potential for use as indicators of human fecal contamination. Environmental Science and Technology 39 (14), 5157–5169.

Hakil, M., Denis, S., Viniegra-González, G., Augur, C., 1998. Degradation and product analysis of caffeine and related dimethylxanthines by filamentous fungi. Enzyme and Microbial Technology 22 (5), 355–359.

Heinz, B., Birk, S., Liedl, R., Geyer, T., Straub, K.L., Bester, K., Kappler, A., 2006. Vulnerability of a Karst Spring to wastewater infiltration (Gallusquelle, southwest Germany). Austrian Journal of Earth Sciences 99, 11–17.

Heinz, B., Birk, S., Liedl, R., Geyer, T., Straub, K.L., Andresen, J., Bester, K., Kappler, A., 2009. Water quality deterioration at a karst spring (Gallusquelle, Germany) due to combined sewer overflow: Evidence of bacterial and micro-pollutant contamination. Environmental Geology 57 (4), 797–808.

Hrudey, S.E., Payment, P., Huck, P.M., Gillham, R.W., Hrudey, E.J., 2003. A fatal waterborne disease epidemic in Walkerton, Ontario: comparison with other waterborne outbreaks in the developed world. Water Science and Technology 47 (3), 7–14.

Hunkeler, D., Mudry, J., 2007. In: Methods of karsthydrogeology; Goldscheider, N., Drew, D., (Eds), International contributions to hydrogeology 26, Taylor and Francis group, London, pp. 93–121.

Lelo, A., Miners, J.O., Robson, R.A., Birkett, D.J., 1986. Quantitative assessment of caffeine partial clearances in man. British Journal of Clinical Pharmacology 22, 183–186.

Maloszewski, P., Stichler, W., Zuber, A., Rank, D., 2002. Identifying the flow systems in a karstic-fissured-porous aquifer, the Schneealpe, Austria, by modelling of environmental 18O and 3H isotopes. Journal of Hydrology 256 (1–2), 48–59.

McFeters, G.A., Stuart, D.G., 1972. Survival of coliform bacteria in natural waters: field and laboratory studies with membrane-filter chambers. Applied Microbiology 24 (5), 805–811.

Memon, B.A., Azmeh, M.M., 2001. Failure of an industrial wastewater lagoon in a karst terrain and remedial action. Environmental Geology 40 (11–12), 1424–1432.

Miao, X.-S., Yang, J.-J., Metcalfe, C.D., 2005. Carbamazepine and its metbaolites in wastewater and in biosolids in a municipal wastewater treatment plant. Environmental Science and Technology 39 (19), 7469–7475.

Musolff, A., Leschik, S., Reinstorf, F., Strauch, G., Schirmer, M., 2010. Micropollutant loads in the urban water cycle. Environmental Science and Technology 44 (13), 4877–4883.

Nödler, K., Licha, T., Bester, K., Sauter, M., 2010. Development of a multi-residue analytical method, based on liquid chromatography–tandem mass spectrometry, for the simultaneous determination of 46 micro-contaminants in aqueous samples. Journal of Chromatography A 1217 (42), 6511–6521.

Ogunseitan, O.A., 1996. Removal of caffeine in sewage by Pseudomonas putida: Implications for water pollution index. World Journal of Microbiology and Biotechnology 12 (3), 251–256.

Rutsch. M., Rieckermann, J., Krebs, P., 2006. Quantification of sewer leakage: a review. Water Science and Technology 54 (6–7), 135–144.

Schmidt, G., Schoyerer, R., 1966. On the detection of caffeine and its metabolites in urine (German: Zum Nachweis von Coffein und seinen Metaboliten im Harn). Deutsche Zeitschrift für gerichtliche Medizin 57 (3), 402–409.

Seiler, R.L., Zaugg, S.D., Thomas, J.,M., Howcroft, D.,L., 1999. Caffeine and pharmaceuticals as indicators of wastewater contamination in wells. Ground Water 37 (3), 405–410.

Siegener, R., Chen, R.F., 2002. Caffeine in Boston Harbor seawater. Marine Pollution Bulletin 44 (5), 383–387.

Srdjenovic, B., Djordjevic-Milic, V., Grujic, N., Injac, R., Lepojevic, Z., 2008. Simultaneous HPLC determination of caffeine, theobromine, and theophylline in food, drinks, and herbal products. Journal of Chromatographic Science 46 (2), 144–149.

Stavric, B., 1988. Methylxanthines: Toxicity to humans. 3. Theobromine, paraxanthine and the combined effect of methylxanthines. Food and Chemical Toxicology 26 (8), 725–733.

Swartz, C.H., Reddy, S., Benotti, M.J., Yin, H., Barber, L.B., Brownawell, B.J., Rudel, R.A., 2006. Steroid estrogens, nonylphenol ethoxylate metabolites, and other wastewater contaminants in groundwater affected by a residential septic system on Cape Cod, MA. Environmental Science and Technology 40 (16), 4894–4902.

Takada, H., Satoh, F., Bothner, M.H., Tripp, B.W., Johnson, C.G., Farrington, J.W., 1997. Anthropogenic molecular markers: tools to identify the sources and transport pathways of pollutants. In: Molecular markers in environmental geochemistry; Eganhouse, R.P. (Ed.), ACS Symposium Series 671, American Chemical Society, Washington, DC, pp. 178–195.

Chapter 5

5 Evidence for the microbially mediated abiotic formation of reversible and non-reversible sulfamethoxazole transformation products during denitrification

Karsten Nödler[1], Tobias Licha[1], Manuela Barbieri[2], Sandra Pérez[3]

Citation: Nödler, K., Licha, T., Barbieri, M., Pérez, S., 2012. Evidence for the microbially mediated abiotic formation of reversible and non-reversible sulfamethoxazole transformation products during denitrification. Water Research 46 (7), 2131–2139.

http://dx.doi.org/10.1016/j.watres.2012.01.028

[1]Department Applied Geology, Geoscience Center of the University of Göttingen, Goldschmidtstr. 3, 37077 Göttingen, Germany

[2]Department of Geosciences, Institute of Environmental Assessment and Water Research, IDAEA-CSIC. Jordi Girona 18–26, 08034 Barcelona, Spain

[3]Department of Environmental Chemistry, Institute of Environmental Assessment and Water Research, IDAEA-CSIC. Jordi Girona 18–26, 08034 Barcelona, Spain

Abstract

The antibiotic sulfonamide drug sulfamethoxazole (SMX) is extensively used in both human and veterinary medicine. Since it cannot be completely eliminated by the typical state-of-the-art wastewater treatment technology, it is frequently detected in the water cycle. SMX, as aromatic amine, can undergo abiotic transformations with the under denitrifying conditions produced nitrogen species nitric oxide (NO) and nitrite (NO_2^-). NO and aromatic amines are commonly known to form diazonium cations. Depending on the reaction conditions the diazonium cation disintegrates under cleavage of elementary nitrogen and substitutes its diazo-group by an NO_2-group or by hydrogen. Following this approach, two transformation products (TPs) of the persistent SMX under denitrifying conditions were hypothesized and synthesized: 4-nitro-N-(5-methylisoxazol-3-yl)-benzenesulfonamide (4-nitro-SMX) and N-(5-methylisoxazol-3-yl)-benzenesulfonamide (desamino-SMX). The synthesized compounds were identified by Nuclear Magnetic Resonance (NMR) spectroscopy and used as reference standards for their confirmation and quantification in denitrifying water/sediment batch experiments and in environmental samples. During the denitrifying degradation experiment SMX was no longer detected after 10 days whereas increasing concentrations of the two TPs were observed. However, at day 87 the SMX concentration recovered to 53 ± 16% of the initial concentration after most of the nitrate was consumed. A retransformation of 4-nitro-SMX to SMX was postulated and confirmed by another anoxic water/sediment test in the absence of nitrate as electron acceptor. Both TPs were also detected in karst spring samples, highlighting the need and benefit of focusing on transformation products in environmental studies. Furthermore, the consideration of the retransformation potential of 4-nitro-SMX can substantially improve the understanding of SMX behavior during processes such as bank filtration and artificial recharge.

5.1 Introduction

The antibiotic sulfonamide drug 4-amino-N-(5-methylisoxazol-3-yl)-benzenesulfonamide (sulfamethoxazole, SMX) is extensively used in both human and veterinary medicine (García-Galán et al, 2009; Watanabe *et al.*, 2010). Since it cannot be completely eliminated by the typical state-of-the-art wastewater treatment technology, it is frequently detected in the water cycle (Heberer, 2002; Kolpin *et al.*, 2002; Nödler *et al.*, 2011; Pérez *et al.*, 2005).

Various studies at laboratory and field scale were conducted to investigate the fate of SMX in sediment and soil including bank filtration processes (i.e. Baumgarten *et al.*, 2011; Drillia *et al.*, 2005a; Grünheid *et al.*, 2005; Heberer *et al.*, 2008; Höltge and Kreuzig, 2007; Radke *et al.*, 2009). At the pH range encountered in natural systems, i.e rivers and aquifers, sorption of SMX to organic matter and minerals can be considered to be of minor importance (Drillia *et al.*, 2005b; Gao and Pedersen, 2005; Li and Zhang, 2010; Radke *et al.*, 2009). Investigations on biodegradation rates of SMX clearly demonstrate its dependency on residence time, the dominating redox condition, the adaptation of microorganisms, the availability of an alternative carbon source, the entry routes (accompanying matrix) of SMX, and its initial concentration (Baumgarten *et al.*, 2011; Höltge and Kreuzig, 2007; Radke *et al.*, 2009). In degradation studies, the elimination or removal of SMX is commonly identified by a decreasing concentration of SMX but transformation products (TPs) are not described (Baumgarten *et al.*, 2011; Drillia *et al.*, 2005a; Grünheid *et al.*, 2005; Höltge and Kreuzig, 2007; Radke *et al.*, 2009). However, reported data on removal rates under anoxic conditions are not consistent, ranging from an insignificant removal in soil column tests to almost full removal in field studies (Baumgarten *et al.*, 2011; Heberer *et al.*, 2008). Moreover, a study by Conn *et al.* (2010) on soil treatment of wastewater reported a sporadic and irregular occurrence of SMX in the pore water, further emphasizing the complexity of its environmental behavior. The mineralization of SMX by microorganisms is very limited (Höltge and Kreuzig, 2007). Therefore, the identification and quantification of TPs must be considered as a major issue for a realistic assessment of its environmental fate and associated risk.

In general, aromatic amines react readily with nitric oxide (NO) to form diazonium cations (Brückner, 2004; Itoh *et al.*, 1996). Diazonium cations are not stable under ambient conditions and thus can undergo numerous consecutive reactions, strongly depending on reaction conditions and available reaction partners (Brückner, 2004; Itoh *et al.*, 1996; Opgenorth and Rüchardt, 1974). Following the diazotization reaction, in the presence of excess nitrite, which also exists in significant concentrations as part of the microbial denitrification process (Hollocher, 1982), the diazonium cation can form aromatic nitro- (NO_2) species in water at room temperature and near neutral pH under cleavage of elementary nitrogen (Opgenorth and Rüchardt, 1974). Further, in *in vivo* studies with rats, Woolley and Sigel (1982) observed the reductive deamination of the sulfonamide drug sulfadiazine (SDZ, an aromatic amine structurally related to SMX) resulting in the substitution of the aromatic NH_2 by hydrogen. The TP was observed when rats were orally administered (stomach tube) with SDZ and sodium nitrite. However, the deamination product was not detected in the absence of nitrite, which clearly underlines the significance of nitrite in the deamination process. In acidic environments (i.e. the stomach), nitrite is chemically reduced to NO (McKnight *et al.*, 1997). Thus, a diazonium cation formed by SDZ and NO was also suggested as a probable intermediate molecule during the reductive deamination of the sulfonamide antibiotic (Woolley and Sigel, 1982). Pereira *et al.* (2011) observed a fast abiotic transformation of aniline and sulfanilic acid in the presence of nitrite. In an attempt to identify the TPs by liquid chromatography-mass spectrometry (LC-MS) they suggested the formation of nitro-aryl and diazonium compounds. In experiments with anilines, Lammerding *et al.* (1982) concluded that the actual diazotization reaction of the aromatic amines is independent from biological assistance. As NO and nitrite are formed enzymatically during the microbial denitrification process (Hollocher, 1982; Knowles, 1982), SMX may also undergo the aforementioned reactions under denitrifying conditions. However, to the authors' knowledge no study has yet evaluated this possible reaction pathway for SMX.

In the presented work, a set of anoxic water/sediment tests containing SMX, high concentrations of nitrate, and easily biodegradable organic carbon were conducted in order to investigate the potential reactions of SMX with microbially formed NO and nitrite. Based on the early findings by Woolley and Sigel (1982) on the transformation of SDZ and to the reaction mechanism postulated by Opgenorth and Rüchardt (1974), the formation of deaminated SMX and a nitro-species of SMX were hypothesized. To test this hypothesis the respective TPs were synthesized and used for their quantitative determination in the microcosms and in environmental samples.

5.2 Experimental methods

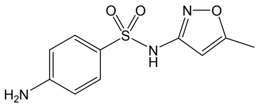
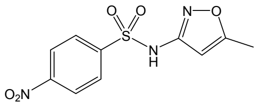
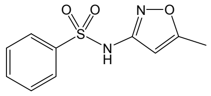
5.2.1 Chemicals

LC-MS grade methanol, analytical grade toluene, and analytical grade ethyl acetate were purchased from Fisher Scientific (Schwerte, Germany). Ammonium acetate and formic acid were obtained from VWR (Darmstadt, Germany). Sulfamethoxazole, benzenesulfonyl chloride (≥99%), 4-nitrobenzenesulfonyl chloride (97%), 3-amino-5-methylisoxazole (≥98%), and pyridine (≥99%) were purchased from Sigma Aldrich (Steinheim, Germany). The internal standard sulfamethoxazole-$^{13}C_6$ (SMX-$^{13}C_6$) was obtained from LGC Promochem (Wesel, Germany). Organic and inorganic salts for the preparation of the water/sediment tests (ammonium phosphate, iron(III) chloride, sodium acetate, sodium bicarbonate, sodium chloride, potassium dihydrogen phosphate, magnesium chloride hexahydrate, magnesium nitrate hexahydrate, potassium chloride, calcium chloride dihydrate, sodium sulphate, ammonium chloride and mercury(II) chloride) were all purchased from Merck KGaA (Darmstadt, Germany). Ultrapure water was obtained from a combined water purification system from Millipore (Schwalbach, Germany). It consisted of Elix 5 (Progard 1 silver cartridge) and Milli-Q Gradient A10 (Quantum Ex Ultrapure Organex + Q-Gard 1 cartridge).

5.2.2 Synthesis of SMX-transformation products as reference substances

The TP 4-nitro-N-(5-methylisoxazol-3-yl)-benzenesulfonamide (4-nitro-SMX) was synthesized as described by Rieder *et al.* (1988). Six grams of 4-nitrobenzenesulfonyl chloride was mixed with 2.4 g of 3-amino-5-methylisoxazole in 10 mL of pyridine at 0 °C. After a reaction time of 24 h at room temperature the crude product was precipitated in 200 mL of ultrapure water, filtered, and washed twice with 25 mL of ultrapure water. The product was then recrystallized using an ethyl acetate:toluene (1:3, v/v) mixture, filtered, and washed with ethyl acetate. For the synthesis of the TP N-(5-methylisoxazol-3-yl)-benzenesulfonamide (desamino-SMX) 4.8 g of benzenesulfonyl chloride was used instead of the 4-nitrobenzenesulfonyl chloride. The synthesis was similar to 4-nitro-SMX. Structures of both compounds are presented in Table 5.1. The identity and purity of the TPs were confirmed by 1H-Nuclear Magnetic Resonance (1H-NMR) spectroscopy. The purity was >95% for 4-nitro-SMX and 88% for desamino-SMX.

Table 5.1: Structures, pKa and log K_{OW} values, (−)ESI-MS/MS quantifier and qualifier transitions of the analytes.

Compound (CAS)	Structure	pKa[a]	Log K_{OW}[a]	Quantifier (CE)[d]	Qualifier (CE)[d]
Sulfamethoxazole, SMX (723-46-6)		5.81 ± 0.5[b] 1.4 ± 0.1[c]	0.66 ± 0.41	252 → 154 (13.5 V)	252 → 106 (17.5 V)
4-nitro-SMX (29699-89-6)		5.65 ± 0.4	1.27 ± 0.41	282 → 138 (20.5 V)	282 → 186 (13.0 V)
Desamino-SMX (13053-79-7)		6.92 ± 0.5	1.34 ± 0.40	237 → 141 (13.0 V)	237 → 77 (25.5 V)

[a] Scifinder predicted values.

[b] pKa of the secondary amine.

[c] pKa of the primary amine (conjugate acid).

[d] Collision energy.

5.2.3 Anoxic water/sediment batch experiment with SMX and high nitrate concentration

Aim of this water/sediment batch experiment was the evaluation of the fate of SMX and its TPs under denitrifying conditions over time. The sediment was obtained from a test site for artificial groundwater recharge located in Sant Vicenç dels Horts (Barcelona, Spain) on the banks of the Llobregat River. The aquifer consisted of quaternary alluvial sediments, mainly gravel and sand containing small fractions of lutites. Sediment samples were collected from an oxic unsaturated horizon at about 1 m depth under the bottom of the infiltration pond prior to its operation. The sediment was sieved to < 1 mm and homogenized in steel containers. Sieving was conducted to remove the coarse fraction, which was expected to be less active for surface and microbially mediated reactions (Barbieri *et al.*, 2011). The air-dried sediment contained <0.2% of total nitrogen and total organic carbon (TOC) in the bulk. The total carbon content (TC) of the air-dried sediment was 2.5%. Concentrations of manganese and ferric iron associated to oxide-hydroxides and oxides in the air-dried sediment were 0.07 and 5.8 mg g^{-1}, respectively.

The aqueous test medium for the water/sediment test was prepared to mimic the Llobregat River water (used for artificial recharge) except for nitrate and the dissolved organic carbon (DOC). High concentrations of nitrate and easily biodegradable DOC were selected to stimulate denitrifying activity (Knowles, 1982; McCarty *et al.*, 2007; Myers and McGarity, 1971), and to maintain it during a significant lapse of time. The following amounts of inorganic salts were thus dissolved per liter ultrapure water: 43 mg sodium bicarbonate, 184 mg sodium chloride, 2.2 mg potassium dihydrogen phosphate, 251 mg magnesium chloride hexahydrate, 8477 mg magnesium nitrate hexahydrate, 76 mg potassium chloride, 441 mg calcium chloride dihydrate, 296 mg sodium sulphate, and 5.9 mg ammonium chloride. In order to remove dissolved oxygen the solution was purged with argon 5.0 for 1 h. Finally, 171 mg sodium acetate and 3 mL of an SMX stock solution in methanol (0.333 mg mL^{-1}) were added per liter solution, resulting in an SMX concentration of 1 mg L^{-1} and a DOC concentration of 970 mg L^{-1} (consisting of acetate and the methanol from the SMX stock solution). Although cell growth initiation is delayed by trace concentrations of SMX (Underwood *et al.*, 2011), a complete inhibition of microbial growth at the given SMX concentration was not suspected (Letzel, 2008; Mohatt *et al.*, 2011).

The assembling of the batches was conducted under argon atmosphere in a glove box. Fourteen glass bottles (300 mL) were all filled with 120 g of the air-dried and homogenized sediment and 240 mL of the liquid test medium. A headspace of 15 mL argon was left in each bottle. Screw caps with PTFE seals were used to close the bottles. The batches were shaken and wrapped in aluminum foil to prevent photochemical reactions for the duration of the experiment (Boreen *et al.*, 2004). A fraction of the liquid test medium (10 mL) was frozen as a reference of the initial concentration of SMX. All batches were incubated at 25 ± 1 °C and expended according to the sampling schedule. On a regular basis, the batches were shaken carefully to homogenize the water solution while avoiding the destruction of the developed biofilms.

To distinguish biological from non-biological effects, 14 abiotic control batches were prepared analogous to the biotic batches. The sediment and the liquid test medium for the controls were autoclaved three times for 20 min (T=121 °C and 1 atm overpressure, once a day for three consecutive days). Prior to assembling, SMX and sodium acetate were then added to the sterilized liquid test medium. Furthermore, mercury(II) chloride (60 mg L^{-1}) was applied to prevent microbial activity (Davis *et al.*, 2006). A fraction of the liquid test medium (10 mL) was frozen as a reference of the initial concentration of SMX. Aliquots of water from the disassembled microcosms were incubated on tryptic soy agar (TSA) plates twice during the first 41 days of the experiment. The plates were incubated in duplicate at 25 °C for one week under aerobic conditions and two weeks under anaerobic conditions. None of the plates demonstrated a growth of microorganisms. Furthermore, the successful inhibition of denitrifying activity was confirmed by the constant nitrate concentration until the end of the experiment (data not shown).

Duplicate biotic batches were utilized after 2, 5, 10, 14, 25, 41 and 87 days, and duplicates of abiotic batches after 2, 5, 9, 15, 26, 41 and 89 days. For the quantification of SMX and the TPs, 10 mL aliquots of each batch supernatant were stored at −18 °C in amber glass vials until analysis. Samples for nitrite and nitrate analysis were filtered prior to freezing (0.45 µm PALL Acrodisc® Sterile Syringe Filters with Supor® membrane).

5.2.4 Anoxic water/sediment batch experiment with 4-nitro-SMX without nitrate

To confirm the suspected retransformation of 4-nitro-SMX to SMX in the absence of nitrate-reduction, a further water/sediment experiment was conducted. For this experiment the outflow of an anoxic soil column was used as the aqueous phase. The absence of nitrate (< 0.1 mg L^{-1}) and nitrite (< 0.01 mg L^{-1}) in this aqueous phase were confirmed by ion chromatographic and photometric analytical methods (DIN EN 26 777), respectively. Sixty mg L^{-1} sulphate was detected by ion chromatography. Fourteen glass bottles (100 mL) were all filled with 40 g of sediment and 100 mL of the anoxic water. Prior to assembling of the batches, the water was purged with argon 5.0 for 1 h in order to remove residues of dissolved oxygen. Prior to assembly, the liquid was spiked with 1 mg L^{-1} of iron(III) chloride and ammonium phosphate, respectively. Furthermore, 40 mg L^{-1} of sodium acetate and 1 mg L^{-1} of the synthesized 4-nitro-SMX were added. The batches were shaken and incubated at 25 ± 1 °C submerged under water to maintain anoxic conditions. After one hour, two batches were directly sampled. Aliquots of the water phase were frozen and used for the determination of the initial SMX and 4-nitro-SMX concentrations. The remaining batches were carefully shaken once a day. After 3, 7, 14, 21, 28 and 35 days, two batches were expended and aliquots of 5 mL supernatant were frozen until analysis.

5.2.5 Chemical analysis of the batch experiments

Nitrate, nitrite and sulphate determination of the water/sediment batches were conducted by ion chromatography (IC). The pH, dissolved oxygen and the redox potential were determined by electrode measurements. The reference electrode used for the determination of the redox potential was $Ag/AgCl_2$ (4 M KCl, 200 mV). SMX and its TPs were analyzed by high performance liquid chromatography-electrospray-tandem mass spectrometry (HPLC-ESI-MS/MS). Details regarding the instrumentation have been published previously (Nödler *et al.*, 2010) and can be found in Appendix A (Text A1). A flow rate of 200 µL min^{-1} and an injection volume of 100 µL were applied. The column oven was operated at 30 °C. The eluents consisted of 0.015% formic acid + 5% methanol (eluent A) and methanol (eluent B). To avoid adverse interference during the ionization process with the high load of inorganic sample matrix (Bester, 2008), a slow gradient was chosen over a rapid analysis. The elution started with 5% B followed by a gradient of 27 minutes to 65% B. This was followed by a sharp gradient of 1 min to 95% B, which was held for 5 min. After a gradient of 1 min to 5% B the system was allowed to equilibrate for 11 min.

As an additional method to minimize matrix effects, samples were diluted 1:4 (v/v) with aqueous 5 mM ammonium acetate solution, containing 4% methanol. Furthermore, SMX-$^{13}C_6$ (125 ng mL^{-1}) was used as the internal standard and the standard solutions for the calibration were prepared in an inorganic matrix according to 50% of the aqueous test medium's initial concentration. Since the nitrate concentration in the water/sediment batches decreased during the experiment, the 50% of the inorganic matrix was applied as a compromise. Afterwards, the standards were also diluted 1:4 (v/v) with aqueous 5 mM ammonium acetate solution, containing 4% methanol. Before analysis, all samples and standard solutions were centrifuged at 1500 rpm (Christ RVC 2-18, purchased from Fisher Scientific, Schwerte, Germany) for 30 min at room temperature.

The quantification of analytes was performed in multiple reaction monitoring (MRM) mode and two transitions per analyte were monitored. The TPs in the batch experiments were confirmed if the following criteria were met for standards and unknowns: a) identical retention time in the chromatogram, b) identical quantifier and qualifier mass transitions and c) intensity ratios of quantifier and qualifier match (EU Commission Decision No. 657/2002).

In a previous study using an acidic mobile phase, SMX demonstrated the highest sensitivity in the positive ionization mode (Nödler *et al.*, 2010). However, as 4-nitro-SMX and desamino-SMX demonstrated only poor ionization efficiencies in this mode, all compounds were analyzed in the negative ion mode. The compounds were quantified by using their most intense mass transition. The applied capillary voltages were −40 V for 4-nitro-SMX and

−35 V for all other compounds, respectively. Individual parameters are presented in Table 5.1. The internal standard SMX-$^{13}C_6$ was quantified by using the mass transition m/z 258 → 160 (13.5 V collision energy).

5.2.6 Environmental samples

Sixty-two water samples were collected from the karst spring "Gallusquelle" (Swabian Alb, Germany) between May and October 2010. The irregular and event-based inflow of wastewater related micro-contaminants was already demonstrated in other studies (Heinz *et al.*, 2009; Hillebrand *et al.*, 2012).

The determination of nitrate was conducted by IC. Sample preparation for the determination of SMX and the TPs was conducted by solid phase extraction (SPE) according to Nödler *et al.* (2010). However, the method quantification limit (MQL) of SMX reported therein was improved upon by using of 0.8 mL final extract volume instead of 1 mL. Therefore, the applied preconcentration factor was higher and thus the MQL of SMX in the presented study was 2.1 ng L^{-1}. The individual MS-parameters of 4-nitro-SMX and desamino-SMX given in the previous chapter were included with the cited methodology. Further information can be found in Appendix A (Text A2). Eight concentration levels (1–250 ng L^{-1}) were used for the calibration of the three analytes and included the preconcentration step. The linear correlation coefficients exceeded 0.99 and the MQL according to a signal-to-noise ratio (S/N) of 10 were 1.0 and 1.5 ng L^{-1} for 4-nitro-SMX and desamino-SMX, respectively.

5.3 Results and discussion

5.3.1 SMX transformation under denitrifying conditions

During the first 41 days of the denitrifying experiment, dissimilative denitrification (the use of nitrate as electron acceptor in energy metabolism) was clearly indicated by an increasing nitrite concentration while the nitrate concentration decreased simultaneously (Figure 5.1b). No dissolved oxygen was detected in any of the microcosms. The ranges of the pH (7.6–8.3) and the redox potential (Eh, −200 mV to −100 mV, determined in soil suspension) indicated suitable conditions for (bacterial) denitrification in the biotic batch experiments (Knowles, 1982; Seo and DeLaune, 2010).

No significant removal of SMX was observed in the abiotic control of the water/sediment test (Figure 5.1a). Despite the high initial SMX concentration of 1 mg L^{-1} no SMX was detected after 10 days in the biotic test (Figure 5.1a). As all batches were wrapped in aluminum foil for the duration of the experiment, a photochemical degradation of SMX could be excluded (Boreen *et al.*, 2004). The fast degradation of SMX was initially in contrast with results recently published (Mohatt *et al.*, 2011). Mohatt *et al.* (2011) did not observe a significant biological degradation of SMX in a denitrifying water/sediment batch experiment. However, the molar excess of the initial nitrate spike relative to the used SMX concentration was two orders of magnitude lower in their study than in the presented study. Furthermore, their (Mohatt *et al.*, 2011) experiments were designed to investigate the degradation of SMX as a primary substrate whereas in the presented study high concentrations of alternative carbon and energy sources (acetate and methanol) were applied. Thus, the ratio of initial nitrate and SMX concentrations and/or the presence of alternative carbon or energy sources may be important factors for the observed reactions. The high nitrate and easily degradable DOC concentrations applied in the presented study effectively supported the growth of denitrifying microorganisms. Thus, the rapid decrease of the SMX concentration was most likely triggered by rapidly increasing denitrifying microbial activity (Figure 5.1b) with the production of NO and nitrite resulting in a simple diazotization reaction followed by the formation of the hypothesized TPs (Figure 5.1a).

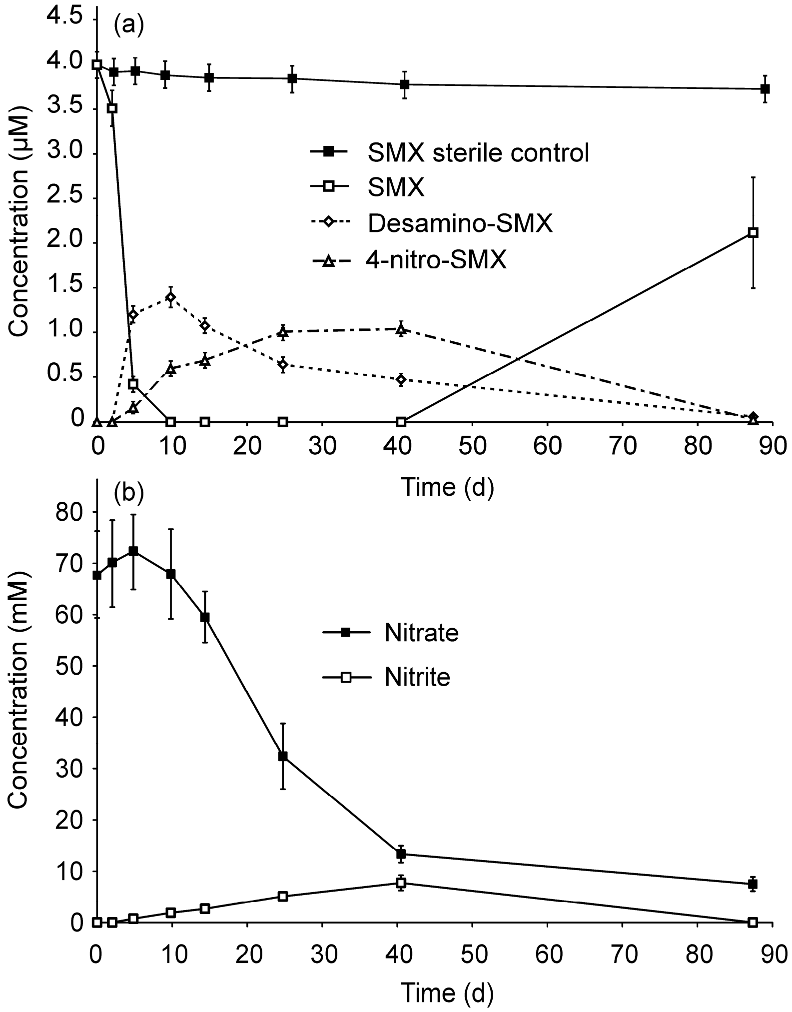


Figure 5.1: Water/sediment experiment on SMX transformation under denitrifying conditions, chart (a): concentrations of SMX (biotic and sterile control), desamino-SMX and 4-nitro-SMX. Chart (b): concentrations of nitrate and nitrite. Mean values of two batches per time point, error bars represent the respective concentration range of duplicates plus the analytical error.

In contrast to the fast and initially complete disappearance of the applied SMX, increasing concentrations of the TPs 4-nitro-SMX and desamino-SMX, hypothesized from the literature (Opgenorth and Rüchardt, 1974; Woolley and Sigel, 1982), were observed in the denitrifying water/sediment test. A typical MRM chromatogram of a standard solution and a sample from the nitrate-reducing microcosm can be found in Appendix A (Figure A.1). The abiotic formation of the TPs without sediment was demonstrated in preliminary abiotic experiments with aqueous solutions of SMX and nitrite at different pH values (data not shown, more information can be found in Appendix A (Text A3). At favorable pH conditions, the exclusively abiotic formation of both TPs was observed within hours. As a conclusion of these preliminary experiments, the chemical transformation process of SMX to desamino-SMX and 4-nitro-SMX itself can indeed occur without microbial assistance. However, in contrast to the aqueous abiotic experiments, the reactions in the denitrifying water/sediment test were microbially mediated, as the reactants NO and nitrite were enzymatically formed (Hollocher, 1982; Knowles, 1982). The recent findings of Mohatt *et al.* (2011) on the transformation of SMX under iron-reducing conditions followed a similar principle: The observed rapid transformation of SMX was attributed to abiotic reactions of SMX and Fe(II) generated by the microbial reduction of Fe(III) soil minerals.

As presented in Figure 5.1a, the concentration of 4-nitro-SMX correlated very well with the nitrite concentration. Opgenorth and Rüchardt (1974) incubated an aqueous solution (pH 6.5, 20 °C) of m-nitrobenzene-diazonium cation with different nitrite concentrations. They observed increasing yields of the resulting dinitrobenzene with an increasing molar ratio of nitrite to the diazonium cation (experimental range of 40:1 to 250:1), which is generally in accordance with the results in the presented study.

At first sight, the concentration profiles of the TPs may be interpreted in terms of the desamino-SMX serving as a precursor for 4-nitro-SMX formation. However, during a degradation experiment with desamino-SMX under conditions similar to the denitrification experiment with SMX (data not shown), no 4-nitro-SMX was formed within 42 days. Therefore, the compound can be excluded to be an intermediate TP of SMX to 4-nitro-SMX.

As demonstrated in Figure 5.1a, a molar imbalance of SMX and the observed TPs was noticeable from day 2 until the end of the experiment and may be attributed to additionally formed and not yet identified TPs. Furthermore, although SMX was no longer detected, the concentration of 4-nitro-SMX still increased from experiment day 10 to 25. This gives further indication for the presence of intermediate transformation products of SMX to 4-nitro-SMX. However, the diazonium cation of SMX and any other intermediates could not clearly be identified. Lammerding *et al.* (1982) proposed that cellular compounds may trap a significant amount of diazonium ions, which could explain the failure to detect other TPs in the presented study. Furthermore, due to the fast reaction kinetics of substituted aromatic diazonium cations with nitrite (Opgenorth and Rüchardt, 1974), it is unlikely to detect this very reactive compound at all.

As additional or complementary explanation of the molar imbalance, sorption to the sediment may be more relevant for 4-nitro-SMX and desamino-SMX than for SMX and the sediment may have retained a considerable amount of the two compounds. As predicted by Scifinder®, both TPs demonstrate higher log K_{OW}

values than SMX (Table 5.1). Furthermore, nitroaromatic compounds may sorb specifically to natural clay minerals (Haderlein *et al.*, 1996). The pKa of desamino-SMX is predicted to be 7 (Table 5.1). This would increase the amount of the neutral species susceptible to hydrophobic sorption at neutral water pH.

5.3.2 Retransformation of 4-nitro-SMX

At day 87 of the nitrate-reduction experiment with SMX, nitrate was still present but nitrite was no longer detected (Figure 5.1b). Surprisingly, SMX reappeared at the same time and its concentration recovered to 53 ± 16% of the initial concentration. Due to the high effort to minimize adverse matrix effects during ESI-MS/MS analysis (isotope-labeled internal standard, matrix calibration, slow gradient and sample dilution) analytical issues as a reason for this observation can virtually be excluded.

The reduction of 4-nitro-SMX to its corresponding amino-compound and thus the retransformation of 4-nitro-SMX to SMX was postulated. To verify the hypothesized retransformation, an additional anoxic water/sediment test with 4-nitro-SMX in absence of the denitrifying conditions described in section 5.2.4 was conducted. The results presented in Figure 5.2 clearly demonstrate the retransformation potential of 4-nitro-SMX. No dissolved oxygen was detected in any of the microcosms. The pH was 7.2–7.6 and the redox potential (Eh) was −200 mV during the experiment.

The formation of aromatic amines from nitroaromatic compounds under reducing conditions was already demonstrated in different studies (Heijman *et al.*, 1995; Rügge *et al.*, 1998). It is assumed that the reduction of aromatic nitro groups (Ph-NO_2) occurs abiotically (Macalady *et al.*, 1986). Heijman *et al.* (1995) demonstrated the reduction of ten different monosubstituted nitrobenzenes in laboratory aquifer columns with all compounds being stoichiometrically reduced to their corresponding amino compounds. The molar imbalance of 4-nitro-SMX and SMX (Figure 5.2) may be attributed to a degradation of SMX to other TPs (Baumgarten *et al.*, 2011; Mohatt *et al.*, 2011) or a different pathway of 4-nitro-SMX degradation.

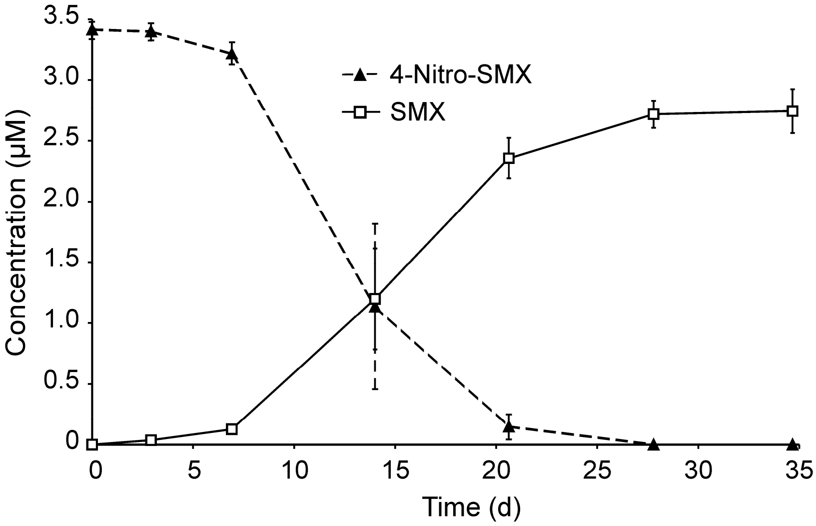


Figure 5.2: Water/sediment experiment on the 4-nitro-SMX retransformation: concentrations of 4-nitro-SMX and SMX. Mean values of two batches per time point, error bars represent the respective concentration range plus the analytical error.

5.3.3 Environmental monitoring and relevance of the presented study

In total, 62 spring water samples in a time series spanning six months (May to October 2010) from the same spring were analyzed. Desamino-SMX, 4-nitro-SMX and SMX were detected in 4, 6 and 3 samples, respectively. The respective concentration ranges were 2.9–7.7 ng L^{-1}, 3.4–5.4 ng L^{-1} and 2.1–9.6 ng L^{-1}.

The spring water analysis clearly demonstrated that the TPs, which were detected in the batch experiment, are also present in the environment. During the period of investigation the concentration ranges of SMX and its TPs in the spring water were in the same order of magnitude, highlighting the need and benefit of including TPs in environmental studies. This measure could substantially improve the understanding of the environmental occurrence, fate and behavior of SMX. In the investigated period the nitrate concentration range was 12.2–14.9 mg L^{-1}. However, there was no significant correlation of nitrate and the occurrence of any of the organic contaminants. Nitrite was not determined. It is yet unclear if the TPs were formed within the karst aquifer or if they were introduced into the system. Furthermore, due to the heterogeneity of the aquifer (Geyer *et al.*, 2007) it is difficult to specify the location of transformation. Einsiedl *et al.* (2005), for example, observed the occurrence of significant microbial denitrification processes already above the soil/bedrock interface. Therefore, as one compound may be more reactive than the other, a negative or positive correlation of nitrate, nitrite, and any of the TPs at the spring may not necessarily be observed, not even if the formation occurred within the aquifer.

Interestingly, a co-occurrence of SMX with any of the TPs was not observed, which was also demonstrated in the denitrifying water/sediment test between day 10 and 87 (Figure 5.1a). The concentration ratio of the TPs in the environmental samples was not constant. This was also valid for the denitrifying water/sediment experiment. These observations were possibly related to the residence time of the contamination, the input scenario, the ratio of the initial concentrations of nitrate and SMX, or the hydraulic connectivity of regions with enhanced denitrifying activity within the complex and heterogeneous karst aquifer. The denitrifying activity and thus the yields of NO and nitrite are influenced, for example, by the type of organic substrates, nutrients, and the microorganism species (Blaszczyk, 1993; Knowles, 1982). Therefore, different environments and input scenarios may lead to different concentration patterns of SMX and the TPs and further studies need to be undertaken to investigate the controlling factors of the transformation.

The TP 4-nitro-SMX has the potential to be reduced to the parent compound SMX. During artificial recharge and riverbank filtration the redox conditions in the adjacent aquifer may be variable with time (Massmann *et al.*, 2006). Accordingly, monitoring of SMX and 4-nitro-SMX potentially simplifies and further improves the evaluation of sites for artificial recharge and bank filtration. Furthermore, discrepancies of laboratory and field studies as described by Baumgarten *et al.* (2011) and even a sporadical and irregular occurrence of SMX (Conn *et al.*, 2010) could possibly be explained by the demonstrated retransformation.

A serious aspect derives from the supposed reaction mechanism with NO and nitrite. The structural element 4-amino-benzenesulfonamide is a common feature in many sulfonamide antibiotics and a behavior similar to SMX is expected. A large number of these compounds are used in veterinary medicine. The subsequent use of manure from medicated animals in agriculture bears the risk of groundwater contamination with these compounds together with nitrate and a high organic carbon load (García-Galán *et al.*, 2010).

Regarding the toxicological relevance of SMX in potable water, adverse health effects are usually not expected at the typically encountered ng L^{-1} concentrations (Bruce *et al.*, 2010). However, adverse effects on microbial growth were demonstrated for environmentally relevant concentrations (Underwood *et al.*, 2011). To the authors' knowledge there is currently no data available on the toxicity of the presented TPs. In comparison with the corresponding aromatic amines a variety of different nitro-aromatics are clearly more toxic to methanogenic bacteria (Razo-Flores *et al.*, 1997). As 4-nitro-SMX and desamino-SMX were also identified in the environment, a careful toxicological evaluation of both compounds and studies about their behavior in drinking water treatment is essential.

5.4 Conclusions

- For the first time, corresponding TPs suggested by known abiotic reactions of aromatic amines with nitric oxide (NO) and nitrite (NO_2^-) were also observed being formed from sulfamethoxazole (SMX) under nitrate-reducing conditions.
- The observed TPs desamino-SMX and 4-nitro-SMX were also detected in environmental samples. This emphasizes the significance of the presented study and the study of environmental TPs in general. However, further studies are necessary to investigate, if the formation of the presented TPs also takes place in the environment and/or if there are additional significant sources. Furthermore, other sites need to be investigated for the presence of these compounds.
- Up to now there is no information on the toxic effects and the behavior of the observed TPs in drinking water treatment.
- The TP 4-nitro-SMX has the potential to retransform to SMX. The controversy of SMX degradation in the literature under anoxic conditions might be explained by retransformable TPs like the 4-nitro-SMX. It should be investigated, if there are additional TPs with retransformation potential. Furthermore, there is strong reason to suspect that other sulfonamide antibiotics produce similar TPs and further studies are needed to determine their occurrence and effects in the environment.

5.5 Acknowledgments

The authors are very grateful to Peter Knaus from the water supply company Hermentingen for his assistance in sampling and to Reinhard Machinek (University of Göttingen, Chemistry Department) for NMR product analysis. The presented study was funded by the European Commission (contract number

518118-1, GABARDINE, "Groundwater Artificial Recharge Based on Alternative Sources of Water: Advanced Integrated Technologies and Management"), and by the German Federal Ministry of Education and Research (promotional references No. 02WM0802 & 02WM1081, SMART, "Sustainable and Integral Management of Available Water Resources Using Innovative Technologies" and promotional reference No. 02WRS1277A, AGRO, "Risikomanagement von Spurenstoffen und Krankheitserregern in ländlichen Karsteinzugsgebieten"). S.P. acknowledges the contract from the Ramón y Cajal Program of the Spanish Ministry of Science and Innovation. M.B. acknowledges AGAUR (Generalitat de Catalunya, Spain) for the financial support through an FI pre-doctoral grant.

5.6 References

Barbieri, M., Carrera, J., Sanchez-Vila, X., Ayora, C., Cama, J., Köck-Schulmeyer, M., López de Alda, M., Barceló, D., Tobella Brunet, J., Hernández García, M., 2011. Microcosm experiments to control anaerobic redox conditions when studying the fate of organic micropollutants in aquifer material. Journal of Contaminant Hydrology 126 (3–4), 330–345.

Baumgarten, B., Jährig, J., Reemtsma, T., Jekel, M., 2011. Long term laboratory column experiments to simulate bank filtration: factors controlling removal of sulfamethoxazole. Water Research 45 (1), 211–220.

Bester, K., 2008. Quantification with HPLC–MS/MS for environmental issues: quality assurance and quality assessment. Analytical and Bioanalytical Chemistry 391 (1), 15–20.

Blaszczyk, M., 1993. Effect of medium composition on the denitrification of nitrate by Paracoccus denitrificans. Applied and Environmental Microbiology 59 (11), 3951–3953.

Boreen, A.L., Arnold, W.A., McNeill, K., 2004. Photochemical fate of sulfa drugs in the aquatic environment: sulfa drugs containing five-membered heterocyclic groups. Environmental Science and Technology 38 (14), 3933–3940.

Bruce, G.M., Pleus, R.C., Snyder, S.A., 2010. Toxicological relevance of pharmaceuticals in drinking water. Environmental Science and Technology 44 (14), 5619–5626.

Brückner, R., 2004. Reaktionsmechanismen, 3rd, ed.; Elsevier GmbH: München, p. 247.

Conn, K.E., Siegrist, R.L., Barber, L.B., Meyer, M.T., 2010. Fate of trace organic compounds during vadose zone soil treatment in an onsite wastewater system. Environmental Toxicology and Chemistry 29 (2), 285–293.

Davis, J.W., Gonsior, S.J., Markham, D.A., Friederich, U., Hunziker, R.W., Ariano, J.M., 2006. Biodegradation and product identification of [14C]hexabromocyclododecane in wastewater sludge and freshwater aquatic sediment. Environmental Science and Technology 40 (17), 5395–5401.

Drillia, P., Dokianakis, S.N., Fountoulakis, M.S., Kornaros, M., Stamatelatou, K., Lyberatos, G., 2005a. On the occasional biodegradation of pharmaceuticals in the activated sludge process: the example of the antibiotic sulfamethoxazole. Journal of Hazardous Materials 122 (3), 259–265.

Drillia, P., Stamatelatou, K., Lyberatos, G., 2005b. Fate and mobility of pharmaceuticals in solid matrices. Chemosphere 60 (8), 1034–1044.

Einsiedl, F., Maloszewski, P., Stichler, W., 2005. Estimation of denitrification potential in a karst aquifer using the 15N and 18O isotopes of NO_3^-. Biogeochemistry 72 (1), 67–86.

European Commision (2002). Commision Decision 2002/657/EC implementing Council Directive 96/23/EC concerning performance of analytical methods and the interpretation of results. Official Journal of the European Community L221/8.

Gao, J., Pedersen, J.A., 2005. Adsorption of sulfonamide antimicrobial agents to clay minerals. Environmental Science and Technology 39 (24), 9509–9516.

García-Galán, M.J., Díaz-Cruz, M.S., Barceló, D., 2009. Combining chemical analysis and ecotoxicity to determine environmental exposure and to assess risk from sulfonamides. Trends in Analytical Chemistry 28 (6), 804–819.

García-Galán, M.J., Garrido, T., Fraile, J., Ginebreda, A., Díaz-Cruz, M.S., Barceló, D., 2010. Simultaneous occurrence of nitrates and sulfonamide antibiotics in two ground water bodies of Catalonia (Spain). Journal of Hydrology 383 (1–2), 93–101.

Geyer, T., Birk, S., Licha, T., Liedl, R., Sauter, M., 2007. Multitracer test approach to characterize reactive transport in karst aquifers. Ground Water 45 (1), 36–45.

Grünheid, S., Amy, G., Jekel, M., 2005. Removal of bulk dissolved organic carbon (DOC) and trace organic compounds by bank filtration and artificial recharge. Water Research 39 (14), 3219–3228.

Haderlein, S.B., Weissmahr, K.W., Schwarzenbach, R.P., 1996. Specific adsorption of nitroaromatic explosives and pesticides to clay minerals. Environmental Science and Technology 30 (2), 612–622.

Heberer, T., 2002. Occurrence, fate, and removal of pharmaceutical residues in the aquatic environment: a review of recent research data. Toxicology Letters 131 (1–2), 5–17.

Heberer, T., Massmann, G., Fanck, B., Taute, T., Dünnbier, U., 2008. Behaviour and redox sensitivity of antimicrobial residues during bank filtration. Chemosphere 73 (4), 451–460.

Heijman, C.G., Grieder, E., Holliger, C., Schwarzenbach, R.P., 1995. Reduction of nitroaromatic compounds coupled to microbial iron reduction in laboratory aquifer columns. Environmental Science and Technology 29 (3), 775–783.

Heinz, B., Birk, S., Liedl, R., Geyer, T., Straub, K.L., Andresen, J., Bester, K., Kappler, A., 2009. Water quality deterioration at a karst spring (Gallusquelle, Germany) due to combined sewer overflow: evidence of bacterial and micro-pollutant contamination. Environmental Geology 57 (4), 797–808.

Hillebrand, O., Nödler, K., Licha, T., Sauter, M., Geyer, T., 2012. Caffeine as an indicator for the quantification of untreated wastewater in karst systems. Water Research 46 (2), 395–402.

Hollocher, T.C., 1982. The pathway of nitrogen and reductive enzymes of denitrification. Antonie van Leeuwenhoek International Journal of General and Molecular Microbiology 48 (6), 531–544.

Höltge, S., Kreuzig, R., 2007. Laboratory testing of sulfamethoxazole and its metabolite acetyl-sulfamethoxazole in soil. Clean: Soil, Air, Water 35 (1), 104–110.

Itoh, T., Matsuya, Y., Nagata, K., Ohsawa, A., 1996. Reductive deamination of aromatic amines with nitric oxide (NO). Tetrahedron Letters 37 (24), 4165–4168.

Knowles, R., 1982. Denitrification. Microbiology and Molecular Biology Reviews 46 (1), 43–70.

Kolpin, D.W., Furlong, E.T., Meyer, M.T., Thurman, E.M., Zaugg, S.D., Barber, L.B., Buxton, H.T., 2002. Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999–2000: a national reconnaissance. Environmental Science and Technology 36 (6), 1202–1211.

Lammerding, A.M., Bunce, N.J., Merrick, R.L., Corke, C.T., 1982. Structural effects on the microbial diazotization of anilines. Journal of Agricultural and Food Chemistry 30 (4), 644–647.

Letzel, M., 2008. Behavior of priority organic compounds included in the European Water Framework Directive in wastwater treatment plants and in surface waters (original title in German: Verhalten prioritärer organischer Stoffe der Wasserrahmenrichtlinie in Kläranlagen und beim Eintrag in oberirdische Gewässer); Report by the Bavarian Environment Agency: Munich, Germany.

Li, B., Zhang, T., 2010. Biodegradation and adsorption of antibiotics in the activated sludge process. Environmental Science and Technology 44 (9), 3468–3473.

Macalady, D.L., Tratnyek, P.G., Grundl, T.J., 1986. Abiotic reduction reactions of anthropogenic organic chemicals in anaerobic systems: a critical review. Journal of Contaminant Hydrology 1 (1–2), 1–28.

Massmann, G., Greskowiak, J., Dünnbier, U., Zuehlke, S., Knappe, A., Pekdeger, A., 2006. The impact of variable temperatures on the redox conditions and the behaviour of pharmaceutical residues during artificial recharge. Journal of Hydrology 328 (1–2), 141–156.

McCarty, G.W., Mookherji, S., Angier, J.T., 2007. Characterization of denitrifying activity in zones of groundwater exfiltration within a riparian wetland ecosystem. Biology and Fertility of Soils 43 (6), 691–698.

McKnight, G.M., Smith, L.M., Drummond, R.S., Duncan, C.W., Golden, M., Benjamin, N., 1997. Chemical synthesis of nitric oxide in the stomach from dietary nitrate in humans. Gut 40 (2), 211–214.

Mohatt, J.L., Hu, L., Finneran, K.T., Strathmann, T.J., 2011. Microbially mediated abiotic transformation of the antimicrobial agent sulfamethoxazole under iron-reducing soil conditions. Environmental Science and Technology 45 (11), 4793–4801.

Myers, R.J.K., McGarity, J.W., 1971. Factors influencing high denitrifying activity in the subsoil of solodized solonetz. Plant and Soil 35 (1–3), 145–160.

Nödler, K., Licha, T., Bester, K., Sauter, M., 2010. Development of a multi-residue analytical method, based on liquid chromatography–tandem mass spectrometry, for the simultaneous determination of 46 micro-contaminants in aqueous samples. Journal of Chromatography A 1217 (42), 6511–6521.

Nödler, K., Licha, T., Fischer, S., Wagner, B., Sauter, M., 2011. A case study on the correlation of micro-contaminants and potassium in the Leine River (Germany). Applied Geochemistry 26 (12), 2172–2180.

Opgenorth, H.-J., Rüchardt, C., 1974. Aromatische Diazoverbindungen, V1) Reaktion aromatischer Diazoniumsalze mit Nitritionen2). Justus Liebigs Annalen der Chemie 8, 1333–1347.

Pereira, R., Pereira, L., van der Zee, F.P., Madalena Alves, M., 2011. Fate of aniline and sulfanilic acid in UASB bioreactors under denitrifying conditions. Water Research 45 (1), 191–200.

Pérez, S., Eichhorn, P., Aga, D.S., 2005. Evaluating the biodegradability of sulfamethazine, sulfamethoxazole, sulfathiazole, and trimethoprim at different stages of sewage treatment. Environmental Toxicology and Chemistry 24 (6), 1361–1367.

Radke, M., Lauwigi, C., Heinkele, G., Mürdter, T.E., Letzel, M., 2009. Fate of the antibiotic sulfamethoxazole and its two major human metabolites in a water sediment test. Environmental Science and Technology 43 (9), 3135–3141.

Razo-Flores, E., Donlon, B., Lettinga, G., Field, J.A., 1997. Biotransformation and biodegradation of N-substituted aromatics in methanogenic granular sludge. FEMS Microbiology Reviews 20 (3–4), 525–538.

Rieder, M.J., Uetrecht, J., Shear, N.H., Spielberg, S.P., 1988. Synthesis and *in vitro* toxicity of hydroxylamine metabolites of sulfonamides. Journal of Pharmacology and Experimental Therapeutics 244 (2), 724–728.

Rügge, K., Hofstetter, T.B., Haderlein, S.B., Bjerg, P.L., Knudsen, S., Zraunig, C., Mosbæk, H., Christensen, T.H., 1998. Characterization of predominant reductants in an anaerobic leachate-contaminated aquifer by nitroaromatic probe compounds. Environmental Science and Technology 32 (1), 23–31.

Seo, D.C., DeLaune, R.D., 2010. Fungal and bacterial mediated denitrification in wetlands: influence of sediment redox conditions. Water Research 44 (8), 2441–2450.

Underwood, J.C., Harvey, R.W., Metge, D.W., Repert, D.A., Baumgartner, L.K., Smith, R.L., Roane, T.M., Barber, L.B., 2011. Effects of the antimicrobial sulfamethoxazole on groundwater bacterial enrichment. Environmental Science and Technology 45 (7), 3096–3101.

Watanabe, N., Bergamaschi, B.A., Loftin, K.A., Meyer, M.T., Harter, T., 2010. Use and environmental occurrence of antibiotics in freestall dairy farms with manured forage fields. Environmental Science and Technology 44 (17), 6591–6600.

Woolley Jr., J.L., Sigel, C.W., 1982. The role of dietary nitrate and nitrite in the reductive deamination of sulfadiazine by the rat, guinea pig, and neonatal calf. Life Sciences 30 (25), 2229–2234.

Chapter 6

6 General conclusions and perspectives

Drinking water suppliers need to be aware of potential contaminations in their raw water resources in order to provide high water quality and to minimize water treatment efforts. The use of micro-contaminants as water quality indicators has aroused great interest in the scientific community and selected compounds such as caffeine and carbamazepine were already used for the sensitive detection and even the quantification of a wastewater contamination in groundwater and surface water. Micro-contaminants are suggested to be source specific and thus offer the potential to be employed as indicators for source delineation in monitoring groundwater and surface water quality, its decline or improvement. Following this approach, indicators for different land uses can provide comprehensive information about a water body on the catchment scale. The applications of reactive indicators to detect a wider range of groundwater processes on the catchment scale are still scarce. However, laboratory experiments on the redox-specific transformation of micro-contaminants convincingly demonstrated the great potential of using selective reaction products for the indication of the corresponding boundary conditions. Therefore, the identification and selection of appropriate indicator compounds need intensive research to prove that the selected compounds meet the decisive indicator criteria.

6.1 Analysis of micro-contaminants

In order to be applied as indicators, sensitive and selective detection methods for micro-contaminant quantification must be readily available. The recent developments in extraction, separation, and detection techniques enabled the detection of trace concentrations of highly polar organic compounds and the detection of concentrations in the ng L^{-1} range. While they are still impressive, these detection limits have become standard in organic analysis. However, true multi-residue analytical methods covering indicators for a high variety of compounds are still an exception.

In contrast to the vast majority of analytical methods focusing on micro-contaminants, the switching electrospray ionization technique, a generic eluent composition (methanol and 0.015% formic acid), and one single extraction protocol were used in the in this work developed chemical analytical method. This enabled the simultaneous extraction and determination of 46 analytes belonging to a high variety of contributing sources within a catchment and therefore, a striking amount of information on the geological/natural system can be obtained from just one single injection. Furthermore, the hereby-achieved intercomparability of the individual analyte concentrations also allows for the interpretation of analyte ratios (e.g. atrazine/desethylatrazine) for source delineation and the distinction between attenuation and dilution/mixing processes.

However, it is recommended to adapt the method according to local characteristics. After using the developed method for the analysis of more than 3000 samples from very different regions (Brazil, Germany, Greece, Italy, the Middle East, Spain, USA) it became apparent that local prescription practice leads to significantly different and characteristic analyte patterns (fingerprints). Considering equipment-specific limitations, analytical methods are usually not static constructions and other compounds can further be integrated as analytes. This was demonstrated in Chapter 5 where the transformation products (TP) of sulfamethoxazole (SMX) desamino-SMX and 4-nitro-SMX were successfully integrated. In connection with two consecutive and recently completed studies atenololic acid, a TP of the β-blockers atenolol and metoprolol, was also included within the in this work developed methodology (Barbieri *et al.*, 2012a; Schaffer *et al.*, 2012).

6.2 Laboratory studies on the transformation of micro-contaminants

In general, the identification of (environmental) TPs of already known micro-contaminants is one of the most important and challenging tasks in cutting-edge environmental science. A degradation study considering only the decreasing concentration of an exposed compound but not further elucidating the true fate (transformation, mineralization) of the substance is usually of very limited significance. The development of high-resolution mass spectrometers and sophisticated databases allows for the identification of unknown compounds such as TPs. However, both techniques are not essential if a selective reaction pathway can be hypothesized and reference standards of the derived TPs are either commercially available or can be synthesized with acceptable effort. In the presented work the latter approach was followed to investigate the transformation of the antibiotic SMX under denitrifying conditions.

Both hypothesized compounds (desamino-SMX and 4-nitro-SMX) were detected in the microcosms. These findings were new and it is very likely that other sulfonamide antibiotics behave in a similar way. The entire scope of these findings is not yet foreseeable. However, as the environmental impact of this type of emerging contaminants is yet unknown, it is highly recommend to include them into environmental monitoring programs. Furthermore, the surprising retransformation potential of the 4-nitro-SMX is a striking example for how complex the environmental behavior of micro-contaminants can be. In a recently completed and published study it was observed that diclofenac behaves in a similar way (Barbieri *et al.*, 2012b).

Selective reaction products such as the TPs of SMX produced under denitrifying conditions offer the potential to be used as indicators for the respective redox condition (reactive tracer). However, in order to associate unambiguous indicator functionalities to these compounds the understanding of the controlling boundary conditions is essential. Denitrification is a significant redox-process in natural aquifers. However, if processes others than denitrification releasing the reactants NO and NO_2^- (e.g. anammox) dominate, a clear assignment to denitrifying conditions is not possible. Furthermore, there is suspicion that the initial concentrations of easily degradable organic carbon and NO_3^- are controlling factors of the transformation under denitrifying conditions. Current column studies with SMX already demonstrated the significant influence of the initial NO_3^- concentration on the SMX concentration in the column outflow (Banzhaf *et al.*, 2012). The research on this type of TPs is in its early stages and further studies to investigate all controlling boundary conditions are needed.

6.3 Field studies

A field study at the German karst spring Gallusquelle (Swabian Alb) demonstrated that the in this work discovered TPs for SMX are also present in the environment. As the controlling boundary conditions for the transformation are not yet identified, a clear indication for a certain redox-process cannot be drawn from the results. However, as the SMX and TP-fingerprint of the microcosms and the environmental samples showed remarkable similarities, the attempt of using this new type of emerging contaminants as indicators is highly promising.

Fecal contaminations bear a serious threat to water quality and public health. Drinking water suppliers must be aware of the risk associated with the input of untreated wastewater in their raw water resources and sensitive detection methods are required to minimize the risk. Here, a method for the estimation of the untreated wastewater amount in a karst aquifer based on the caffeine concentration at the spring was introduced. The high detection frequency of caffeine demonstrated a frequent wastewater input. In combination with hydrogeological data, information about the general water chemistry, and bulk parameters (e.g. turbidity) the introduced approach is highly valuable for the development and evaluation of risk assessment strategies. Consequently, contamination sources can be detected and eliminated or at least efficient early warning systems can be implemented.

The estimation of untreated wastewater is based on different assumptions. One assumption is that due to the rapid transport and low water temperatures caffeine is not degraded in the flow path to the spring. If this assumption is not correct, the true amount of wastewater is underestimated. Therefore, the same team of authors performed an artificial tracer experiment with caffeine and uranine to identify the half-life of caffeine in this karst aquifer (Hillebrand *et al.*, 2012). The results demonstrated that the degradation of caffeine and thus the amount of wastewater was underestimated in the previous work. This again shows, on the one hand, the importance of knowledge on the controlling boundary conditions in order to use a compound as a (quantitative) indicator. On the other hand, artificial tracer tests with trace concentrations of micro-contaminants can be used to identify the natural attenuation potential of an aquifer. Similar conclusions can be drawn from a recently completed study by Barbieri *et al.* (2012a; and references therein), in which the enzymatic hydrolysis of atenolol was suggested to be independent from the redox-condition. The possibly involved amidases and proteolytic enzymes are ubiquitous in the environment. Therefore, the production of atenololic acid from atenolol could be suggested as an indication for microbial activity in general.

For evaluating the natural attenuation potential of aquifers, surface water bodies, and sites for artificial recharge the input function of the investigated compound must be known. Apart from artificial tracer experiments, the input functions of micro-contaminants are commonly vague. The extensive monitoring program on the temporal and spatial correlation quality between selected micro-contaminants and K^+ in the Leine River clearly demonstrates, that the concentrations of several compounds correlate excellently with K^+-concentrations. A correlation with K^+ indicates that the concentration of the respective micro-contaminant depends only on river discharge. Following this assumption, the prediction of micro-contaminant concentrations at certain locations could be substantially simplified. Regarding bank filtration and the interaction of surface water and groundwater in general, it may be possible to derive input functions of the correlating micro-contaminants. Furthermore, the approach can be used for source delineation. An interesting subsidiary effect in this context was the observation that the concentrations of readily biodegradable compounds (especially caffeine and caffeine metabolites) were often significantly different regarding the investigated riverbank. Depending on various criteria (flow velocity, morphology, etc.) complete mixing of the contamination and the recipient water body requires a certain time and flow path. If these boundary conditions are known, source delineation may be possible from the riverbank-dependent concentration differences.

There may be limitations for the correlation approach with K^+ such as a high geogenic background concentration of K^+, which was proposed for the Wipper River (Germany) and confirmed in the meantime. However, compounds, which correlate with K^+ in a river system, correlate also with each other. Therefore, by using the concentration ratios of correlating micro-contaminants in the introduced 'raw water', the attenuation potential (including both sorption and degradation/transformation) of natural and technical processes for one compound can be evaluated by the comparison with the more stable substance. As confirmed in this work, the highly persistent anticonvulsant carbamazepine is an excellent quantitative wastewater indicator. Therefore, a comparison with carbamazepine would be a very promising option for this approach.

6.4 References

Banzhaf, S. Nödler, K., Licha, T., Krein, A., Scheytt, T., 2012. Redox-sensitivity and mobility of selected pharmaceutical compounds in a low flow column experiment. Science of the Total Environment 438, 113–121.

Barbieri, M., Licha, T., Nödler, K., Carrera, J., Ayora, C., Sanchez-Vila, X., 2012a. Fate of β-blockers in aquifer material under nitrate reducing conditions: batch experiments. Chemosphere 89 (11), 1272–1277.

Barbieri, M., Carrera, J., Ayora, C., Sanchez-Vila, X., Licha, T., Nödler, K., Osorio, V., Pérez, S., Köck-Schulmeyer, M., López de Alda, Barceló, D., 2012b. Formation of diclofenac and sulfamethoxazole reversible transformation products in aquifer material under denitrifying conditions: batch experiments. Science of the Total Environment 426, 256–263.

Hillebrand, O., Nödler, K., Licha, T., Sauter, M., Geyer, T., 2012. Identification of the natural attenuation potential of karst aquifers by an artificial multitracer experiment with caffeine. Water Research 46 (16), 5381–5388.

Schaffer, M., Börnick, H., Nödler, K., Licha, T., Worch, E., 2012. Role of cation exchange processes on the sorption influenced transport of cationic β-blockers in aquifer sediments. Water Research 46 (17), 5472–5482.

Appendix A

Text A1. High performance liquid chromatography-electrospray-tandem mass spectrometry (HPLC-ESI-MS/MS) instrumentation and general instrumental parameters (Nödler et al., 2010).

All instruments were purchased from Varian, Darmstadt, Germany. The HPLC system consisted of a ProStar 410 autosampler and two ProStar 210 pumps. A Polaris C18-Ether column 150 mm×2 mm i.d., 3 µm particle was used for chromatographic separation. An L1200 triple quadrupole with electrospray interface (ESI) was used for detection and quantification. The drying and nebulizing gas pressures were 180×10^3 and 386×10^3 Pa, respectively. The drying gas temperature was set to 280 °C. Argon 5.0 with a pressure of 0.27 Pa was used as the collision gas. The spray and shield voltages were −4.5 kV and −0.5 kV, respectively.

Text A2. Extraction and analysis of the environmental samples (Nödler et al., 2010).

Samples were allowed to settle in the refrigerator (4 °C) for no more than 12 h before extraction. 500 mL of the supernatant was spiked with 100 ng of the internal standard (sulfamethoxazole-$^{13}C_6$) and 5 mL of a pH buffer concentrate (13.4 g L^{-1} potassium dihydrogen phosphate and 6.22 g L^{-1} disodium hydrogen phosphate dihydrate in ultrapure water). OASIS HLB 500 mg from Waters, Eschborn, Germany was used for the extraction. Prior to the extraction the sorbent was conditioned by subsequently flushing with 4 mL methanol and 2×4 mL ultrapure water. An extraction flow rate of 15 mL min^{-1} was applied. In order to remove inorganic salt matrix after the extraction, the sorbent was rinsed twice with 1.5 mL ultrapure water. The sorbent was dried by drawing air through the cartridge under vacuum for 30 min. The cartridge was stored at −18 °C until the elution of analytes. The analytes were subsequently eluted with 2×2 mL methanol and 2×2 mL ethyl acetate. The solvents were evaporated at 40 °C with a gentle stream of nitrogen. The analytes were re-dissolved in 0.8 mL of aqueous 5 mM ammonium acetate solution, containing

4% methanol. Before analysis, the extract was centrifuged at 1500 rpm for 30 min at room temperature.

The instrumentation and individual parameters given in Text A1 were used for analysis. Eluent A was 0.015% formic acid + 5% methanol in ultrapure water and eluent B was methanol. The elution started isocratically for 50 s with 100% A, which was followed by a gradient of 10 s to 95% A. This step was followed by a 39-min linear gradient to 95% B, which was held for 5 min. After a 1 min gradient to 100% A, the system was allowed to equilibrate for 11 min. A flow rate of 0.2 mL min^{-1} and an injection volume of 0.1 mL were applied. The separation was operated at 30 °C.

Text A3. Abiotic aqueous experiments on SMX transformation with nitrite at different pH values.

A sterile aqueous solution of SMX and nitrite was incubated at neutral and acidic pH values. At neutral pH no nitrogen oxide (NO) is produced from nitrite under abiotic conditions (McKnight *et al.*, 1997). Therefore, no transformation of SMX should be observed. However, at pH values below the acid dissociation constant of nitrous acid (HNO_2, pKa ~3.2–3.4) NO is formed and the formation of desamino-SMX should be observed (McKnight *et al.*, 1997; Woolley and Sigel, 1982). After neutralization of this solution nitrite is formed. With nitrite the under acidic conditions proposed diazonium cation of SMX should transform to 4-nitro-SMX (Opgenorth and Rüchardt, 1974).

Experiment at neutral pH: Ultrapure water was purged with argon 5.0 for 1 h to remove dissolved oxygen. After this step, the water was buffered to pH 7.2 (855 mg L^{-1} disodium hydrogen phosphate dihydrate and 240 mg L^{-1} potassium dihydrogen phosphate). The pH value was confirmed by using a pH electrode. Then, 250 µg L^{-1} SMX, 45 mg L^{-1} sodium nitrite (30 mg L^{-1} nitrite), and 60 mg L^{-1} mercury(II) chloride were added. Seven 50-mL volumetric flasks were completely filled (without headspace) with the liquid. The flasks were closed by a glass plug and incubated at 20 ± 1 °C in the dark. On a weekly basis one of these solutions was qualitatively analyzed according to the method described in chapter 2.5 of the main manuscript. According to the hypothesis, none of the TPs were detected over the whole period of 49 days.

Experiment at acidic pH: The buffered solution from the previous experiment was titrated to pH 2.1 by using concentrated orthophosphoric acid. The pH value was confirmed by using a pH electrode. Then, 250 µg L^{-1} SMX, 45 mg L^{-1}

sodium nitrite (30 mg L^{-1} nitrite), and 60 mg L^{-1} mercury(II) chloride were added. After one hour the solution was qualitatively analyzed according to the method described in chapter 2.5 of the main manuscript and as suspected, the presence of desamino-SMX was confirmed. The TP 4-nitro-SMX was not detected. The solution was neutralized (pH 7–7.4) by using 1 M sodium hydroxide, allowed to react for one hour, and analyzed by the LC-MS method. The TP 4-nitro-SMX was now detected.

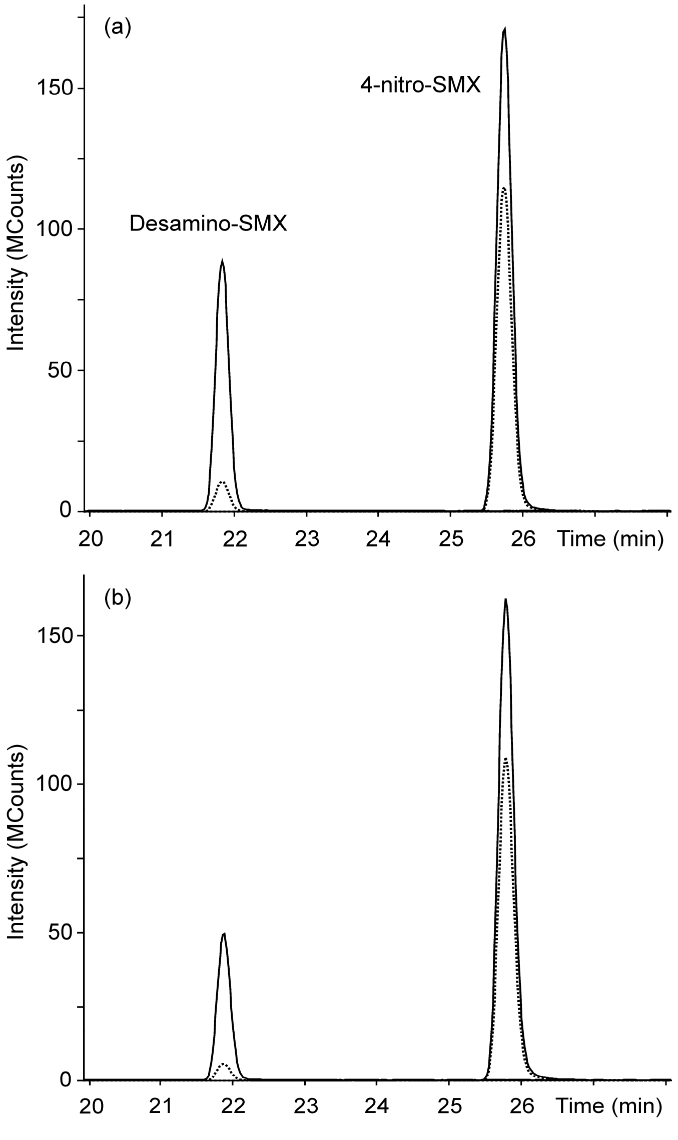


Figure A.1: Chromatogram of a standard (a) and a sample from the denitrifying degradation experiment of SMX (b). Identical retention times and intensity ratios of quantifier (continuous line) and qualifier transitions (dashed line) confirmed the formation of desamino-SMX and 4-nitro-SMX.

References

McKnight, G.M., Smith, L.M., Drummond, R.S., Duncan, C.W., Golden, M., Benjamin, N., 1997. Chemical synthesis of nitric oxide in the stomach from dietary nitrate in humans. Gut 40 (2), 211–214.

Nödler, K., Licha, T., Bester, K., Sauter, M., 2010. Development of a multi-residue analytical method, based on liquid chromatography–tandem mass spectrometry, for the simultaneous determination of 46 micro-contaminants in aqueous samples. Journal of Chromatography A 1217 (42), 6511–6521.

Opgenorth, H.-J., Rüchardt, C., 1974. Aromatische Diazoverbindungen, V1) Reaktion aromatischer Diazoniumsalze mit Nitritionen2). Justus Liebigs Annalen der Chemie 8, 1333–1347.

Woolley Jr., J.L., Sigel, C.W., 1982. The role of dietary nitrate and nitrite in the reductive deamination of sulfadiazine by the rat, guinea pig, and neonatal calf. Life Sciences 30 (25), 2229–2234.

Appendix B

List of all journal articles, conference abstracts, and miscellaneous publications authored or co-authored by me and directly related to the presented work (latest update 01/2013).

Journals (Peer-Reviewed)

Reh R, Licha T, Geyer T, **Nödler K**, Sauter M (2013): Occurrence and distribution of organic micro-pollutants in a complex hydrogeological karst system, results of a two-year intensive study. Science of the Total Environment 443: 438–445.

Niedbala A, Schaffer M, Licha T, **Nödler K**, Börnick H, Worch E, Ruppert H (2013): Influence of competing inorganic cations on the ion exchange of the monovalent organic cation metoprolol on natural sediment. Chemosphere 90 (6): 1945–1951.

Banzhaf S, **Nödler K**, Licha T, Krein A, Scheytt T (2012): Redox-sensitivity and mobility of selected pharmaceutical compounds in a low flow column experiment. Science of the Total Environment 438: 113–121.

Schaffer M, Börnick H, **Nödler K**, Licha T, Worch E (2012): Role of cation exchange processes on the sorption influenced transport of cationic β-blockers in aquifer sediments. Water Research 46 (17): 5472–5482.

Barbieri M, Carrera J, Ayora C, Sanchez-Vila X, Licha T, **Nödler K**, Osorio V, Pérez S, Köck-Schulmeyer M, López de Alda M, Barceló D (2012): Effect of denitrifying conditions on the fate of the pharmaceuticals diclofenac and sulfamethoxazole in aquifer material: batch experiments. Science of the Total Environment 426: 256–263.

Barbieri M, Licha T, **Nödler K**, Carrera J, Ayora C, Sanchez-Vila X (2012): Fate of β-blockers in aquifer material under nitrate reducing conditions: batch experiments. Chemosphere 89 (11): 1272–1277.

Hillebrand O, **Nödler K**, Licha T, Sauter M, Geyer T (2012): Caffeine as an indicator for the quantification of untreated wastewater in karst systems. Water Research 46 (2): 395–402.

Hillebrand O, **Nödler K**, Licha T, Sauter M, Geyer T (2012): Identification of the attenuation potential of a karst aquifer by an artificial dualtracer experiment with caffeine. Water Research 46 (16): 5381–5388.

Nödler K, Licha T, Barbieri M, Pérez S (2012): Evidence for the microbially mediated abiotic formation of reversible and non-reversible sulfamethoxazole transformation products during denitrification. Water Research 46 (7): 2131–2139.

Nödler K, Licha T, Fischer S, Wagner B, Sauter M (2011): A case study on the correlation of micro-contaminants and potassium in the Leine River (Germany). Applied Geochemistry 26 (12): 2172–2180.

Nödler K, Licha T, Bester K, Sauter M (2010): Development of a multi-residue analytical method, based on liquid chromatography–tandem mass spectrometry, for the simultaneous determination of 46 micro-contaminants in aqueous samples. Journal of Chromatography A 1217 (42): 6511–6521.

Conference Abstracts

Nödler K, Licha T, Barbieri M, Pérez S (2012): Nachweis eines reversiblen und eines irreversiblen Transformationsproduktes des Sulfamethoxazols unter denitrifizierenden Bedingungen. Gemeinsame Jahrestagung der SETAC GLB und der Fachgruppe Umweltchemie und Ökotoxikologie der GDCh, Leipzig, Germany, p. 144.

Reh R, Geyer T, Licha T, **Nödler K**, Sauter M (2012): Organische Spurenstoffe als Transport-Indikatoren in einem urban geprägten komplexen Karstsystem. Schriftenreihe der Deutschen Gesellschaft für Geowissenschaften Heft 78 anlässlich der Jahrestagung 2012 der Fachsektion Hydrogeologie in der Deutschen Gesellschaft für Geowissenschaften (FH-DGG), Dresden, Germany, p. 49.

Hillebrand O, **Nödler K**, Licha T, Geyer T (2012): Multitracer-Test zur Bestimmung von Transport und *in-situ* Abbau organischer Spurenstoffe am Beispiel von Coffein. Schriftenreihe der Deutschen Gesellschaft für Geowissenschaften Heft 78 anlässlich der Jahrestagung 2012 der Fachsektion Hydrogeologie in der Deutschen Gesellschaft für Geowissenschaften (FH-DGG), Dresden, Germany, p. 105.

Hillebrand O, **Nödler K**, Licha T, Sauter M, Geyer T (2012): Anwendung von Coffein als Indikator zur Quantifizirung von versickerndem Abwasser in Karstsystemen – Ein Fallbeispiel. Schriftenreihe der Deutschen Gesellschaft für Geowissenschaften Heft 78 anlässlich der Jahrestagung 2012 der Fachsektion Hydrogeologie in der Deutschen Gesellschaft für Geowissenschaften (FH-DGG), Dresden, Germany, p. 145.

Schaffer M, Niedbala A, **Nödler K**, Licha T, Börnick H, Fona Z, Worch E (2012): Untersuchungen zum Kationenaustauschverhalten des basischen Beta-Blockers Metoprolol an natürlichen Sorbentien. Schriftenreihe der Deutschen Gesellschaft für Geowissenschaften Heft 78 anlässlich der Jahrestagung 2012 der Fachsektion Hydrogeologie in der Deutschen Gesellschaft für Geowissenschaften (FH-DGG), Dresden, Germany, p. 187.

Banzhaf S, **Nödler K**, Licha T, Krein A, Scheytt T (2012): Säulenversuche zur Redoxsensitivität und zum Transportverhalten ausgewählter Pharmazeutika. Schriftenreihe der Deutschen Gesellschaft für Geowissenschaften Heft 78 anlässlich der Jahrestagung 2012 der Fachsektion Hydrogeologie in der Deutschen Gesellschaft für Geowissenschaften (FH-DGG), Dresden, Germany, p. 192.

Nödler K, Licha T, Barbieri M, Pérez S (2012): Nachweis eines reversiblen und eines irreversiblen Transformationsproduktes des Sulfamethoxazols unter denitrifizierenden Bedingungen. Referate zur Jahrestagung 2012 der Wasserchemischen Gesellschaft, Neu-Ulm, pp. 139–143.

Schaffer M, Niedbala A, **Nödler K**, Licha T, Börnick H, Worch E (2012): Sorptionsverhalten organischer Kationen an natürlichem Sediment am Beispiel des Beta-Blockers Metoprolol. Referate zur Jahrestagung 2012 der Wasserchemischen Gesellschaft, Neu-Ulm, pp. 410–414.

Hillebrand O, **Nödler K**, Licha T, Geyer T (2012): Abschätzungen des Transportverhaltens und der *in-situ* Degradation von Coffein in einem Karstgrundwasserleiter mithilfe eines Multitracer-Versuchs. Referate zur Jahrestagung 2012 der Wasserchemischen Gesellschaft, Neu-Ulm, pp. 365–369.

Steiniger B, Sachse R, Börnick H, **Nödler K**, Licha T, Cavalcanti CGB, Cavalcanti CP, Worch E (2012): Untersuchungen zum Verbleib organischer Spurenstoffe im Paranoá-See, Brasília, Brasilien . ein Beispiel für die Anwendung einfacher Seemodelle. Referate zur Jahrestagung 2012 der Wasserchemischen Gesellschaft, Neu-Ulm, pp. 370–374.

Banzhaf S, **Nödler K**, Licha T, Krein A, Scheytt T (2012): Redox-sensitivity and mobility of selected pharmaceutical compounds in a laboratory column experiment. Geophysical Research Abstracts 14, EGU2012-70, EGU General Assembly 2012, Vienna, Austria.

Doummar J, Baierl M, **Nödler K**, Licha T, Sauter M, Geyer T (2012): Carbamazepine as indicator for potential short-term contamination of karst springs. Geophysical Research Abstracts 14, EGU2012-11158, EGU General Assembly 2012, Vienna, Austria.

Hillebrand O, **Nödler K**, Licha T, Geyer T (2012): Multitracer test for the determination of transport and *in-situ* degradation of organic micro-contaminants in karst aquifers on the example of caffeine. Geophysical Research Abstracts 14, EGU2012-17-1, EGU General Assembly 2012, Vienna, Austria.

Reh R, Licha T, **Nödler K**, Geyer T, Sauter M (2012): Organic compounds as indicators for transport in an urban characterized complex karst system. Geophysical Research Abstracts 14, EGU2012-9440-1, EGU General Assembly 2012, Vienna, Austria.

Valhondo C, **Nödler K**, Köck-Schulmeyer M, Hernandez M, Licha T, Ayora C, Carrera J (2012): Removal of organic micropollutants in an artificial recharge system. Geophysical Research Abstracts 14, EGU2012-12123, EGU General Assembly 2012, Vienna, Austria.

Barbieri M, Carrera J, Ayora C, Sanchez-Vila X, Licha T, **Nödler K**, Osorio V, Pérez S, Köck Schulmeyer M, López de Alda M, Barceló D (2011): Entender el destino y el comportamiento de productos farmacéuticos seleccionados en el material del suelo-acuífero antes de la recarga artificial (Understanding the fate and behaviour of selected pharmaceuticals in soil-aquifer material prior to artificial recharge)" La Segunda Conferencia SCARCE, 28—29.11.2011, Madrid, Spain.

Hillebrand O, **Nödler K**, Licha T, Geyer T, Sauter M (2011): Application of caffeine as indicator for the quantification of recharging wastewater in karst systems - A case study. Geophysical Research Abstracts 13, p. 3377.

Licha T, **Nödler K**, Geyer T, Reh R, Hillebrand O (2011): Spurenorganika im Wasserkreislauf – neue Perspektiven in der Hydrogeologie. Unterlagen zum Seminar Altlasten und Schadensfälle – Neue Entwicklungen, Hessisches Landesamt für Umwelt und Geologie, Wetzlar, Germany, May 24.–25.2011.

Licha T, **Nödler K**, Voutsa D, Samara C (2011): Emerging pollutants fort he evaluation of the quality status of marine environment and identification of pollution sources. Proceedings 4th Environmental Conference of Macedonia, Thessaloniki, Greece, March 18.–20.2011.

Nödler K, Licha T, Fischer S, Wagner B, Sauter M (2011): A case study on the correlation of micro-contaminants and potassium in river Leine (Germany). Geophysical Research Abstracts 13, p. 5929.

Nödler K, Licha T, Sauter M (2011): Anthropogene organische Spurenstoffe in der Bucht von San Francisco. Referate zur Jahrestagung 2011 der Wasserchemischen Gesellschaft, Norderney, Germany, pp. 218–220.

Nödler K, Licha T, Sauter M (2011): Identification of significant transport processes for organic micropollutant classes during soil aquifer treatment (SAT) – a field study. International Conference on Integrated Water Resources Management, Dresden, Germany.

Schaffer M, Börnick H, Licha T, **Nödler K**, Worch E (2011): Column experiments to simulate the transport of the basic beta-blocker atenolol in sandy aquifers under competitive conditions. Geophysical Research Abstracts 13, p. 3018.

Schaffer M, Börnick H, Licha T, **Nödler K**, Worch E, Sauter M (2011): Säulenversuche zum Stofftransport des basischen Betablockers Atenolol unter gesättigten, kompetitiven Bedingungen. Referate zur Jahrestagung 2011 der Wasserchemischen Gesellschaft, Norderney, Germany, pp. 294–298.

Steiniger B, Sachse R, Börnick H, **Nödler K**, Licha T, Cavalcanti CGB, Cavalcanti CP, Worch E (2011): Modeling the fate of organic micropollutants in a tropical reservoir in Brasilia,

Brazil. International Conference on Integrated Water Resources Management Dresden, Germany.

Steiniger B, Börnick H, Hebben N, Abbt-Braun G, Frimmel F, **Nödler K**, Licha T, Cavalcanti CGB, Cavalcanti CP, Worch E (2011): Investigations on the distribution of emerging organic pollutants in Lake Paranoá - Contributions to the management of the lake. The 12th international specialized conference on watershed & river basin management, Recife, Brazil.

Steiniger B, Börnick H, Hebben N, Abbt-Braun G, Frimmel F, **Nödler K**, Licha T, Cavalcanti CGB, Cavalcanti CP, Worch E (2011): The occurrence of emerging organic pollutants in a tropical reservoir in Brazil - contributions to the management of Lake Paranoá. International Conference on Integrated Water Resources Management Dresden, Germany.

Voutsa D, Licha T, **Nödler K**, Samara C (2011): Emerging pollutants in marine environment. 13th EuCheMS International Conference on Chemistry and the Environment, Zurich, Switzerland, September 11.–15.2011.

Nödler K, Licha T, Fischer S, Sauter M (2010): Zeitliche und räumliche Verteilung anthropogener organischer Spurenstoffe im Einzugsgebiet der Leine. Schriftenreihe der Deutschen Gesellschaft für Geowissenschaften Heft 67 anlässlich der Jahrestagung 2010 der Fachsektion Hydrogeologie in der Deutschen Gesellschaft für Geowissenschaften (FH-DGG), Tübingen, Germany, p. 148.

Nödler K, Licha T, Sauter M (2010): Anthropogene organische Spurenstoffe im küstennahen Bereich mariner Systeme. Schriftenreihe der Deutschen Gesellschaft für Geowissenschaften Heft 67 anlässlich der Jahrestagung 2010 der Fachsektion Hydrogeologie in der Deutschen Gesellschaft für Geowissenschaften (FH-DGG), Tübingen, Germany, p. 149.

Nödler K, Licha T, Fischer S, Sauter M (2010): Zeitliche und räumliche Verteilung anthropogener organischer Spurenstoffe im Einzugsgebiet der Leine. Referate zur Jahrestagung 2010 der Wasserchemischen Gesellschaft, Bayreuth, Germany, pp. 379–383.

Nödler K, Licha T, Sauter M (2010): Anthropogene organische Spurenstoffe im küstennahen Bereich mariner Systeme. Referate zur Jahrestagung 2010 der Wasserchemischen Gesellschaft, Bayreuth, Germany, pp. 401–404.

Nödler K, Licha T, Fischer S, Sauter M (2010): Temporal & spatial distribution patterns of 46 micro-contaminants in a small river catchment. 7th Int. Groundwater Quality (GQ10) Conference 'Groundwater Quality Management in a Rapidly Changing World', Zurich, Switzerland.

Nödler K, Licha T, Sauter M (2010): Identification of significant transport processes for organic micropollutant classes during soil aquifer treatment (SAT) - a controlled field experiment. Geophysical Research Abstracts12, p. 8660.

Nödler K, Licha T, Fischer S, Sauter M (2010): Zeitliche und räumliche Verteilung anthropogener organischer Spurenstoffe im Einzugsgebiet der Leine. Zusammenfassung der Beiträge der 26. Jahrestagung der Deutschen Gesellschaft für Limnologie und der deutschen Sektion der SIL, Bayreuth, Germany, pp. 88–89.

Rahman MA, Ptak T, **Nödler K**, Licha T, Dimitriadis K, Soupilas A, Sauter M (2010): Influence of aquifer properties on water quality changes during infiltration of treated effluent. 7th International Symposium on Managed Aquifer Recharge (ISMAR7), Abu Dhabi, UAE.

Nödler K, Licha T (2009): Maßgeschneiderte LC-MS/MS Analytik für die Indikatorfunktionalität von Organika in Gewässern. Kurzreferate zur Tagung der

Fachgruppe Analytische Chemie der Gesellschaft Deutscher Chemiker (ANAKON 2009), Berlin, Germany, p. 224.

Nödler K, Licha T (2009): Maßgeschneiderte LC-MS/MS Analytik für die Indikatorfunktionalität von Organika in Gewässern. Referate zur Jahrestagung 2009 der Wasserchemischen Gesellschaft, Stralsund, Germany, pp. 145–148.

Barbieri M, Carrera J, Sànchez-Vila X, Ayora C, Cama J, Lopez de Alda M, Barceló D, Köck M, Tobella Brunet J, Valhondo C, Licha T, **Nödler K** (2009): Batch tests on the biodegradation of emerging organic micropollutants. Micropol & Ecohazard 2009, San Francisco, California.

Karsten Nödler

PERSÖNLICHE DATEN

geboren am	15. April 1977
in	Witzenhausen
Familienstand	ledig
Staatsangehörigkeit	Deutsch

HOCHSCHULAUSBILDUNG

01/2008 – 07/2012	**Georg-August-Universität Göttingen** *Promotionsstudium am Geowissenschaftlichen Zentrum Göttingen, Abteilung Angewandte Geologie*
10/2005 – 09/2007	**Universität Duisburg-Essen** *Master-Studium „Water Science“, abgeschlossen mit der Note 1,3* Thema der Masterarbeit: „*Asellus aquaticus* als Passivsammler lipophiler organischer Schadstoffe in belasteten Gewässern“, durchgeführt im Department Wirkungsorientierte Analytik am Helmholtz-Zentrum für Umweltforschung Leipzig und ausgezeichnet mit dem Sachtleben-Award sowie dem Preis für die beste Masterarbeit 2007 im Studiengang „Water Science“.
10/2002 – 09/2005	**Universität Duisburg-Essen** *Bachelor-Studium „Water Science“, abgeschlossen mit der Note 1,3* Thema der Bachelorarbeit: „Monitoring organischer Schadstoffbelastung von Fließ- und Oberflächengewässern mittels SPME und GC/MS – Entwicklung einer Multimethode“, durchgeführt am Institut für Lebensmittelchemie- und technologie der Technischen Universität Graz.

BERUFSAUSBILDUNG & BERUFSERFAHRUNG

08/2001 – 09/2002	**Vesuvius Becker & Piscantor, Großalmeroder Schmelztiegelwerke GmbH** *Anstellung als Chemielaborant*
08/2000 – 07/2001	**Labor für Umweltanalytik UmLab GmbH, Kassel** *Anstellung als Chemielaborant*
09/1997 – 07/2000	**Labor für Umweltanalytik UmLab GmbH, Kassel** *Berufsausbildung als Chemielaborant, abgeschlossen mit Auszeichnung (Note 1,0)*