Fakulta rybářství
a ochrany vod
Faculty of Fisheries
and Protection
of Waters

Jihočeská univerzita
v Českých Budějovicích
University of South Bohemia
in České Budějovice

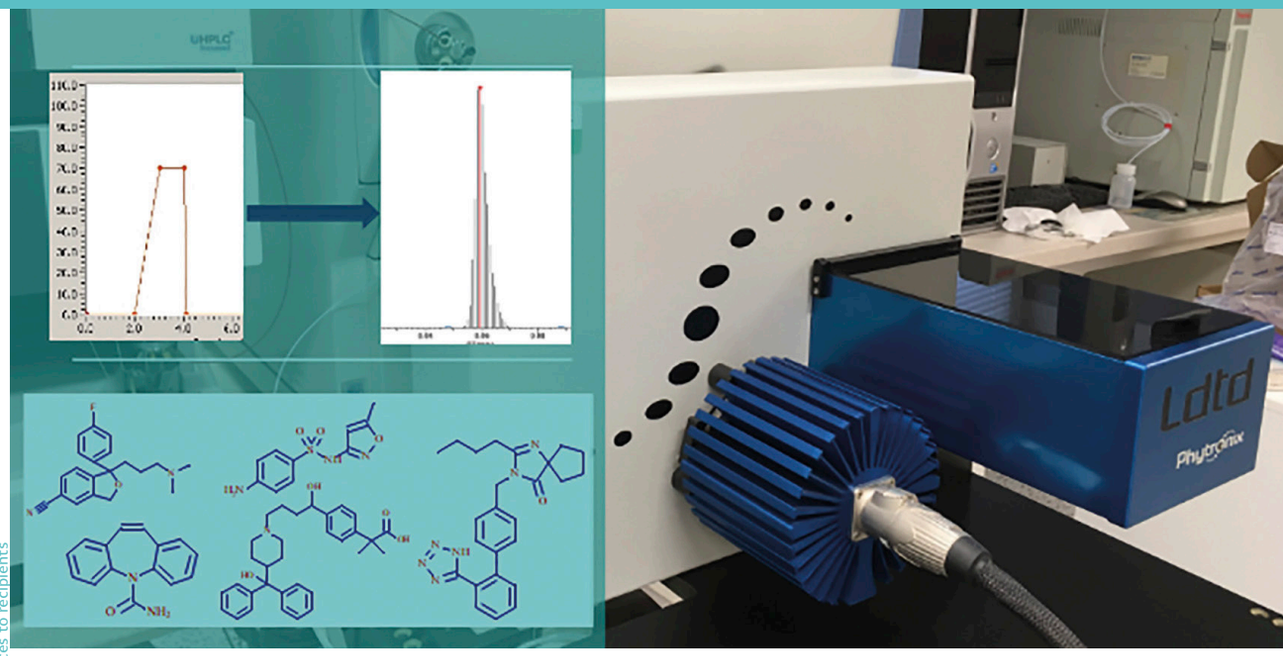
2020

Fakulta rybářství
a ochrany vod
Faculty of Fisheries
and Protection
of Waters

Jihočeská univerzita
v Českých Budějovicích
University of South Bohemia
in České Budějovice

Tracing pharmaceuticals and personal care products (PPCPs) from sources to recipients

Sledování farmaceutik a produktů osobní péče od zdrojů až po recipienty



Adam Bořík

Tracing pharmaceuticals and personal care products (PPCPs) from sources to recipients

Adam Bořík

ISBN 978-80-7514-116-3

Czech Republic, Vodňany, 2020

Fakulta rybářství
a ochrany vod
Faculty of Fisheries
and Protection
of Waters

Jihočeská univerzita
v Českých Budějovicích
University of South Bohemia
in České Budějovice

Tracing pharmaceuticals and personal care products (PPCPs) from sources to recipients

Sledování farmaceutik a produktů osobní péče od zdrojů až po recipienty

Adam Bořík

Czech Republic, Vodňany, 2020

I, Adam Bořík, thereby declare that I wrote the Ph.D. thesis myself using results of my own work or collaborative work of me and colleagues and with help of other publication resources which are properly cited.

I hereby declare that, in accordance with the § 47b Act No. 111/1998 Coll., as amended, I agree with publicizing of my Ph.D thesis in full version electronically in a publicly accessible part of the STAG database operated by the University of South Bohemia in České Budějovice on its web sites, with keeping my copyright to the submitted text of this Ph.D. thesis. I also agree so that the same electronic way, in accordance with above mentioned provision of the Act No. 111/1998 Coll., was used for publicizing reviews of supervisor and reviewers of the thesis as well as record about the progress and result of the thesis defence. I also agree with compering the text of my Ph.D. thesis with a database of theses "Theses.cz" operated by National Register of university theses and system for detecting of plagiarisms.

In Vodňany 14th May, 2020

Supervisor:
Assoc. Prof. Roman Grabic

Consultants:
Kateřina Grabicová, Ph.D., Ganna Fedorova, Ph.D.

Head of Laboratory of Laboratory of Environmental Chemistry and Biochemistry:
Prof. Tomáš Randák

Dean of Faculty of Fisheries and Protection of Waters:
Prof. Pavel Kozák

Board of doctorate study defence with reviewers:
Assoc. Prof. Josef Matěna – head of the board
Prof. Lukáš Kalous – board member
Prof. Petr Ráb – board member
Prof. Ondřej Slavík – board member
Assoc. Prof. Martin Kocour – board member
Assoc. Prof. Tomáš Policar – board member
Assoc. Prof. Zdeněk Adámek – board member

Assoc. Prof. Zdeněk Šimek, Masaryk University, Brno, Czech Republic – thesis reviewer
Assoc. Prof. Jerker Fick, Umea University, Sweden – thesis reviewer

Date, hour and place of Ph.D. defence:
17th September 2020, at 1 p.m., in USB, FFPW, RIFCH, Vodňany, Czech Republic

Name: Adam Bořík

Title of thesis:
Tracing pharmaceuticals and personal care products (PPCPs) from sources to recipients
Sledování farmaceutik a produktů osobní péče od zdrojů až po recipienty

Ph.D. thesis, USB FFPW, RIFCH, Vodňany, 2020, 76 pages, with the summary in English and Czech.
Graphic design & technical realisation: JENA Šumperk, www.jenasumperk.cz
ISBN 978-80-7514-116-3

CONTENT

CHAPTER 1 **7**

General introduction

CHAPTER 2 **23**

Development of a robust extraction procedure for the HPLC-ESI-HRPS determination of multi-residual pharmaceuticals in biota samples

CHAPTER 3 **33**

Determination of citalopram in fish brain tissue: benefits of coupling laser diode thermal desorption with low- and high-resolution mass spectrometry

CHAPTER 4 **45**

Ultrafast laser diode thermal desorption method for analysis of representative pharmaceuticals in soil leachate samples

CHAPTER 5 **55**

General discussion 57

English summary 71

Czech summary 72

Acknowledgements 73

List of publications 74

Training and supervision plan during study 75

Curriculum vitae 76

CHAPTER 1

GENERAL INTRODUCTION

PPCPs definition and potential risk overview

Pharmaceutical and personal care products (PPCPs) represent wide class of compounds including extensively used medicals for human and animal treatment, active compounds of cosmetic products. The PPCPs classification can be based upon their purpose, chemical structure, or physical-chemical properties. By the purpose, PPCPs can be categorized into following groups- antibiotics, antidepressants, anticonvulsants, antineoplastics, beta-blockers, contrast media, disinfectants, fragrances, hormones, lipid regulators, nonsteroidal anti-inflammatory drugs, preservatives, sunscreen agents (Wang and Wang, 2016). Physico-chemical properties of PPCPs are largely diverse (Tarpani and Azapagic, 2018) and consequently it is problematic to generalized that across the pollutant category. Most PPCPs relevant for recipients, respectively for water as investigated sample matrix, are low volatile, moderately polar, and thermally unstable (Ohoro et al., 2019).

Pollution is one of the key aspects resulting in negative changes in environment. Contamination of water ecosystem by PPCPs raised to be an actual topic during nineties (Holm et al., 1995). Previous investigations of water pollution aimed mainly on persistent organic pollutants (POPs), because of their limited degradation, lipid solubility corresponding with bioaccumulation potential and consequent toxicological effects (Wenning and Martello, 2013). Whereas, pharmaceutical compounds are typically water-soluble with lower bioaccumulation rates. The major concerns of pharmaceuticals and other PPCPs are extensive use, increasing production and mostly unknown impact on nontarget organisms in environment.

According to purpose, way of application and consumption on daily base, the most relevant source of PPCPs is human consumption and subsequent production of household wastewater. Despite to wastewater treatment process, up to $\mu g\ L^{-1}$ of these compounds can be found in recipients as consequence of technological limitation of conventional wastewater treatment plant (WWTP) (Golovko et al., 2014a). Although such concentrations seem to be negligible and do not induce acute toxicity, negative impact on water ecosystem has been reported. Active pharmaceuticals ingredients are designed to alter specific physiological pathways which can potentially affect non-target organisms (Petersen, Heiaas and Tollefsen, 2014). The known concern is synthetic estrogens as ethinylestradiol (Jackson and Klerks, 2019) less investigated and more attention deserving group of birth control medication are progestins (Šauer et al., 2018). The ability to alter endocrine regulation of fish reproduction system and consequent effects have been observed within environmentally relevant concentrations (Fent, 2015; A. Lange et al., 2009). Investigation of psychoactive compound effects on aquatic fauna consider occurrence of similar nervous system response as for target organisms (humans and other mammals). This corresponds with some behavioral studies. For example, therapeutic plasma levels of selective serotonin reuptake inhibitor sertraline decrease shelter-seeking behavior of *Pimephales promales* and cause potentially inappropriate reaction on predators (Valenti et al., 2012). Reduction of defense ability was observed also for *Procambarus clarkii* exposed to env. relevant concentration of methamphetamine when individuals exhibit significant reduction of depth and volume of constructed burrows (Guo et al., 2020). Brodin et al. 2017 reported alteration of boldness after exposing *Rutilus rutilus* to env. relevant concentration of oxazepam (Brodin et al. 2017). Moreover, pharmaceuticals occurred as complex mixture of different classes of compounds, parent compounds, and their metabolites and transformation products. It is important to note that multi-component mixture can exhibit synergistic, antagonistic or additive effects compare to individual compound expose (Vasquez et al., 2014).

Sources and of PPCPs

The main source of recipient contamination by PPCPs is considered household wastewater via WWTPs. After usage, these compounds are released into wastewater and WWTPS in form of parent compound or metabolite (especially in case of pharmaceuticals).

The individual wastewater treatment steps are designated according to their order in treatment plant facility. Primary treatment is mechanical process removes floated or settled particles. This first stage can be enhanced with addition of metal salts or other compounds causing agglomeration of suspended solids and colloids. Consequently, ability to eliminate PPCPs is mainly defined by compound sorption to these particles. Absorption and adsorption are two major mechanisms affecting compound affinity. The absorption is related to compound lipophilicity, characterized by the octanol-water partition coefficient (K_{ow}) (Behera et al., 2011), whereas adsorption is characterized by dissociation constant (pKa) related to compound disposition to be dissociated in aqueous phase. Positively charged compounds can be than adsorbed with negatively charged surface of particles (Ternes et al., 2004). Sorption elimination mechanism is also relevant in secondary or so-called biologically treatment with conventional sewage sludge, where PPCPs are sorbed on biological floc. This microbial aggregate is composed of protozoa and bacteria organisms which can assimilate only low molecular weight compound, large molecules must be firstly hydrolyzed by enzymes. Organic matter used for microbial growth is then decomposed along with PPCPs. Biodegradability can be than determined and categorized through pseudo first-order degradation kinetics published by Joss et al. (2006).

PPCPs degradation by microbial extracellular enzymes is limited by some factors. The low PPCPs concentrations at enzyme sub-saturating levels and organisms in nutrient rich environment have limited stimulation initiated adaptation of enzymatic system for low concentrated compounds (Daughton and Ternes, 1999).

The limited ability of conventional WWTP to remove PPCPs is well known and reported in many research papers. For example, study published in 2004 by Carballa et al., investigated removal efficiency of eight pharmaceutical (carbamazepine, diazepam, diclofenac, ibuprofen, naproxen, roxithromycin, sulfamethoxazole and iopromide), three hormones (estrone, 17β-estradiol and 17α-ethinylestradiol) and fragrances (galaxolide, tonalide). Primary treatment was effective only for galaxolide, tonalide 30–50% and 17β-estradiol (20%) according to their higher K_{ow}. The rest of compounds was eliminated particularly during secondary treatment 40–65% except stable contrast media iopromide (Carballa et al., 2004). It has to be mentioned that biodegradation products of PPCPs can exhibit similar or even higher toxicological risk then parent compound. Octyphenol is product of bacterial degradation of nonionic surfactant octylphenol polyethoxylate which oestrogenic activity is ten times lower compare to its product (Kagle et al., 2009). The elimination efficiency is not absolutely constant and can be influenced by factors like hydraulic retention time (Ejhed et al., 2018) physical and chemical conditions- pH (Paul et al., 2014) and temperature. The PPCPs concentration variability on WWTP effluent is then affected by microbial activity (which is temperature depend) and sessional specific consumption trends of some pharmaceuticals. Consequently, during cold season can be observed overall higher concentrations of some micropollutants (Golovko et al., 2014b).

Moreover, negative PPCPs removal efficiency (concentration increase after treatment) of conventional WWTPs was reported for some PPCPs. A work published by Blair et al., 2015 concluded two potential mechanisms of the negative mass balance of PPCPs i) the compounds are released from particles during biological treatment process ii) microbial activity retransforms the compounds into active form (Blair et al., 2015).

Tertiary treatment is additional step that improves water quality before it is discharged to recipients. This process possibly involves reduction of organics, turbidity, nitrogen, phosphorus, metals and pathogens (Gerba and Pepper, 2015). The advanced oxidation process such as ozonation, UV radiation have relatively good elimination efficiency of PPCPs that in many cases overcome 80% (Alharbi et al., 2017; Ternes et al., 2003; de Wilt et al., 2018). Ozonation of water environment produces hydroxyl radical which together with ozone act as oxidizing agents. Ozonation was considered as technique reducing biological activity of PPCPs due to its selective hydroxylation of their functional groups (Oulton, Kohn, and Cwiertny 2010). Unfortunately, PPCPs transformation products can exhibit reduction (X. Chen et al., 2012) as well as enhancement (Hamdi El Najjar et al., 2013; Schlüter-Vorberg et al., 2015) of toxicity compare to precursor. Similarly to ozonation, UV based oxidation is effective technique of PPCPs degradation, but concurrently source of PPCPs by-products with potential toxicity effect (Ellepola et al., 2020).

Sewage sludge is by-product of wastewater treatment process contains nutrients and organic carbon matter make it suitable for agriculture application. Almost 52% of sludge produced in EU are spread on land and 40% for agriculture purpose (Ivanová et al., 2018). Despite good fertilizer properties treated sewage sludge contains ng to μg g^{-1} PPCPs (Tran, Reinhard, and Gin 2018) that make it relevant contamination source of surface water during precipitation events and subsequent runoff (Sabourin et al., 2009). According to application of swine slurry and dairy cattle manure as part of agriculture management, these two matrices can be considered as sources of veterinary pharmaceuticals (Gros et al., 2019) and runoff related vector to recipient as in previous case.

Fate of PPCPs in recipients

PPCPs are not categorized as persistent contaminants but their incomplete elimination and extensive use cause continuous contamination of aquatic environment. These so-called ¨pseudo persistent¨ compounds are therefore constantly present in recipient (as parent compound or transformation products), where they may affect non-target organisms and undergo elimination and/or transformation process.

Photolysis is important process of PPCPs degradation in surface water reported in extensive number of research papers (Buser, Poiger and, Müller 1998; Calisto, Domingues and Esteves, 2011; Dabić, Babić and Škorić, 2019; Lin and Reinhard, 2005; Santoke and Cooper, 2017). Ability of compound to by directly photolyzed is depended on their structural feature able to absorb solar UV radiation- conjugated π systems, heteroatoms aromatic rings and structural moieties, such as phenol, nitro, and napthoxyl groups (Boreen, Arnold and McNeill, 2003). Indirect photolysis is significant for overall PPCPs and most relevant for compounds unable to absorb wavelength above 290 nm. This degradation mechanism involves reactive species ($^1O_2$, O_2^-, ROO•, HO_2• and HO•) accelerating compounds oxidation. Such reactive species can be formed by direct photolysis of dissolved organic matter or nitrate ions (photosensitizers) (Albaigés, Bayona and Radović, 2016; Bai et al., 2018).

Hydrolysis represents abiotic degradation process influenced by temperature, pH value (Loftin et al., 2008) and water-reactive functional group like amine and esters in micropollutant structure (Zhou, Porter and Zhang, 2017). This degradation pathway has been proven as relevant for antibiotic pharmaceutical oxytetracycline (Xuan et al., 2010). Unlike to hydrolysis unavailable sulfonamides (Białk-Bielińska et al., 2012), structure of oxytetracycline included readily hydrolyzed structural features, which make it degradable under environmental conditions (Loftin et al., 2008).

Partitioning is a process of pharmaceutical transition between water and sediment phases. It is well known that major number of PPCPs are weak acid, bases or zwiterions, some of them posses in addition lipophilic moiety in the molecule. Their interactions with solid particles are more complex than sorption of traditional pollutants e.g. POPs. It has been described that electrostatic and hydrophilic soil properties (ion exchange capacity, clay content etc.) play more important role than sorption to organic carbon representing prevailing mechanism of partitioning for POPs (Kodešová et al., 2015). The PPCPs sorption onto sediment and other particles plays important role for compound bioavailability and corresponding effects on non-target organisms. Higher microorganism density compare to water body makes sediments major biodegradation space of PPCPs in recipient, where elimination efficiency depends mainly on aerobic conditions (Kunkel and Radke, 2008). Although biodegradation in surface water does not have same relevance as in wastewater treatment a synergic elimination effect with other degradation pathways can occur (Gan et al., 2014), or can be more significant for compounds resistant to photo and hydrolysis (Kunkel and Radke, 2008).

Efficient analytical approaches are essential for determination of relevant PPCPs contamination pathways, quantitative monitoring and identification of transformation products. Therefore, development of advanced methods and their evaluation is important part of environmental research.

Analytical methods in environmental research

During the last decades, environmental scientists were able to describe and link dangerous potential of PPCPs for water ecosystems. This breakthrough can be achieved due to rapid development of sensitive analytical instruments and data systems. Ability to detect trace level concentrations of complex compounds with their structural confirmation is essential for environmental research and compound prioritization for risk assessment, monitoring and legislation establishment (Caldwell et al., 2014).

The number of analytical approaches in environmental research field use chromatography-based techniques. Gas chromatography with mass spectrometry (GC-MS) is well-established separation and detection method usually applied for investigation of volatile compounds (e.g. POPs) (Y. J. Chen et al., 2020). This technique offers accessibility, selectivity (Mandalakis, Tsapakis and Stephanou, 2001) and electron ionization mass spectra libraries (Sparkman, 1996). However, the PPCPs are categorized mostly as non-volatile compounds, therefore additional sample treatment like derivatization is required for GC-MS analysis (Zwiener, Glauner and Frimmel, 2000). The more suitable alternative for polar and temperature-sensitive compound separation is liquid chromatography (LC). The variable options for stationary and mobile phase selection allow compounds separation within wide polarity range. Relatively low polar compounds like synthetic progestins can be successfully separated on reverse phase with C18 carbon chain (Fedorova et al., 2014), whereas involvement of hydrophilic interaction columns (HILIC) into LC system is appropriate for ionic compound analysis. Moreover, polar organic solvents used along with HILIC stationary phase cause significant ionization efficiency improvement (van Nuijs, Tarcomnicu and Covaci, 2011). LC system can be coupled with several types of detectors such as UV and fluorescent spectrometers or mass spectrometers. The mass spectrometry started to be probably the most desired detection technique for LC since efficient ionization sources were available. Atmospheric pressure chemical ionization (APCI) and electrospray ionization (ESI) are the most spread techniques based on transformation of neutral molecules into ionic species under conventional LC conditions. These methods are suitable for nonvolatile compounds and provide high ionization efficiency and negligible in source fragmentation (Tarr, Zhu and, Cole 2000). During decades different types of mass

spectrometers have been developed and lots of them were successfully used in environmental research. As examples can be given quadrupole, time of flight, ion trap, orbital trap and hybrid instruments combing benefits of individual analyzers. The most frequently used instrument for quantitative target analysis is triple quadrupole mass spectrometer. Ability to operate in selective reaction monitoring mode respectively perform tandem MS experiments enhances method selectivity due to compound structural confirmation via detected fragments (V. Lange et al., 2008). According to overall good performance, this setup has been applied for investigation of PPCPs in different environmentally relevant matrices such as wastewater (Ashfaq et al., 2019), sludge (Li et al., 2016), sediment (Beretta et al., 2014), surface water (Sun et al., 2016) and biota samples (Ramirez et al., 2007a).

Despite to routine application of quadrupole analyzers, this instrumentation possesses limitations. Nowadays, rapidly increasing analytical trends like metabolomics (Brew et al., 2020) and different types of non-target screening (Bletsou et al., 2015) require identification of compound elemental composition formula and detail structural information which cannot be obtained with low-resolution instrument. Therefore, high-resolution instrumentation such as orbital trap and time of flight are appropriate alternatives for new analytical challenges in environmental research.

Modern analytical development process should consider maximization of time efficiency and minimize production of hazardous waste and overall material consumption during sample preparation (chapter 2) and analysis. Suitable instrumentation fulfilling such assumptions are ambient ionization techniques. Direct sample introduction without chromatography compound separation is common property of these techniques. Also, minimal or no sample treatment prior analysis is usually required as a consequent techniques like desorption electrospray ionization (DESI) (Strittmatter, Düring and Takáts, 2012), direct analysis in real-time (DART) (Haunschmidt et al., 2010) and laser diode thermal desorption (LDTD) (Viglino, Prévost and Sauvé, 2011) have found their applications in PPCPs investigation. The development, applicability and advantages of LDTD for determination of target compounds in complex matrices are reported in chapter 3 and 4 of the thesis. The application of LDTD in environmental studies is discussed in chapter 5.

The scientific aims of the thesis

The main subject of this study was development and evaluation of novelty advanced analytical methods applicable for environmental research of PPCPs in complex environmental matrices relevant for micropollutant's fate in aquatic environment.

- Establishment of sample preparation method.
- Development of targeted analytical methods with ambient ionization instrumentation.
- Evaluation of applicability of ambient ionization mass spectrometry for non-target screening purpose.

References

Albaigés, Joan, Josep M. Bayona, and Jagoš R. Radović. 2016. "Photochemical Effects on Oil Spill Fingerprinting." In *Standard Handbook Oil Spill Environmental Forensics*, Academic Press, 917–59. https://www.sciencedirect.com/science/article/pii/B9780128038321000209#bib0555 (May 22, 2020).

Alharbi, Sultan K. et al., 2017. "Photolysis and UV/H2O2 of Diclofenac, Sulfamethoxazole, Carbamazepine, and Trimethoprim: Identification of Their Major Degradation Products by ESI–LC–MS and Assessment of the Toxicity of Reaction Mixtures." *Process Safety and Environmental Protection* 112: 222–34. https://www.sciencedirect.com/science/article/pii/S095758201730229X?casa_token=EQKUXVEYX7IAAAAA:rL47zdnPXvFnnWcTdV2ufQSOQyJA3dYzl1nHus3D0FKUBl7DNbrcfrj9Q8d9zM2Il6YhA2eYkBQ (May 17, 2020).

Ashfaq, Muhammad et al., 2019. "Occurrence, Spatial Variation and Risk Assessment of Pharmaceuticals and Personal Care Products in Urban Wastewater, Canal Surface Water, and Their Sediments: A Case Study of Lahore, Pakistan." *Science of the Total Environment* 688: 653–63.

Badami, Bharati V. 2008. "Concept of Green Chemistry." *Resonance* 13(11): 1041–48.

Bai, Ying, Zhengguo Cui, Rongguo Su, and Keming Qu. 2018. "Influence of DOM Components, Salinity, PH, Nitrate, and Bicarbonate on the Indirect Photodegradation of Acetaminophen in Simulated Coastal Waters." *Chemosphere* 205: 108–17. https://www.sciencedirect.com/science/article/pii/S0045653518307367 (May 22, 2020).

Beattie, Iain et al., 2012. "Evaluation of Laser Diode Thermal Desorption (LDTD) Coupled with Tandem Mass Spectrometry (MS/MS) for Support of *in Vitro* Drug Discovery Assays: Increasing Scope, Robustness and Throughput of the LDTD Technique for Use with Chemically Diverse Compound Libraries." *Journal of Pharmaceutical and Biomedical Analysis* 59: 18–28. https://www.sciencedirect.com/science/article/pii/S0731708511005905 (March 27, 2019).

Behera, Shishir Kumar, Hyeong Woo Kim, Jeong Eun Oh, and Hung Suck Park. 2011. "Occurrence and Removal of Antibiotics, Hormones and Several Other Pharmaceuticals in Wastewater Treatment Plants of the Largest Industrial City of Korea." *Science of the Total Environment* 409(20): 4351–60. https://www.sciencedirect.com/science/article/pii/S0048969711007327 (May 16, 2020).

Beretta, Magda et al., 2014. "Occurrence of Pharmaceutical and Personal Care Products (PPCPs) in Marine Sediments in the Todos Os Santos Bay and the North Coast of Salvador, Bahia, Brazil." *Journal of Soils and Sediments* 14(7): 1278–86.

Białk-Bielińska, Anna et al., 2012. "Hydrolysis of Sulphonamides in Aqueous Solutions." *Journal of Hazardous Materials* 221–222: 264–74.

Blair, Benjamin et al., 2015. "Evaluating the Degradation, Sorption, and Negative Mass Balances of Pharmaceuticals and Personal Care Products during Wastewater Treatment." *Chemosphere* 134: 395–401. https://www.sciencedirect.com/science/article/pii/S0045653515004245?via%3Dihub (May 19, 2020).

Bletsou, Anna A. et al., 2015. "Targeted and Non-Targeted Liquid Chromatography-Mass Spectrometric Workflows for Identification of Transformation Products of Emerging Pollutants in the Aquatic Environment." *TrAC - Trends in Analytical Chemistry* 66: 32–44.

Boreen, Anne L, William A Arnold, and Kristopher McNeill. 2003. "Photodegradation of Pharmaceuticals in the Aquatic Environment: A Review." *Aquatic Sciences* 65(4): 320–41. https://link.springer.com/content/pdf/10.1007/s00027-003-0672-7.pdf (May 21, 2020).

Borik, Adam et al., 2020. "Determination of Citalopram in Fish Brain Tissue: Benefits of Coupling Laser Diode Thermal Desorption with Low- and High-Resolution Mass Spectrometry." *Analytical and Bioanalytical Chemistry* 412:4353–4361.

Brew, David W. et al., 2020. "Metabolomic Investigations of the Temporal Effects of Exposure to Pharmaceuticals and Personal Care Products and Their Mixture in the Eastern Oyster (Crassostrea Virginica)." *Environmental Toxicology and Chemistry* 39(2): 419–36.

Brodin, Tomas et al., 2017. "Environmental Relevant Levels of a Benzodiazepine (Oxazepam) Alters Important Behavioral Traits in a Common Planktivorous Fish, (Rutilus Rutilus)." *Journal of Toxicology and Environmental Health - Part A: Current Issues* 80(16–18): 963–70.

Brunner, Andrea Mizzi et al., 2019. "Monitoring Transformation Product Formation in the Drinking Water Treatments Rapid Sand Filtration and Ozonation." *Chemosphere* 214: 801–11.

Buser, Hans Rudolf, Thomas Poiger, and Markus D. Müller. 1998. "Occurrence and Fate of the Pharmaceutical Drug Diclofenac in Surface Waters: Rapid Photodegradation in a Lake." *Environmental Science and Technology* 32(22): 3449–56. https://pubs.acs.org/doi/abs/10.1021/es980301x (May 22, 2020).

Bynum, Nichole D., Katherine N. Moore, and Megan Grabenauer. 2014. "Evaluation of Laser Diode Thermal Desorption-Tandem Mass Spectrometry (LDTD-MS-MS) in Forensic Toxicology." *Journal of Analytical Toxicology* 38(8): 528–35. https://academic.oup.com/jat/article-lookup/doi/10.1093/jat/bku084 (May 30, 2020).

Caldwell, Daniel J., Frank Mastrocco, Luigi Margiotta-Casaluci, and Bryan W. Brooks. 2014. "An Integrated Approach for Prioritizing Pharmaceuticals Found in the Environment for Risk Assessment, Monitoring and Advanced Research." *Chemosphere* 115(1): 4–12.

Calisto, Vânia, M. Rosário M. Domingues, and Valdemar I. Esteves. 2011. "Photodegradation of Psychiatric Pharmaceuticals in Aquatic Environments - Kinetics and Photodegradation Products." *Water Research* 45(18): 6097–6106. https://www.sciencedirect.com/science/article/pii/S0043135411005276 (May 22, 2020).

Carballa, Marta et al., 2004. "Behavior of Pharmaceuticals, Cosmetics and Hormones in a Sewage Treatment Plant." *Water Research* 38(12): 2918–26.

Chen, Xijuan et al., 2012. "Ozonation Products of Triclosan in Advanced Wastewater Treatment." *Water Research* 46(7): 2247–56.

Chen, Yi Jie et al., 2020. "GC-MS/MS Analysis for Source Identification of Emerging POPs in PM2.5." *Ecotoxicology and Environmental Safety* 193: 110368.

Dabić, Dario, Sandra Babić, and Irena Škorić. 2019. "The Role of Photodegradation in the Environmental Fate of Hydroxychloroquine." *Chemosphere* 230: 268–77. https://www.sciencedirect.com/science/article/pii/S0045653519309294 (May 22, 2020).

Daughton, Christian G., and Thomas A. Ternes. 1999. "Pharmaceuticals and Personal Care Products in the Environment: Agents of Subtle Change?" *Environmental Health Perspectives* 107(SUPPL. 6): 907–38.

Ding, Jiannan et al., 2015. "Biological Fate and Effects of Propranolol in an Experimental Aquatic Food Chain." *Science of the Total Environment* 532: 31–39.

Dion-Fortier, Annick et al., 2019. "Signal Enhancement in Laser Diode Thermal Desorption-Triple Quadrupole Mass Spectrometry Analysis Using Microwell Surface Coatings." *Journal of Mass Spectrometry* 54(2): 167–77.

Ejhed, H. et al., 2018. "The Effect of Hydraulic Retention Time in Onsite Wastewater Treatment and Removal of Pharmaceuticals, Hormones and Phenolic Utility Substances." *Science of the Total Environment* 618: 250–61. https://www.sciencedirect.com/science/article/pii/S004896971733070X?casa_token=jHKWjtfVwXwAAAAA:N9nXq18wNMIITZ1XI-t1j-8onxeq72QYiLC1Y5Bu9Q85kHiDcvGm8stmVJoRIm9iyc0c-5dWNFA (May 18, 2020).

Ellepola, Nishanthi et al., 2020. "A Toxicological Study on Photo-Degradation Products of Environmental Ibuprofen: Ecological and Human Health Implications." *Ecotoxicology and Environmental Safety* 188: 109892. https://www.sciencedirect.com/science/article/pii/S0147651319312230 (May 22, 2020).

Facey, Sandra J. et al., 2018. "Rapid and Complete Degradation of Diclofenac by Native Soil Microorganisms." *Environmental Technology and Innovation* 10: 55–61.

Fayad, Paul B., Michèle Prévost, and Sébastien Sauvé. 2010. "Laser Diode Thermal Desorption/Atmospheric Pressure Chemical Ionization Tandem Mass Spectrometry Analysis of Selected Steroid Hormones in Wastewater: Method Optimization and Application." *Analytical Chemistry* 82(2): 639–45. http://www.ncbi.nlm.nih.gov/pubmed/20000768 (February 12, 2019).

Fedorova, Ganna, Oksana Golovko, Tomas Randak, and Roman Grabic. 2014. "Storage Effect on the Analysis of Pharmaceuticals and Personal Care Products in Wastewater." *Chemosphere* 111: 55–60. https://ac.els-cdn.com/S0045653514003361/1-s2.0-S0045653514003361-main.pdf?_tid=96085d26-b337-11e7-a055-00000aab0f02&acdnat=1508243878_f9c26bc7c6e78030c8cacb4563a64e1a (October 17, 2017).

Fent, Karl. 2015. "Progestins as Endocrine Disrupters in Aquatic Ecosystems: Concentrations, Effects and Risk Assessment." *Environment International* 84: 115–30.

Gan, Zhiwei et al., 2014. "Transformation of Acesulfame in Water under Natural Sunlight: Joint Effect of Photolysis and Biodegradation." *Water Research* 64: 113–22. https://www.sciencedirect.com/science/article/pii/S0043135414004862?via%3Dihub#bib40 (May 23, 2020).

Gerba, Charles P., and Ian L. Pepper. 2015. "Municipal Wastewater Treatment." In *Environmental Microbiology: Third Edition*, Academic Press, 583–606. https://www.sciencedirect.com/science/article/pii/B9780128147191000227 (May 17, 2020).

Golovko, Oksana et al., 2014a. "Seasonal Changes in Antibiotics, Antidepressants/Psychiatric Drugs, Antihistamines and Lipid Regulators in a Wastewater Treatment Plant." *Chemosphere* 111: 418–26. https://www.sciencedirect.com/science/article/pii/S0045653514005104#t0005 (November 21, 2018).

Golovko, Oksana et al., 2014b. "Seasonal Changes in Antibiotics, Antidepressants/Psychiatric Drugs, Antihistamines and Lipid Regulators in a Wastewater Treatment Plant." *Chemosphere* 111: 418–26. https://www.sciencedirect.com/science/article/pii/S0045653514005104 (May 19, 2020).

Gros, Meritxell et al., 2019. "Veterinary Pharmaceuticals and Antibiotics in Manure and Slurry and Their Fate in Amended Agricultural Soils: Findings from an Experimental Field Site (Baix Empordà, NE Catalonia)." *Science of the Total Environment* 654: 1337–49.

Guo, Wei et al., 2020. "Psychoactive Compounds at Environmental Concentration Alter Burrowing Behavior in the Freshwater Crayfish." *Science of the Total Environment* 711: 135138.

Hamdi El Najjar, Nasma et al., 2013. "Levofloxacin Oxidation by Ozone and Hydroxyl Radicals: Kinetic Study, Transformation Products and Toxicity." *Chemosphere* 93(4): 604–11.

Harahap, Yahdiana, Innet Maysyarah, and Herman Suryadi. 2017. "Analytical Validation of Clopidogrel in Human Plasma through Ultrahigh-Performance Liquid Chromatography-Tandem Mass Spectrometry." *International Journal of Applied Pharmaceutics* 9: 163–67.

Hashemi, Beshare, Parvin Zohrabi, and Mojtaba Shamsipur. 2018. "Recent Developments and Applications of Different Sorbents for SPE and SPME from Biological Samples." *Talanta* 187: 337–47.

Haunschmidt, Manuela, Christian W. Klampfl, Wolfgang Buchberger, and Robert Hertsens. 2010. "Determination of Organic UV Filters in Water by Stir Bar Sorptive Extraction and Direct Analysis in Real-Time Mass Spectrometry." In *Analytical and Bioanalytical Chemistry*, Springer, 269–75.

Heudi, Olivier et al., 2011. "Laser Diode Thermal Desorption-Positive Mode Atmospheric Pressure Chemical Ionization Tandem Mass Spectrometry for the Ultra-Fast Quantification of a Pharmaceutical Compound in Human Plasma." *Journal of Pharmaceutical and Biomedical Analysis* 54(5): 1088–95. https://www.sciencedirect.com/science/article/pii/S0731708510006680 (May 30, 2020).

Holm, John V., Kirsten Rugge, Poul L. Bjerg, and Thomas H. Christensen. 1995. "Occurrence and Distribution of Pharmaceutical Organic Compounds in the Groundwater Downgradient of a Landfill (Grindsted, Denmark)." *Environmental Science and Technology* 29(5): 1415–20. https://pubs.acs.org/doi/abs/10.1021/es00005a039 (May 10, 2020).

Huber, Sandra et al., 2016. "A First Screening and Risk Assessment of Pharmaceuticals and Additives in Personal Care Products in Waste Water, Sludge, Recipient Water and Sediment from Faroe Islands, Iceland and Greenland." *Science of the Total Environment* 562: 13–25.

Ivanová, Lucia et al., 2018. "Pharmaceuticals and Illicit Drugs – A New Threat to the Application of Sewage Sludge in Agriculture." *Science of The Total Environment* 634: 606–15. https://www.sciencedirect.com/science/article/pii/S0048969718311720 (November 21, 2018).

Jackson, Latonya M., and Paul L. Klerks. 2019. "Impact of Long-Term Exposure to 17A-Ethinylestradiol in the Live-Bearing Fish Heterandria Formosa." *Archives of Environmental Contamination and Toxicology* 77(1): 51–61.

Joss, Adriano et al., 2006. "Biological Degradation of Pharmaceuticals in Municipal Wastewater Treatment: Proposing a Classification Scheme." *Water Research* 40(8): 1686–96. https://www.sciencedirect.com/science/article/pii/S0043135406001126 (January 22, 2019).

Kagle, Jeanne et al., 2009. "Chapter 3 Biodegradation of Pharmaceutical and Personal Care Products." *Advances in Applied Microbiology* 67(C): 65–108. https://pubmed.ncbi.nlm.nih.gov/19245937/ (May 20, 2020).

Kibuye, Faith A. et al., 2019. "Fate of Pharmaceuticals in a Spray-Irrigation System: From Wastewater to Groundwater." *Science of the Total Environment* 654: 197–208.

Klement, Aleš et al., 2018. "Sorption of Citalopram, Irbesartan and Fexofenadine in Soils: Estimation of Sorption Coefficients from Soil Properties." *Chemosphere* 195: 615–23. https://www.sciencedirect.com/science/article/pii/S0045653517320684 (June 7, 2019).

Kodešová, Radka et al., 2015. "Pharmaceuticals' Sorptions Relative to Properties of Thirteen Different Soils." *Science of The Total Environment* 511: 435–43. https://www.sciencedirect.com/science/article/pii/S0048969714017999 (April 30, 2019).

Košinová, Anna. 2019. 56 "Odpověď Půdního Mikrobního Společenstva Na Aplikaci Farmak Přítomných v Zavlažovacích Vodách Se Zaměřením Na Irbesartan." University of South Bohemia, České Budějovice, Czech Republic.

Kunkel, Uwe, and Michael Radke. 2008. "Biodegradation of Acidic Pharmaceuticals in Bed Sediments: Insight from a Laboratory Experiment." *Environmental Science and Technology* 42(19): 7273–79.

Lange, Anke et al., 2009. "Sexual Reprogramming and Estrogenic Sensitization in Wild Fish Exposed to Ethinylestradiol." *Environmental Science and Technology* 43(4): 1219–25.

Lange, Vinzenz, Paola Picotti, Bruno Domon, and Ruedi Aebersold. 2008. "Selected Reaction Monitoring for Quantitative Proteomics: A Tutorial." *Molecular Systems Biology* 4(1): 222. https://onlinelibrary.wiley.com/doi/abs/10.1038/msb.2008.61 (May 25, 2020).

Lanshoeft, Christian et al., 2014. "Laser Diode Thermal Desorption Atmospheric Pressure Chemical Ionization Tandem Mass Spectrometry Applied for the Ultra-Fast Quantitative Analysis of BKM120 in Human Plasma." *Analytical and Bioanalytical Chemistry* 406(22): 5413–23. http://link.springer.com/10.1007/s00216-014-7966-6 (May 30, 2020).

Li, Mingyue et al., 2016. "Simultaneous Analysis of 45 Pharmaceuticals and Personal Care Products in Sludge by Matrix Solid-Phase Dispersion and Liquid Chromatography Tandem Mass Spectrometry." *Analytical and Bioanalytical Chemistry* 408(18): 4953–64.

Lin, Angela Yu Chen, and Martin Reinhard. 2005. "Photodegradation of Common Environmental Pharmaceuticals and Estrogens in River Water." *Environmental Toxicology and Chemistry* 24(6): 1303–9. http://doi.wiley.com/10.1897/04-236R.1 (May 22, 2020).

Loftin, Keith A., Craig D. Adams, Michael T. Meyer, and Rao Surampalli. 2008. "Effects of Ionic Strength, Temperature, and PH on Degradation of Selected Antibiotics." *Journal of Environmental Quality* 37(2): 378–86.

Lohne, Jack J. et al., 2012. "Laser Diode Thermal Desorption Mass Spectrometry for the Analysis of Quinolone Antibiotic Residues in Aquacultured Seafood." *Rapid Communications in Mass Spectrometry* 26(24): 2854–64. http://www.ncbi.nlm.nih.gov/pubmed/23136016 (March 27, 2019).

Lonappan, Linson et al., 2016. "Diclofenac in Municipal Wastewater Treatment Plant: Quantification Using Laser Diode Thermal Desorption—Atmospheric Pressure Chemical Ionization—Tandem Mass Spectrometry Approach in Comparison with an Established Liquid Chromatography-Electrospray Ionization–Tandem Mass Spectrometry Method." *Journal of Chromatography A* 1433: 106–13. https://www.sciencedirect.com/science/article/pii/S0021967316000625 (April 9, 2018).

Mandalakis, Manolis, Manolis Tsapakis, and Euripides G. Stephanou. 2001. "Optimization and Application of High-Resolution Gas Chromatography with Ion Trap Tandem Mass Spectrometry to the Determination of Polychlorinated Biphenyls in Atmospheric Aerosols." *Journal of Chromatography A* 925(1–2): 183–96.

Mohapatra, D.P. et al., 2012. "Carbamazepine in Municipal Wastewater and Wastewater Sludge: Ultrafast Quantification by Laser Diode Thermal Desorption-Atmospheric Pressure Chemical Ionization Coupled with Tandem Mass Spectrometry." *Talanta* 99: 247–55. https://www.sciencedirect.com/science/article/pii/S0039914012004274 (March 27, 2019).

van Nuijs, Alexander L.N., Isabela Tarcomnicu, and Adrian Covaci. 2011. "Application of Hydrophilic Interaction Chromatography for the Analysis of Polar Contaminants in Food and Environmental Samples." *Journal of Chromatography A* 1218(35): 5964–74.

Ohoro, C. R., A. O. Adeniji, A. I. Okoh, and O. O. Okoh. 2019. "Distribution and Chemical Analysis of Pharmaceuticals and Personal Care Products (PPCPs) in the Environmental Systems: A Review." *International Journal of Environmental Research and Public Health* 16(17). / pmc/articles/PMC6747491/?report=abstract (July 22, 2020).

Van der Oost, Ron, Jonny Beyer, and Nico P.E. Vermeulen. 2003. "Fish Bioaccumulation and Biomarkers in Environmental Risk Assessment: A Review." *Environmental Toxicology and Pharmacology* 13(2): 57–149.

Oulton, Rebekah L., Tamar Kohn, and David M. Cwiertny. 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): 1956–78.

Paul, Samir C. et al., 2014. "Sorption Behavior of Ibuprofen and Naproxen in Simulated Domestic Wastewater." *Water, Air, and Soil Pollution* 225(1): 1821. http://link.springer.com/10.1007/s11270-013-1821-9 (May 18, 2020).

Petersen, Karina, Harald Hasle Heiaas, and Knut Erik Tollefsen. 2014. "Combined Effects of Pharmaceuticals, Personal Care Products, Biocides and Organic Contaminants on the Growth of Skeletonema Pseudocostatum." *Aquatic Toxicology* 150: 450–54.

Prosser, R. S., and P. K. Sibley. 2015. "Human Health Risk Assessment of Pharmaceuticals and Personal Care Products in Plant Tissue Due to Biosolids and Manure Amendments, and Wastewater Irrigation." *Environment International* 75: 223–33.

Ramirez, Alejandro J., Mohammad A. Mottaleb, Bryan W. Brooks, and C. Kevin Chambliss. 2007a. "Analysis of Pharmaceuticals in Fish Using Liquid Chromatography-Tandem Mass Spectrometry." *Analytical Chemistry* 79(8): 3155–63.

Ramirez, Alejandro J., Mohammad A. Mottaleb, Bryan W. Brooks, and C. Kevin Chambliss. 2007b. "Analysis of Pharmaceuticals in Fish Using Liquid Chromatography-Tandem Mass Spectrometry." *Analytical Chemistry* 79(8): 3155–63.

Sabourin, Lyne et al., 2009. "Runoff of Pharmaceuticals and Personal Care Products Following Application of Dewatered Municipal Biosolids to an Agricultural Field." *Science of the Total Environment* 407(16): 4596–4604.

Santoke, Hanoz, and William J. Cooper. 2017. "Environmental Photochemical Fate of Selected Pharmaceutical Compounds in Natural and Reconstituted Suwannee River Water: Role of Reactive Species in Indirect Photolysis." *Science of the Total Environment* 580: 626–31. https://www.sciencedirect.com/science/article/pii/S0048969716326948 (May 22, 2020).

Šauer, Pavel et al., 2018. "Two Synthetic Progestins and Natural Progesterone Are Responsible for Most of the Progestagenic Activities in Municipal Wastewater Treatment Plant Effluents in the Czech and Slovak Republics." *Water Research* 137: 64–71. https://www.sciencedirect.com/science/article/pii/S0043135418301787 (September 18, 2018).

Schlüter-Vorberg, Lisa et al., 2015. "Toxification by Transformation in Conventional and Advanced Wastewater Treatment: The Antiviral Drug Acyclovir." *Environmental Science and Technology Letters* 2(12): 342–46.

Solliec, Morgan, Daniel Massé, and Sébastien Sauvé. 2014. "Analysis of Trimethoprim, Lincomycin, Sulfadoxin and Tylosin in Swine Manure Using Laser Diode Thermal Desorption-Atmospheric Pressure Chemical Ionization-Tandem Mass Spectrometry." *Talanta* 128: 23–30.

Sparkman, O. David. 1996. "Evaluating Electron Ionization Mass Spectral Library Search Results." *Journal of the American Society for Mass Spectrometry* 7(4): 313–18.

Strittmatter, Nicole, Rolf Alexander Düring, and Zoltán Takáts. 2012. "Analysis of Wastewater Samples by Direct Combination of Thin-Film Microextraction and Desorption Electrospray Ionization Mass Spectrometry." *Analyst* 137(17): 4037–44.

Subedi, Bikram, Mohammad A. Mottaleb, C. Kevin Chambliss, and Sascha Usenko. 2011. "Simultaneous Analysis of Select Pharmaceuticals and Personal Care Products in Fish Tissue Using Pressurized Liquid Extraction Combined with Silica Gel Cleanup." *Journal of Chromatography A* 1218(37): 6278–84.

Sun, Qian et al., 2016. "PPCPs in Jiulong River Estuary (China): Spatiotemporal Distributions, Fate, and Their Use as Chemical Markers of Wastewater." *Chemosphere* 150: 596–604.

Tarpani, Raphael Ricardo Zepon, and Adisa Azapagic. 2018. "A Methodology for Estimating Concentrations of Pharmaceuticals and Personal Care Products (PPCPs) in Wastewater Treatment Plants and in Freshwaters." *Science of the Total Environment* 622–623: 1417–30.

Tarr, Matthew A., Junhua Zhu, and Richard B. Cole. 2000. "Atmospheric Pressure Ionization Mass Spectrometry." In *Encyclopedia of Analytical Chemistry*, Chichester, UK: John Wiley & Sons, Ltd. http://doi.wiley.com/10.1002/9780470027318.a6003 (May 25, 2020).

Ternes, Thomas A. et al., 2004. "A Rapid Method to Measure the Solid-Water Distribution Coefficient (K d) for Pharmaceuticals and Musk Fragrances in Sewage Sludge." *Water Research* 38(19): 4075–84.

Ternes, Thomas A et al., 2003. "Ozonation: A Tool for Removal of Pharmaceuticals, Contrast Media and Musk Fragrances from Wastewater?" *Water Research* 37(8): 1976–82. https://www.sciencedirect.com/science/article/pii/S0043135402005705 (May 17, 2020).

Tran, Ngoc Han, Martin Reinhard, and Karina Yew Hoong Gin. 2018. "Occurrence and Fate of Emerging Contaminants in Municipal Wastewater Treatment Plants from Different Geographical Regions-a Review." *Water Research* 133: 182–207.

Valenti, Theodore W. et al., 2012. "Human Therapeutic Plasma Levels of the Selective Serotonin Reuptake Inhibitor (SSRI) Sertraline Decrease Serotonin Reuptake Transporter Binding and Shelter-Seeking Behavior in Adult Male Fathead Minnows." *Environmental Science and Technology* 46(4): 2427–35.

Vasquez, M. I. et al., 2014. "Environmental Side Effects of Pharmaceutical Cocktails: What We Know and What We Should Know." *Journal of Hazardous Materials* 279: 169–89.

Viglino, Liza, Michèle Prévost, and Sébastien Sauvé. 2011. "High Throughput Analysis of Solid-Bound Endocrine Disruptors by LDTD-APCI-MS/MS." *Journal of Environmental Monitoring* 13(3): 583–90.

Wang, Jianlong, and Shizong Wang. 2016. "Removal of Pharmaceuticals and Personal Care Products (PPCPs) from Wastewater: A Review." *Journal of Environmental Management* 182: 620–40.

Wenning, Richard J., and Linda Martello. 2013. "POPs in Marine and Freshwater Environments." In *Environmental Forensics for Persistent Organic Pollutants*, Elsevier B.V., 357–90.

de Wilt, Arnoud et al., 2018. "Enhanced Pharmaceutical Removal from Water in a Three Step Bio-Ozone-Bio Process." *Water Research* 138: 97–105. https://www.sciencedirect.com/science/article/pii/S0043135418302240 (May 17, 2020).

Xuan, Richeng et al., 2010. "Hydrolysis and Photolysis of Oxytetracycline in Aqueous Solution." *Journal of Environmental Science and Health - Part B Pesticides, Food Contaminants, and Agricultural Wastes* 45(1): 73–81.

Zhang, Yi et al., 2017. "Rapid Determination of 54 Pharmaceutical and Personal Care Products in Fish Samples Using Microwave-Assisted Extraction—Hollow Fiber—Liquid/Solid Phase Microextraction." *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences* 1051: 41–53.

Zhou, D., W. R. Porter, and G. G.Z. Zhang. 2017. "Drug Stability and Degradation Studies." In *Developing Solid Oral Dosage Forms: Pharmaceutical Theory and Practice: Second Edition*, Academic Press, 113–49. https://www.sciencedirect.com/science/article/pii/B9780128024478000054 (May 22, 2020).

Zwiener, Christian, Thomas Glauner, and Fritz H. Frimmel. 2000. "Biodegradation of Pharmaceutical Residues Investigated by SPE-GC/ITD-MS and on-Line Derivatization." *HRC Journal of High Resolution Chromatography* 23(7–8): 474–78.

CHAPTER 2

DEVELOPMENT OF A ROBUST EXTRACTION PROCEDURE FOR THE HPLC-ESI-HRPS DETERMINATION OF MULTI-RESIDUAL PHARMACEUTICALS IN BIOTA SAMPLES

Grabicova, K.; Vojs Staňová, A.; Koba Ucun, O.; Borik, A.; Randak, T.; Grabic, R., 2018. Development of a robust extraction procedure for the HPLC-ESI-HRPS determination of multi-residual pharmaceuticals in biota samples. Analytica Chimica Acta 1022, 53–60.

My share on this work was about 10%

Analytica Chimica Acta 1022 (2018) 53–60

Contents lists available at ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

Development of a robust extraction procedure for the HPLC-ESI-HRPS determination of multi-residual pharmaceuticals in biota samples

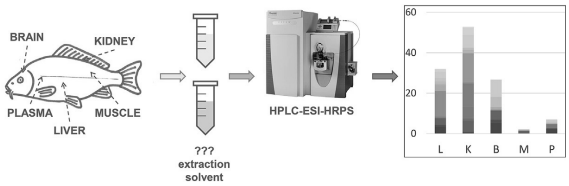
Katerina Grabicova [a, *], Andrea Vojs Staňová [a, b], Olga Koba Ucun [a], Adam Borik [a], Tomas Randak [a], Roman Grabic [a]

[a] University of South Bohemia in Ceske Budejovice, Faculty of Fisheries and Protection of Waters, South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses, Zatisi 728/II, CZ-389 25, Vodnany, Czech Republic
[b] Comenius University in Bratislava, Faculty of Natural Sciences, Department of Analytical Chemistry, Ilkovicova 6, SK-842 15, Bratislava, Slovak Republic

HIGHLIGHTS

- One step solvent extraction for five different fish tissues was developed.
- Acceptable recoveries together with lowering of matrix effect were achieved.
- This method was validated for determination of 74 pharmaceuticals by LC-ESI/HRPS.
- LOQs at sub ng g^{-1}concentration levels were reached for most analytes.
- The method was successfully applied for field fish experiments.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 25 January 2018
Received in revised form
6 April 2018
Accepted 13 April 2018
Available online 18 April 2018

Keywords:
Fish tissues
Micropollutants
Multicomponent analysis
Sample pre-treatment

ABSTRACT

A simple, robust and effective extraction procedure for the determination of 74 pharmaceuticals in different fish tissues by ultrasensitive high performance liquid chromatography with electrospray high resolution product scan (HPLC-ESI-HRPS) was developed and validated. Different extraction solvent mixtures were tested to achieve the highest recoveries of the selected analytes, to minimize the influence of a complex matrix and to reduce the total analysis time as well as cost of analysis.

A mixture of acetonitrile + isopropanol (3:1 v/v) acidified with 0.1% formic acid was the best extraction solvent among the five solvents tested for most of the tissues with the exception of plasma samples, where only acidified acetonitrile exhibited the best performance. The developed method was validated at three concentration levels (5, 20 and 50 ng g-1) in five different fish tissues (liver, kidney, brain, muscle and plasma). Most of the target analytes were extracted with a recovery between 60 and 130%. Very low limits of quantification (LOQs) were obtained for the majority of the pharmaceuticals in all of the studied matrices. The developed analytical method was successfully applied for the analysis of common carp (*Cyprinus carpio*) originating from the waste water effluent-dominated pond Cezarka (Czech Republic). The results confirmed the importance of multi-tissue analysis to obtain complex information on the distribution of pharmaceuticals in fish.

1. Introduction

The pollution of waste and surface waters by various organics/

* Corresponding author.
E-mail address: grabicova@frov.jcu.cz (K. Grabicova).

https://doi.org/10.1016/j.aca.2018.04.011
0003-2670/

chemical contaminants (for example pharmaceuticals and their metabolites) is generally known nowadays [1–3].

The pharmaceuticals and their metabolites present in polluted waters could negatively affect water organisms, with behaviour changes, the alteration of histological and biochemical parameters and reproduction modifications observed [4–10]. From ecotoxicological, ecological and food safety's points of view, it is necessary to know if the pharmaceuticals enter fish and other water organisms [11,12].

These compounds are designed to have a biological effect, and this effect on water organisms has already been proven at environmentally relevant concentrations. The development of ultrasensitive and highly selective analytical methods for their identification and quantification is necessary [13,14]. The combination of high performance liquid chromatography-mass spectrometry with electrospray ionization (HPLC-ESI-MS) is generally regarded as the most sensitive and selective analytical technique for the identification and determination of different pharmaceuticals as well as their metabolites present in complex samples of biological and environmental origin [15,16]. Nevertheless, difficulties with regards to the reproducibility, precision and accuracy of the determination of the target analytes caused by interferences in these kinds of samples at ultra-low concentration levels have been reported [17,18]. Even the implementation of high-resolution mass spectrometry (HRMS) in different operational modes is not sufficient to reduce the risk of false/positive results [19]. To obtain a sufficiently selective, reliable and robust multicomponent HPLC-ESI-MS method, it is necessary to eliminate such interferences in each individual step of the analytical procedure (e.g., sample pre-treatment, the chromatographic process, mass spectrometric detection, etc.) [13,20].

The sample pre-treatment is one of the most important steps in a successful multi-residual analytical procedure [21]. The sample pre-treatment of biological or environmental samples consists of several consequent steps, including homogenization, centrifugation, sonication, freeze-drying, extraction, filtration, etc. The main goals of sample pre-treatment are the pre-concentration of target analytes and decreasing the complexity of samples in a short time, with a low cost, and respecting of the green chemistry concept. Nowadays, several different sample pre-treatment techniques based on liquid-liquid extraction (LLE), solid-liquid extraction (SLE), ultrasound-assisted solvent extraction (USE), pressurized liquid extraction (PLE), solid phase extraction (SPE), microextraction (SPME), microwave-assisted extraction (MAE), QuEChERS and their modifications are used for the pre-concentration of different pharmaceuticals in complex biota samples [20,22,23].

The essential purpose of the implementation of an extraction procedure in multicomponent analysis is to obtain the highest recoveries of a broad range of analytes (from polar to non-polar) and sufficient elimination of co-extracts (salts, lipids, amines, peptides, etc.) with respect to the reduction of the total analysis time and labour costs of the routine analysis of large series of samples [24]. In multi-residual analysis of pharmaceuticals, it is also important to obtain as selective as possible of an extraction because it is not possible to use hard or extensive sample clean-up due to the highly variable chemical and physical properties of the target analytes and their stabilities.

The aim of this paper is to develop and validate a method for the extraction of different fish tissues samples (liver, kidney, brain, muscle and plasma) for HPLC-ESI-HRPS determination of more than 70 pharmaceuticals from different therapeutic classes in these tissues at very low concentration levels.

2. Materials and methods

2.1. Chemicals

The list of native and isotope labelled compounds used for the preparation of the stock solution in methanol (LC-MS grade, Sigma-Aldrich) is listed in SM1. Working solutions at concentration levels of 10, 1, and 0.1 μg mL^{-1} were prepared from these stock solutions and used for spiking. Acetonitrile (ACN), isopropanol (ISP) and ethanol (EtOH) used as the extraction solvents and mobile phase were obtained from Sigma-Aldrich (all LC-MS grade). Formic acid (FA) for acidification was also obtained from Sigma-Aldrich. Ultrapure water (mobile phase) was prepared using an AquaMax Basic 360 Series and Ultra 370 Series instrument (Younglin, purchased from Labicom, CR).

2.2. Sample definition

Fish, common carp (*Cyprinus carpio* L.), was caught by electrofishing from the non-polluted Potocny pond (average weight 3750 ± 200 g, average total length 550 ± 20 mm, Kestrany, South Bohemia region, Czech Republic, for testing of the extraction reagents and for validation) and from the Cezarka pond affected by effluent from a sewage treatment plant (STP; average weight 720 ± 120 g, average total length 330 ± 20 mm, Vodnany, South Bohemia region, Czech Republic, for application of the extraction method to real samples). The blood was taken, the fish was killed and then the brain, muscle, kidney and liver were sampled. Blood samples were centrifuged (Eppendorf centrifuge, 837 × g, 10 min, 4 °C) to obtain plasma. All samples were frozen at −20 °C until the extraction and analysis.

This study was performed in accordance with the principals of the EU-harmonized Animal Welfare Act of the Czech Republic. The unit is licensed (No. 53100/2013-MZE-17214) according to the Czech National Directive (the Law against Animal Cruelty, No. 246/1992).

2.3. Methods of extraction – to find the best extraction conditions

For the development of the extraction procedure, fish liver was chosen due to its highest complexity among all of the analysed fish tissues. In liver, we have previously observed the worst matrix effect among tissues [25].

Fish liver (0.5 g) was weighed into Eppendorf tubes. Internal standards (10 ng), 1 mL of extraction solvent, native standards (25 ng) and a stainless steel ball were added to the sample. Samples were homogenized (TissueLyser II, Quiagen, Germany, 1800 min^{-1} for 10 min), centrifuged (Mini spin, Eppendorf, Germany; 10000 rpm for 5 min) and filtered through regenerated cellulose filters (0.45 μm, Labicom, Czech Republic). The samples were frozen at −20 °C for 24 h, centrifuged again (10000 rpm for 3 min) and aliquots were placed into vials for analysis by HPLC-ESI-HRPS. Samples were prepared as pentaplicates.

To obtain the most efficient extraction (recovery) for a broad spectrum of pharmaceuticals from different therapeutic classes, it was necessary to use several extraction reagent mixtures prepared from acidified acetonitrile, isopropanol and ethanol.

2.4. Analytical system and HPLC-ESI-HRPS analysis

Liquid chromatography with a Q-Exactive mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA), an Accela 1250 LC

pump (Thermo Fisher Scientific) and HTS XT-CTC autosampler (CTC Analytics AG) was used for the analysis of fish extracts. For chromatographic separation, a Hypersil Gold aQ analytical column (50 × 2.1 mm; 5 μm particles, Thermo Fisher Scientific) was used. The temperature of the analytical column was set to 23 °C. Acidified water and ACN (both with 0.1% formic acid) were used as the mobile phases. The gradient set-up used for the elution of the target compounds is described in SM2. A heated electrospray ionization (HESI) source was used for the ionization of the target compounds with a spray voltage of 3.5 kV and nitrogen as the sheath gas (40 arbitrary units), auxiliary gas (10 arbitrary units) and collision gas. The high resolution product scan mode was used with the following parameters: isolation window at the quadrupole was 1 *m/z*, resolution of the orbital trap was 17500 FWHM, the AGC target was 1e6, and the maximum filling time was 30 ms. MS transitions for individual compounds are given in SM1. Data acquisition was performed with Xcalibur Software, and data processing was performed using TraceFinder 3.3 Software (both Thermo Fisher Scientific). Internal standard and matrix matching standard methods were used for quantification of target analytes. Internal standard used for calculation is given in SM1.

2.5. Validation of the developed extraction method

Based on the results obtained from the extraction method optimization, the extraction mixture with the best recoveries for the majority of analysed pharmaceuticals was chosen, and the validation of the method was performed at three concentration levels of 5, 20 and 50 ng g^{-1}. Liver, kidney and muscle samples were prepared according to above-mentioned method (see Chapter 2.3.). The samples were analysed at the same conditions as described in Chapter 2.4.

Brain samples were prepared in the same manner but with half of the amount of brain, internal and native standards, and extraction solvent.

Plasma (0.25 g) was placed into Eppendorf tubes. Internal (5 ng) and native (1.25, 5, and 12.5 ng) standards as well as 0.25 mL of extraction solvent were added. The samples were then vortexed and frozen at −20 °C for 24 h. Then, the preparation was the same as the rest of the fish tissue samples.

Spiked samples were prepared as hexaplicates for each concentration level (5, 20 and 50 ng g^{-1}) and each fish tissue (liver, kidney, muscle, brain and plasma). Validation of the method was performed on the basis of calculations from the fortified samples.

Fish caught in the Cezarka pond were used for extraction method verification. Samples from twelve fish were prepared following the above-mentioned method without the addition of native standards.

3. Results and discussion

3.1. Extraction procedure – influence of the reagent

Ecotoxicologically relevant pharmaceuticals from different therapeutic classes (based on the predicted critical environmental concentration and bioconcentration factor) were selected for this study [1,26]. Based on our previous results, we reduced this selection to 74 pharmaceuticals and their metabolites present in the environment of the Central European region [27–33]. Special attention was paid to psychoactive compounds, whose bioaccumulation [25,34–36] and negative influence on aquatic organisms have been confirmed [4,7,8].

The development of the extraction method was based on findings from our previous work focused on the determination of antibiotics in fish and shrimp meat [29]. In the mentioned paper, the best results were obtained using acidified ACN in a two-step extraction method. In the presented study, we aimed to develop a simple, fast, robust, effective and cheap one-step extraction procedure for the larger selection of pharmaceuticals. Taking into account a broader range of physical-chemical properties and variable fish tissues, we tested five selected extraction solvent mixtures initially: **A.** ACN + 0.1% FA; **B.** ACN + ISP (1:1) + 0.1% FA; **C.** ACN + ISP (2:1) + 0.1% FA; **D.** ACN + ISP (3:1) + 0.1% FA; and **E.** ACN + EtOH (1:1) + 0.1% FA. The addition of isopropanol or ethanol to acetonitrile was assumed to improve the extraction efficiency of less polar analytes. The results of relative recoveries for individual pharmaceuticals spiked at a concentration level of 50 ng g^{-1} and individual extraction reagents are given in Table 1.

From the results, it is evident that not all extraction mixtures are good enough. The number of analytes whose recoveries ranged between 60 and 130% differs for individual extraction reagents. From the 74 analysed pharmaceuticals, only 34 were in this range for reagent A, 45 for reagent B and 54 for reagent C. The two best reagents are reagents D and E (61 and 63 pharmaceuticals in this range). Some scientists refer to sufficient recoveries as being in the range between 40 and 140% [37,38], so only six (10,11-dihydrocarbamazepine, cetirizine, cilazapril, orphenadrine, sotalol and verapamil) and four (bisoprolol, donepezil, sulfamethoxazole and sotalol) pharmaceuticals from the 74 analysed ones were out of this range for reagents D and E, respectively.

Reagent D (acetonitrile + isopropanol (3:1) + 0.1% formic acid) was selected for consequent method validation because it had a comparable number of pharmaceuticals with acceptable recoveries to reagent E but the lowest extraction uncertainty within the tested extraction mixtures (the averages of all of the RSDs were 22% for reagent A, 11% for reagent B, 9% for reagent C, 8% for reagent D, and 12% for reagent E).

3.2. Matrix effects

For evaluation of matrix effects on the signals of the target analytes, the response factors from the calibration curve and matrix matched standards were calculated. If the difference in the response factors from the calibration curve and matrix matched standard exceeded 20%, the concentration of the analyte was calculated using the matrix matched standard response factor. The results of the evaluation of matrix effects in different fish tissues are summarised in Fig. 1. The lowest matrix effect was observed for muscle tissue, where only 15 pharmaceuticals were calculated based on matrix response factors and the rest (59) on response factors from calibration curves. Tissues with elevated lipid contents (such as the liver, kidney and brain) showed a higher matrix influence, and calculation based on matrix response factors had to be performed for 44, 43 and 41 pharmaceuticals for the liver, kidney and brain, respectively.

Ion enhancement of the target analytes was observed mainly for plasma and muscle samples. Ion enhancement was higher than 50% for six (clomipramine, erythromycin, roxithromycin, sertraline, telmisartan and terbinafine) and two (diclofenac and glimepiride) pharmaceuticals in plasma and muscle, respectively. The remaining three tissues (liver, kidney and brain) showed mostly ion suppression (SM3).

3.3. Method validation

Method validation is investigating whether the analytical procedure is suitable for routine use with reliable analytical results [39]. Method validation was performed by evaluating the linearity, trueness, LOQ, repeatability and robustness [40–43].

The evaluation of the linear response for the target analytes was

Table 1
Recoveries for pharmaceuticals in fish liver using different extraction reagents. RSD is given in brackets. Samples were fortified at concentration 50 ng g^{-1}.

Pharmaceutical	Recovery [%]									
	Reagent A		Reagent B		Reagent C		Reagent D		Reagent E	
10, 11-trans-dihydroCBZ	203	(43)	120	(9)	131	(10)	96	(8)	92	(11)
10, 11-dihydroCBZ	164	(4)	112	(5)	146	(10)	143	(6)	133	(8)
Alfuzosin	148	(14)	131	(12)	126	(4)	120	(5)	106	(11)
Amitryptiline	129	(8)	123	(11)	120	(14)	124	(9)	123	(13)
Atenolol	129	(14)	116	(4)	115	(7)	118	(8)	128	(12)
Atorvastatin	119	(26)	106	(8)	109	(10)	94	(3)	107	(15)
Azithromycin	154	(27)	141	(13)	162	(10)	128	(14)	131	(45)
Bezafibrate	192	(43)	133	(22)	131	(3)	128	(7)	104	(13)
Biperiden	128	(10)	132	(18)	120	(4)	115	(2)	130	(11)
Bisoprolol	132	(5)	132	(5)	135	(9)	136	(10)	146	(7)
Caffeine	155	(7)	141	(11)	140	(12)	135	(8)	107	(15)
Carbamazepine (CBZ)	155	(5)	124	(7)	134	(11)	137	(4)	101	(11)
CBZ-10, 11-epoxide	136	(12)	125	(12)	123	(10)	118	(4)	107	(13)
Cetirizine	251	(58)	139	(20)	118	(7)	146	(5)	124	(10)
Cilazapril	309	(54)	120	(8)	120	(11)	156	(8)	130	(10)
Citalopram	177	(10)	110	(6)	117	(10)	109	(7)	123	(8)
Clarithromycin	125	(8)	112	(18)	107	(5)	109	(13)	113	(13)
Clemastine	138	(24)	138	(23)	115	(8)	107	(10)	120	(11)
Clindamycin	114	(7)	114	(12)	104	(5)	106	(5)	111	(11)
Clindamycin sulfoxide	145	(26)	109	(14)	122	(5)	97	(7)	99	(11)
Clomipramine	147	(12)	104	(8)	122	(10)	123	(9)	108	(13)
Clonazepam	109	(35)	148	(8)	140	(12)	121	(8)	108	(14)
Diclofenac	102	(34)	102	(15)	107	(10)	97	(15)	83	(13)
Dicycloverine	129	(17)	122	(19)	116	(4)	112	(4)	92	(12)
Diltiazem	181	(15)	109	(6)	120	(10)	115	(8)	131	(10)
Disopyramide	133	(4)	144	(12)	125	(6)	125	(5)	116	(10)
Donepezil	128	(20)	126	(8)	137	(12)	129	(13)	153	(16)
Erythromycin	84	(30)	136	(7)	133	(6)	123	(5)	106	(11)
Fenbendazole	121	(15)	119	(8)	119	(7)	130	(7)	94	(7)
Fenofibrate	75	(66)	134	(16)	108	(9)	110	(4)	86	(10)
Fexofenadine	182	(19)	133	(22)	130	(7)	134	(4)	126	(12)
Glibenclamide	89	(53)	104	(18)	122	(13)	109	(12)	77	(14)
Glimepiride	96	(56)	118	(26)	118	(13)	109	(14)	102	(13)
Haloperidol	147	(13)	119	(7)	125	(10)	117	(7)	132	(8)
Irbesartan	176	(20)	101	(7)	107	(12)	96	(9)	109	(9)
Levamisole	78	(45)	142	(6)	126	(9)	138	(3)	121	(11)
Loperamide	99	(30)	146	(20)	116	(8)	116	(11)	112	(15)
Maprotiline	139	(7)	131	(9)	120	(13)	124	(9)	123	(12)
Mebendazole	191	(25)	110	(7)	117	(7)	111	(8)	127	(4)
Meclozine	107	(49)	129	(14)	111	(6)	115	(4)	108	(10)
Memantine	136	(8)	124	(13)	112	(3)	108	(4)	119	(11)
Metamphetamine	113	(3)	116	(3)	129	(35)	107	(8)	104	(11)
Metoprolol	145	(4)	111	(8)	113	(9)	112	(8)	114	(9)
Metoprolol acid	117	(12)	107	(4)	132	(6)	108	(4)	123	(14)
Mianserin	183	(14)	113	(6)	118	(10)	109	(7)	120	(11)
Miconazole	101	(44)	141	(11)	115	(4)	110	(13)	100	(15)
Mirtazapine	124	(12)	133	(7)	144	(9)	97	(7)	97	(12)
N4-acetylsulfamethoxazole	142	(18)	131	(15)	120	(10)	122	(5)	127	(19)
N-desmethylcitalopram	175	(14)	114	(6)	120	(11)	114	(8)	124	(7)
Norsertraline	79	(27)	93	(13)	94	(40)	80	(38)	69	(27)
O-desmethylvenlafaxine	149	(15)	138	(8)	133	(9)	125	(7)	121	(14)
Orphenadrine	127	(13)	129	(19)	49	(5)	148	(25)	134	(9)
Oseltamivir carboxylate	287	(56)	130	(10)	111	(5)	110	(7)	109	(11)
Oxazepam	149	(7)	144	(7)	135	(11)	99	(8)	98	(16)
Oxcarbazepine	128	(10)	119	(7)	127	(10)	125	(4)	114	(9)
Pizotifen	162	(10)	121	(17)	109	(4)	104	(4)	119	(9)
Propranolol	107	(17)	108	(10)	110	(10)	111	(8)	116	(6)
Ropinirole	124	(6)	97	(6)	129	(10)	125	(10)	101	(10)
Roxithromycin	162	(30)	138	(20)	127	(7)	123	(7)	115	(11)
Sertraline	120	(5)	123	(6)	114	(13)	109	(6)	119	(9)
Sotalol	95	(31)	147	(5)	140	(7)	143	(7)	140	(12)
Sulfaclozine	40	(64)	124	(6)	113	(6)	113	(8)	109	(11)
Sulfamethazine	178	(30)	134	(13)	116	(5)	131	(3)	116	(10)
Sulfamethizole	146	(41)	101	(11)	95	(4)	97	(2)	105	(15)
Sulfamethoxazole	135	(6)	144	(14)	135	(10)	136	(6)	159	(24)
Telmisartan	158	(7)	107	(10)	134	(12)	130	(8)	137	(10)
Terbinafine	118	(35)	141	(24)	114	(7)	105	(10)	93	(11)
Terbutaline	87	(21)	146	(12)	124	(4)	103	(5)	102	(10)
Tramadol	147	(6)	132	(5)	145	(8)	125	(10)	100	(10)
Triamterene	109	(14)	120	(10)	125	(10)	121	(9)	109	(11)
Trimethoprim	120	(8)	122	(7)	110	(3)	111	(8)	114	(11)
Valsartan	151	(36)	82	(12)	85	(15)	78	(13)	93	(13)
Venlafaxine	116	(3)	115	(5)	113	(7)	111	(8)	118	(13)

Table 1 (*continued*)

Pharmaceutical	Recovery [%]									
	Reagent A		Reagent B		Reagent C		Reagent D		Reagent E	
Verapamil	134	(10)	144	(7)	143	(11)	143	(8)	128	(14)

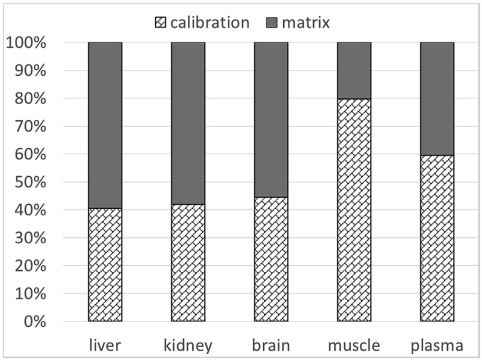


Fig. 1. Evaluation of the matrix effect in different fish tissues as a percentage of compounds quantified based on response factors from calibration curves or matrix matched standards (matrix).

performed at seven concentration levels ranging from 0.1 to 50 ng mL^{-1} (calibration points 0.1, 0.5, 1, 5, 10, 20, 50). Most pharmaceuticals (61) showed very good linearity (squares of residues $r^2 > 0.99$) throughout the entire tested concentration range (0.1–50 ng mL^{-1}) and 11 pharmaceuticals in the concentration range of 0.5–50 ng mL^{-1}. The analytical signals of the last two pharmaceuticals (sulfaclozine and azithromycin) are in general relatively low, which results in linear response ranges of 1–50 and 5–50 ng mL^{-1}, respectively.

The recoveries of all pharmaceuticals at three different concentration levels (5, 20, and 50 ng g^{-1}) are reported in SM4-7 and summarised in Fig. 2 for the liver, kidney, muscle and brain, respectively.

In the case of plasma samples, using reagent D, the recoveries were in the requested range (60–130%) only for 46, 60 and 28 pharmaceuticals of 74 at fortification concentration levels of 5, 20, and 50 ng g^{-1}, respectively. Therefore another extraction reagent (reagent A, acetonitrile + 0.1% formic acid) was then used for the preparation of plasma samples. Corresponding recoveries were then in the acceptable range (60–130%) for 66, 68 and 58 target compounds at concentrations of 5, 20 and 50 ng g^{-1}, respectively. The plasma recovery data are reported in SM8. From the plasma sample results, it is obvious, that the composition of the extraction reagent is a crucial factor in the sample preparation.

The quantification of the target analytes is based on the peak area of the target analyte. Thus, we used the S/N ratio only as an auxiliary parameter for LOQ calculation. The LOQs were calculated from the response at the lowest calibration point, where the relative standard deviation of the average response factor did not deviate more than 30%. The peak area corresponding to this point divided by a factor of four was substituted into the quantification formula instead of the peak area found in the samples to obtain the LOQ for each target analyte in each real sample. The obtained LOQ values for all tissues are given in SM9. The LOQs are the lowest or correspond to the results achieved by HPLC-MS/MS methods used in the determination of target analytes in fish tissue samples in the last 5 years [38,44,45].

A test for the repeatability of the proposed method was performed at three concentration levels (n = 6 for each concentration level). The very good repeatability of the retention times was found for all of the target analytes in all of the measured samples with relative standard deviation (RSD) values below 3.4%. Good peak shape of analytes in a matrix can be documented with comparison

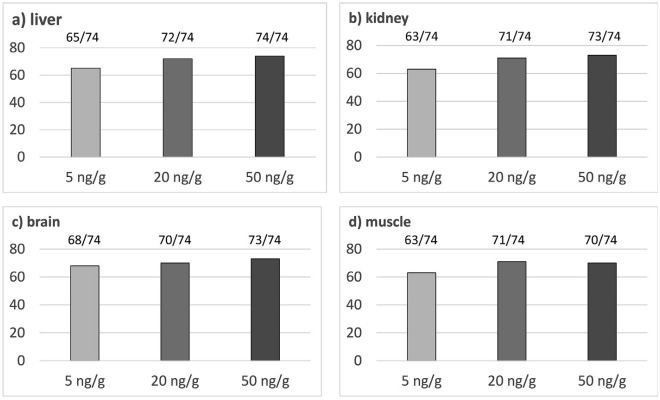


Fig. 2. Extraction performance of the method at different fortification levels: a) liver, b) kidney, c) brain, and d) muscle. The number of pharmaceuticals falling into the recovery range between 60 and 130% is given above the corresponding bars in the graphs.

of chromatograms at fortification level 5 ng g^{-1} with those found in real sample (SM10). The repeatability of quantification can be expressed as the RSD of hexaplicate analysis of fortified samples. It is not surprising that the repeatability of the method is matrix dependent. The median of the RSD of hexaplicates at a concentration level of 20 ng g^{-1}increased in the following order: liver (9%; 0) < brain (9%; 2) < kidney (10%; 6) < plasma (10%; 17) < muscle (13%; 11), where the numbers in brackets correspond to the median value and the number of analytes exceeding 20% of the RSD, respectively.

The most important parameter influencing quantification is the stability of the analytical signal. The stability of HRPS detection can be expressed as the stability of the response factors over time. The biplots of response factors before and after the sequence containing samples of fish liver, muscles and brain (160 samples) are presented in the Fig. 3. We can state that there is no obvious trend between calibration standards measured before and after, as the biplot points are randomly scattered within the 30% interval around the ideal model (where both relative response factor values are identical). Consequently we can conclude that the method is robust for the analysis of large sample sets of complex biota matrices.

3.4. Application of the developed method in the analysis of fish samples

The developed method was successfully applied for the analysis of five tissues from twelve carp (*Cyprinus carpio*) from the Cezarka pond, which is the tertiary treatment of effluent from the municipal STP in Vodnany. The pharmaceuticals found in organs from these fish are presented in Fig. 4, and the average concentrations, detection frequency and LOQs are given in SM9. There were 21 pharmaceuticals present in fish living in the real pond affected by effluent from the STP, with 17 of them found in the liver and kidney (the sums of these concentrations were 32 and 52 ng g^{-1} for the liver and kidney, respectively), 13 in plasma (7 ng g^{-1}), and 7 in the brain (27 ng g^{-1}). The total concentration of all pharmaceuticals in the brain is 4-fold higher than in the plasma, but the number of pharmaceuticals is almost half. The total concentration of present pharmaceuticals is more important than the number of them, especially in the case of psychoactive pharmaceuticals, which have similar or the same mode of action. The muscle was the tissue with the least number of pharmaceuticals found and the lowest total concentration (2.2 ng g^{-1}). In general, these results are in good agreement with our study on brown trout (*Salmo trutta*) living in an effluent-dominated stream [25].

The selective serotonin re-uptake inhibitor (SSRI) citalopram was found in all five matrices in a concentration range of 0.20 (plasma) to 5.3 ng g^{-1} (kidney). Other pharmaceuticals or their metabolites were present only in one or several tissues. Metoprolol acid, a metabolite of metoprolol and atenolol, was found only in the liver at an average concentration of 12 ng g^{-1}. The pharmaceutical with the highest concentration in the kidney was mianserin, which was found only in this tissue. Only psychoactive pharmaceuticals were found in brain tissue. Tramadol, caffeine and sertraline were found in this tissue in average concentrations of 8.9, 5.2 and 5.0 ng g^{-1}, respectively.

Reflecting organ or tissue specific modes of action and the consequent effects of given compounds on fish, it is necessary to analyse the proper tissue indicating the presence of the corresponding pharmaceutical and/or its metabolites. There are pharmaceuticals (or their metabolites) present only in one tissue or two tissues (e.g., clomipramine, metoprolol acid, mianserin and verapamil) as well as pharmaceuticals present in all or in a majority of tissues (citalopram and its metabolite *N*-desmethylcitalopram, sertraline, tramadol and venlafaxine).

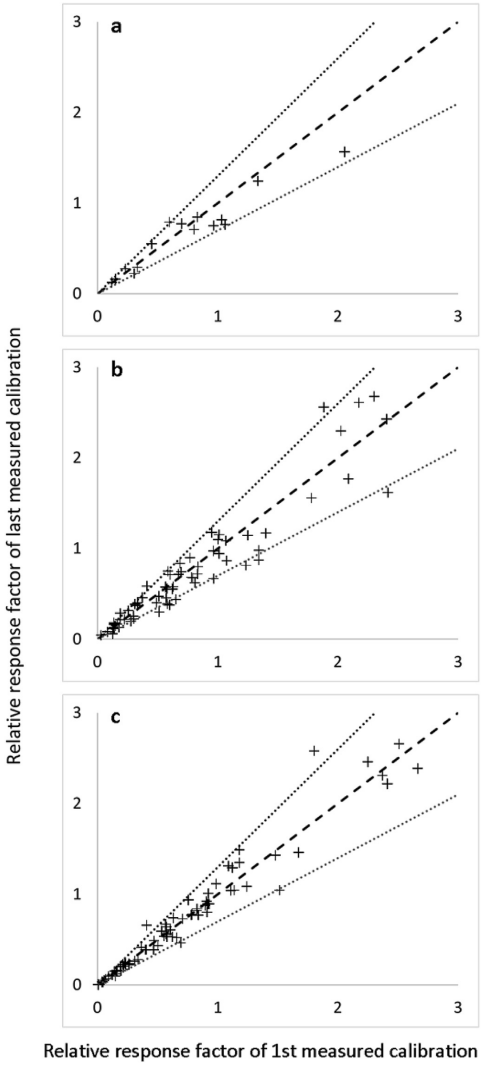


Fig. 3. Stability of the relative response factors at different concentration levels in the sequence of 160 measured samples: a) 0.1 ng mL^{-1}; b) 1 ng mL^{-1}; and c) 10 ng mL^{-1}. Dashed line shows ideal fit with 30% confidence interval represented with dotted line.

4. Conclusions

An ultrasensitive multi-residual HPLC-ESI-HRPS method with a one-step extraction procedure for the determination of 74 pharmaceuticals and their metabolites in different fish tissues was developed.

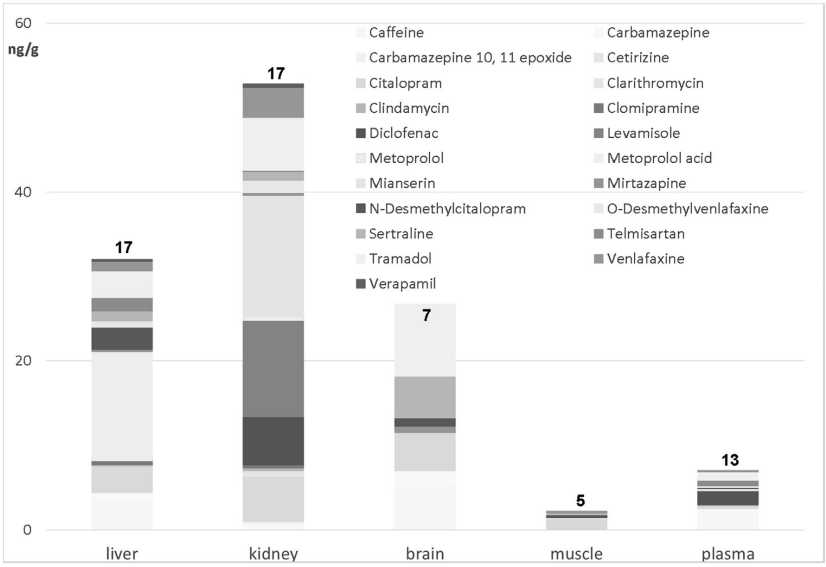


Fig. 4. Concentrations of individual pharmaceuticals in fish from the Cezarka pond.

From the five tested mixtures, the solution of acetonitrile + isopropanol (3:1) acidified with 0.1% formic acid (reagent D) was selected as the best extraction reagent for the extractions of fish liver, kidney, brain and muscle samples. The best extraction reagent for plasma samples was acidified acetonitrile (reagent A). The optimized method using matrix matched standards provides reliable results at environmentally relevant concentrations.

The developed method was applied for the analysis of fish from a pond dominated by STP effluent. The concentrations of the analysed pharmaceuticals ranged from < LOQ to tenths of ng g^{-1}. Different pharmaceuticals and/or their metabolites were found in different fish tissues at varying concentration levels.

This relatively cheap method allows the robust, highly sensitive and highly selective determination of various pharmaceuticals with the sufficient elimination of matrix constituents. It can be used for the routine analyses of a large series of samples.

Such complex information on the presence and levels of pharmaceuticals in different organs can generate significantly better information on the accumulation, transformation and potential effects of the target compounds than single matrix analysis, e.g., conventional plasma samples.

Acknowledgements

This study was supported by the Ministry of Education, Youth and Sports of the Czech Republic, projects CENAKVA (No. CZ.1.05/2.1.00/01.0024), CENAKVA II (No. LO1205 under the NPU I program) and by the Czech Science Foundation (No. 16-06498S). We would also like to thank the Vodnany town municipality for supporting this work. We thank Elsevier for their editing services.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.aca.2018.04.011.

References

[1] R. Grabic, J. Fick, R.H. Lindberg, G. Fedorova, M. Tysklind, Multi-residue method for trace level determination of pharmaceuticals in environmental samples using liquid chromatography coupled to triple quadrupole mass spectrometry, Talanta 100 (2012) 183–195.
[2] E. Gracia-Lor, M. Ibanez, T. Zamora, J.V. Sancho, F. Hernandez, Investigation of pharmaceutical metabolites in environmental waters by LC-MS/MS, Environ. Sci. Pollut. Res. 21 (2014) 5496–5510.
[3] R.H. Lindberg, M. Östman, U. Olofsson, R. Grabic, J. Fick, Occurrence and behaviour of 105 active pharmaceutical ingredients in sewage waters of a municipal sewer collection system, Water Res. 58 (2014) 221–229.
[4] T. Brodin, J. Fick, M. Jonsson, J. Klaminder, Dilute concentrations of a psychiatric drug alter behavior of fish from natural populations, Science 339 (2013) 814–815.
[5] F. Groner, C. Hohne, W. Kleiner, W. Kloas, Chronic diclofenac exposure affects gill integrity and pituitary gene expression and displays estrogenic activity in nile tilapia (Oreochromis niloticus), Chemosphere 166 (2017) 473–481.
[6] K.A. Kidd, P.J. Blanchfield, K.H. Mills, V.P. Palace, R.E. Evans, J.M. Lazorchak, R.W. Flick, Collapse of a fish population after exposure to a synthetic estrogen, Proc. Natl. Acad. Sci. U. S. A 104 (2007) 8897–8901.
[7] J.M. Martin, M. Saaristo, M.G. Bertram, P.J. Lewis, T.L. Coggan, B.O. Clarke, B.B.M. Wong, The psychoactive pollutant fluoxetine compromises antipredator behaviour in fish, Environ. Pollut. 222 (2017) 592–599.
[8] E.S. McCallum, E. Krutzelmann, T. Brodin, J. Fick, A. Sundelin, S. Balshine, Exposure to wastewater effluent affects fish behaviour and tissue-specific uptake of pharmaceuticals, Sci. Total Environ. 605 (2017) 578–588.
[9] S. Rodrigues, A.T. Correia, S.C. Antunes, B. Nunes, Alterations in gills of Lepomis gibbosus, after acute exposure to several xenobiotics (pesticide, detergent and pharmaceuticals): morphometric and biochemical evaluation, Drug Chem. Toxicol. 38 (2015) 126–132.
[10] Y.B. Zhao, S. Castiglioni, K. Fent, Synthetic progestins medroxyprogesterone acetate and dydrogesterone and their binary mixtures adversely affect reproduction and lead to histological and transcriptional alterations in zebrafish (Danio rerio), Environ. Sci. Technol. 49 (2015) 4636–4645.

[11] S. Klatte, H.-C. Schaefer, M. Hempel, Pharmaceuticals in the environment – a short review on options to minimize the exposure of humans, animals and ecosystems, Sustain. Chem. Pharm. 5 (2017) 61–66.
[12] F.A. Weber, T. aus der Beek, A. Bergmann, A. Carius, G. Grüttner, S. Hickmann, I. Ebert, A. Hein, A. Kuester, J. Rose, J. Koch-Jungl, H.C. Stolzenberg, Pharmaceuticals (In the Environment – the Global Perspective), 2014.
[13] W.W. Buchberger, Current approaches to trace analysis of pharmaceuticals and personal care products in the environment, J. Chromatogr. A 1218 (2011) 603–618.
[14] B. Huerta, S. Rodriguez-Mozaz, D. Barcelo, Pharmaceuticals in biota in the aquatic environment: analytical methods and environmental implications, Anal. Bioanal. Chem. 404 (2012) 2611–2624.
[15] U. Bussy, K. Li, W.M. Li, Application of liquid chromatography-tandem mass spectrometry in quantitative bioanalyses of organic molecules in aquatic environment and organisms, Environ. Sci. Pollut. Res. 23 (2016) 9459–9479.
[16] K. Wille, H.F. De Brabander, E. De Wulf, P. Van Caeter, C.R. Janssen, L. Vanhaecke, Coupled chromatographic and mass-spectrometric techniques for the analysis of emerging pollutants in the aquatic environment, Trac. Trends Anal. Chem. 35 (2012) 87–108.
[17] A. Cappiello, G. Famiglini, P. Palma, H. Trufelli, Matrix effects in liquid chromatography-mass spectrometry, J. Liq. Chromatogr. Relat. Technol. 33 (2010) 1067–1081.
[18] P. Panuwet, R.E. Hunter, P.E. D'Souza, X.Y. Chen, S.A. Radford, J.R. Cohen, M.E. Marder, K. Kartavenka, P.B. Ryan, D.B. Barr, Biological matrix effects in quantitative tandem mass spectrometry-based analytical methods: advancing biomonitoring, Crit. Rev. Anal. Chem. 46 (2016) 93–105.
[19] G. Fedorova, T. Randak, R.H. Lindberg, R. Grabic, Comparison of the quantitative performance of a Q-Exactive high-resolution mass spectrometer with that of a triple quadrupole tandem mass spectrometer for the analysis of illicit drugs in wastewater, Rapid Commun. Mass Spectrom. 27 (2013) 1751–1762.
[20] V. Perez-Fernandez, L.M. Rocca, P. Tomai, S. Fanali, A. Gentili, Recent advancements and future trends in environmental analysis: sample preparation, liquid chromatography and mass spectrometry, Anal. Chim. Acta 983 (2017) 9–41.
[21] K.M. Dimpe, P.N. Nomngongo, Current sample preparation methodologies for analysis of emerging pollutants in different environmental matrices, Trac. Trends Anal. Chem. 82 (2016) 199–207.
[22] M. Nunez, F. Borrull, E. Pocurull, N. Fontanals, Sample treatment for the determination of emerging organic contaminants in aquatic organisms, Trac. Trends Anal. Chem. 97 (2017) 136–145.
[23] L. Sanchez-Prado, C. Garcia-Jares, T. Dagnac, M. Llompart, Microwave-assisted extraction of emerging pollutants in environmental and biological samples before chromatographic determination, Trac. Trends Anal. Chem. 71 (2015) 119–143.
[24] C. Baduel, J.F. Mueller, H.H. Tsai, M.J.G. Ramos, Development of sample extraction and clean-up strategies for target and non-target analysis of environmental contaminants in biological matrices, J. Chromatogr. A 1426 (2015) 33–47.
[25] K. Grabicova, R. Grabic, G. Fedorova, J. Fick, D. Cerveny, J. Kolarova, J. Turek, V. Zlabek, T. Randak, Bioaccumulation of psychoactive pharmaceuticals in fish in an effluent dominated stream, Water Res. 124 (2017) 654–662.
[26] J. Fick, R.H. Lindberg, M. Tysklind, D.G.J. Larsson, Predicted critical environmental concentrations for 500 pharmaceuticals, Regul. Toxicol. Pharmacol. 58 (2010) 516–523.
[27] I. Bodik, T. Mackul'ak, M. Faberova, L. Ivanova, Occurrence of illicit drugs and selected pharmaceuticals in Slovak municipal wastewater, Environ. Sci. Pollut. Res. 23 (2016) 21098–21105.
[28] G. Fedorova, O. Golovko, T. Randak, R. Grabic, Storage effect on the analysis of pharmaceuticals and personal care products in wastewater, Chemosphere 111 (2014) 55–60.
[29] G. Fedorova, V. Nebesky, T. Randak, R. Grabic, Simultaneous determination of 32 antibiotics in aquaculture products using LC-MS/MS, Chem. Pap. 68 (2014) 29–36.
[30] G. Fedorova, T. Randak, O. Golovko, V. Kodes, K. Grabicova, R. Grabic, A passive sampling method for detecting analgesics, psycholeptics, antidepressants and illicit drugs in aquatic environments in the Czech Republic, Sci. Total Environ. 487 (2014) 681–687.
[31] O. Golovko, V. Kumar, G. Fedorova, T. Randak, R. Grabic, Seasonal changes in antibiotics, antidepressants/psychiatric drugs, antihistamines and lipid regulators in a wastewater treatment plant, Chemosphere 111 (2014) 418–426.
[32] O. Golovko, O. Koba, R. Kodesova, G. Fedorova, V. Kumar, R. Grabic, Development of fast and robust multiresidual LC-MS/MS method for determination of pharmaceuticals in soils, Environ. Sci. Pollut. Res. 23 (2016) 14068–14077.
[33] T. Mackulak, I. Bodik, J. Hasan, R. Grabic, O. Golovko, A. Vojs-Staňová, M. Gál, M. Naumowitz, J. Tichý, P. Brandeburová, J. Híveš, Dominant psychoactive drugs in the Central European region: a wastewater study, Forensic Sci. Int. 267 (2016) 42–51.
[34] K. Grabicova, R.H. Lindberg, M. Östman, R. Grabic, T. Randak, D.G. Joakim Larsson, J. Fick, Tissue-specific bioconcentration of antidepressants in fish exposed to effluent from a municipal sewage treatment plant, Sci. Total Environ. 488–489 (2014) 46–50.
[35] K. Grabicova, R. Grabic, M. Blaha, V. Kumar, D. Cerveny, G. Fedorova, T. Randak, Presence of pharmaceuticals in benthic fauna living in a small stream affected by effluent from a municipal sewage treatment plant, Water Res. 72 (2015) 145–153.
[36] M. Heynen, T. Brodin, J. Klaminder, M. Jonsson, J. Fick, Tissue-specific uptake of the benzodiazepine oxazepam in adult Eurasian perch (Perca fluviatilis), Environ. Chem. 13 (2016) 849–853.
[37] A.J. Ramirez, M.A. Mottaleb, B.W. Brooks, C.K. Chambliss, Analysis of pharmaceuticals in fish using liquid chromatography-tandem mass spectrometry, Anal. Chem. 79 (2007) 3155–3163.
[38] B. Huerta, A. Jakimska, M. Gros, S. Rodriguez-Mozaz, D. Barcelo, Analysis of multi-class pharmaceuticals in fish tissues by ultra-high-performance liquid chromatography tandem mass spectrometry, J. Chromatogr. A 1288 (2013) 63–72.
[39] B. Magnusson, U. Örnemark (Eds.), Eurachem Guide: the Fitness for Purpose of Analytical Methods – a Laboratory Guide to Method Validation and Related Topics, second ed., 2014.
[40] A. Kruve, R. Rebane, K. Kipper, M.L. Oldekop, H. Evard, K. Herodes, P. Ravio, I. Leito, Tutorial review on validation of liquid chromatography-mass spectrometry methods: Part I, Anal. Chim. Acta 870 (2015) 29–44.
[41] A. Kruve, R. Rebane, K. Kipper, M.L. Oldekop, H. Evard, K. Herodes, P. Ravio, I. Leito, Tutorial review on validation of liquid chromatography-mass spectrometry methods: Part II, Anal. Chim. Acta 870 (2015) 8–28.
[42] I. Taverniers, E. Van Bockstaele, M. De Loose, Trends in quality in the analytical laboratory. I. Traceability and measurement uncertainty of analytical results, Trac. Trends Anal. Chem. 23 (2004) 480–490.
[43] I. Taverniers, M. De Loose, E. Van Bockstaele, Trends in quality in the analytical laboratory. II. Analytical method validation and quality assurance, Trac. Trends Anal. Chem. 23 (2004) 535–552.
[44] R. Tanoue, K. Nomiyama, H. Nakamura, T. Hayashi, J.W. Kim, T. Isobe, R. Shinohara, S. Tanabe, Simultaneous determination of polar pharmaceuticals and personal care products in biological organs and tissues, J. Chromatogr. A 1355 (2014) 193–205.
[45] Y. Zhang, W. Guo, Z. Yue, L. Lin, F. Zhao, P. Chen, W. Wu, H. Zhu, B. Yang, Y. Kuang, J. Wang, Rapid determination of 54 pharmaceutical and personal care products in fish samples using microwave-assisted extraction—hollowfiber—Liquid/solid phase microextraction, J. Chromatogr. B 1051 (2017) 41–53.

CHAPTER 3

DETERMINATION OF CITALOPRAM IN FISH BRAIN TISSUE: BENEFITS OF COUPLING LASER DIODE THERMAL DESORPTION WITH LOW- AND HIGH-RESOLUTION MASS SPECTROMETRY

Borik, A., Staňová, A.V., Brooks, B.W., Grabicová, K., Randák, T., Grabic, R., 2020. Determination of citalopram in fish brain tissue: benefits of coupling laser diode thermal desorption with low- and high-resolution mass spectrometry. Analytical and Bioanalytical Chemistry 412, 4353–4361.

It was allowed by publisher on 25th May, 2020 to include the paper in this Ph.D. thesis

My share on this work was about 50 %

Analytical and Bioanalytical Chemistry (2020) 412:4353–4361
https://doi.org/10.1007/s00216-020-02672-y

RESEARCH PAPER

Determination of citalopram in fish brain tissue: benefits of coupling laser diode thermal desorption with low- and high-resolution mass spectrometry

Adam Borik[1] · Andrea Vojs Staňová[1,2] · Bryan W. Brooks[1,3] · Kateřina Grabicová[1] · Tomáš Randák[1] · Roman Grabic[1]

Received: 18 February 2020 / Revised: 9 April 2020 / Accepted: 20 April 2020 / Published online: 6 May 2020

Abstract
Recent state-of-the-art methods developed for the analysis of polar xenobiotics from different types of biological matrices usually employ liquid chromatography with mass spectrometry. However, there are limitations when a small amount of sample mass is available. For example, individual benthic invertebrates or fish tissue samples often weigh less than 100 mg (e.g., brain, liver) but are necessary to understand environmental fate and bioaccumulation dynamics. We developed ultra-fast methods based on a direct sample introduction technique. This included coupling laser diode thermal desorption with atmospheric pressure chemical ionization mass spectrometry (LDTD-APCI-MS). We then quantitated a common selective serotonin reuptake inhibitor (citalopram) in brain tissues of individual juvenile fish after in vivo exposure to environmentally relevant concentration. Two mass spectrometric methods based on low (LDTD-APCI-triple quadrupole (QqQ)-MS/MS) and high (LDTD-APCI-high-resolution product scan (HRPS)) resolutions were developed and evaluated. Individual instrument conditions were optimized to achieve an accurate and robust analytical method with minimum sample preparation requirements. We achieved very good recovery (97–108%) across the range of 1–100 ng g^{-1} for LDTD-APCI-HRPS. LDTD-APCI-QqQ-MS/MS showed poorer performance due to interferences from the matrix at the lowest concentration level. LDTD-APCI ionization was successfully validated for analysis of non-filtered sample extracts. Evaluation of final methods was performed for a set of real fish brain samples, including comparison of LDTD-APCI-HRPS with a previously validated LC-heated electrospray ionization-HRPS method. This new LDTD-APCI-HRPS method avoids the chromatographic step and provides important benefits such as analysis of limited sample masses, lower total sample volume (typically μL), and reduction in analysis time per sample run to a few seconds.

Keywords Psychoactive pharmaceutical · Juvenile fish · Laser diode thermal desorption · Ambient ionization · Green chemistry

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00216-020-02672-y) contains supplementary material, which is available to authorized users.

✉ Adam Borik
aborik@frov.jcu.cz

[1] Faculty of Fisheries and Protection of Waters, South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses, University of South Bohemia in Ceske Budejovice, Zatisi 728/II, 389 25 Vodnany, Czech Republic

[2] Faculty of Natural Sciences, Department of Analytical Chemistry, Comenius University in Bratislava, Ilkovicova 6, 842 15 Bratislava, Slovak Republic

[3] Department of Environmental Science, Institute of Biomedical Studies, Center for Reservoir and Aquatic Systems Research, Baylor University, Waco, TX 76798, USA

Introduction

Pharmaceuticals are diverse classes of chemical substances that are observed in many environmental compartments around the world [1, 2]. Among the various human medicines, psychoactive pharmaceuticals are characterized by continuously increasing consumption trends [3]. One of the most frequently used psychoactive pharmaceutical is the antidepressant citalopram (CIT), an increasingly common selective serotonin reuptake inhibitor (SSRI). For example, prescription data from England indicated three times higher consumption of CIT in 2010 compared to 1998; this pharmaceutical was the most commonly prescribed antidepressant in England in 2010 [4]. In the Czech Republic, a similar increasing trend in CIT consumption has been observed (from 480 kg in 2011 to 620 kg in 2017) [5].

Psychoactive pharmaceuticals, including CIT, are primarily released to aquatic systems following consumption in hospital and residential settings. Due to relatively insufficient removal during conventional wastewater treatment plan (WWTP) processes, these contaminants of emerging concern enter the aquatic environment [6]. Removal efficiencies for CIT in WWTP are influenced by temperature, varying from 10% in colder months to 40% in warm periods [7, 8]. Consequently, CIT has been found in surface waters ranging from 20 to 430 ng L^{-1} in Europe and the USA, respectively [9–11], and it is one of the most frequently identified SSRI compounds in surface waters at the global scale [12]. Although these concentrations reach ng L^{-1} levels, continuous exposure and subsequent uptake can lead to bioaccumulation in aquatic organisms [13–15] and modulation of reproduction and feeding behavior [16]. Variable distribution of CIT has been observed among fish organs, with the highest concentrations found in liver and brain tissues. These observations are important because this antidepressant elicits neuromodulation within the central nervous system and the liver is the primary organ associated with xenobiotic metabolism [14]. Although some studies have investigated effects of CIT on aquatic organisms, information remains relative, particularly when responses, including behavioral alterations, resulting from molecular initiation events within the central nervous system are considered.

Analytical methods using liquid chromatography combined with different types of detection (mainly mass spectrometry) are commonly used for determination of psychoactive compounds in complex samples [17–20]. Although robust and established methods already exist, there is a need to develop new analytical approaches that reduce sample preparation and analysis time which keep equal selectivity and sensitivity. There is also an increasing need to achieve reliable results when sample masses and volumes are limited, while advancing green chemistry principles [21, 22].

Laser diode thermal desorption with an atmospheric pressure chemical ionization (LDTD-APCI) is a direct sample introduction and ionization technique, where the separation step is avoided. LDTD is based on sample thermal desorption by an infrared diode laser source. The laser energy converts a dried sample to the gaseous phase. The resulting gaseous phase is transported by a carrier gas (synthetic air with defined water content) to the ion source, where neutral compounds are ionized and then reach a mass spectrometer. The main advantages of the LDTD-APCI technique is a low sample volume requirement (1–10 μL), elimination of carryover effect, simplification of sample preparation (e.g., filtration can be omitted), and extremely rapid analysis (approx. 15 s per sample). The LDTD-APCI method has already been successfully applied in analysis of diverse compounds, including cyanotoxins [23], pharmaceuticals [19, 20], and per- and polyfluoroalkyl compounds [24] in water and pharmaceuticals in wastewater sludge [25] and in human and animal plasma samples [26–29].

We aimed to develop high-throughput analytical methods for determination of CIT in fish brain tissues by LDTD-APCI in combination with low- and high-resolution mass spectrometers. We then applied this newly developed method for analysis of juvenile fish brain tissue samples from long-term in vivo exposure experiments. Obtained results were subsequently compared with analyses performed with a previously published method based on the combination of liquid chromatography, heated electrospray ionization, and high-resolution product scan (LC-HESI-HRPS) mass spectrometry [30].

Materials and methods

Chemicals

Methanol and acetonitrile (both LC-MS grade; Merck, Darmstadt, Germany), formic acid (Merck), and ultrapure water (prepared by AquaMax Basic 360 Series and Ultra 370 Series instruments, Young Lin Instruments, purchased from Labicom, Olomouc, Czech Republic) were used in this work.

A citalopram hydrobromide (AK Scientific, Union City, CA, USA) standard stock solution was prepared in methanol at 1 mg mL^{-1}. An isotopically labeled stock solution standard (IS) of D_6-citalopram, which was acquired from Cerillant (Toronto, Canada), was prepared in the same way as the native compound.

Instrumentation

Sample desorption and ionization was performed by a T-960 LDTD-APCI ion source (Phytronix Technologies, Quebec, Canada) with an infrared diode laser source (980 nm, 20 W) controlled via LazSoft 4.0 software (Phytronix Technologies). Heated carrier gas (synthetic air with a certified water content of 35 ppm, flow of 3 L min^{-1}, at 50 °C, obtained from SAID, Bergamo, Italy) was used for transfer of analyte molecules from the desorption spot to the corona discharge region. Ionization parameters such as ion sweep gas pressure (0.3 arbitrary units), discharge current (3 μA), and capillary and vaporizer temperatures (270 °C and 31 °C, respectively) were set up according to the LDTD-APCI manufacturer.

Two different types of mass spectrometers, a triple quadrupole mass spectrometer TSQ Quantum Ultra (LDTD-APCI-triple quadrupole (QqQ)-MS/MS) operated in a selected reaction monitoring mode (0.7 FWHM isolation window) and a hybrid high-resolution mass spectrometer Q Exactive (LDTD-APCI-high-resolution product scan (HRPS)) operated in a high-resolution product scan (1 m/z isolation window and 17,500 FWHM resolution for product scan), were used (both instruments from Thermo Fisher Scientific, San Jose, CA, USA). All analyses were performed in positive ionization mode with mass transitions listed in Electronic

Supplementary Material (ESM) Table S1. Data acquisition and post-processing was performed using Xcalibur 3.0 and TraceFinder 3.3 software, respectively, from Thermo Fisher Scientific. An LC-HESI-HRPS reference method was described previously by Grabicova et al. [30]. Briefly, all LC-HESI-HRPS analyses were performed using an Accela 1250 LC pump (Thermo Fisher Scientific) and a HTS XT-CTC autosampler (CTC Analytics AG) system equipped with a Hypersil Gold aQ column (50 mm × 2.1 mm, 5-μm particles; Thermo Fisher Scientific). LC analyses were performed using gradient elution (ultrapure water and acetonitrile both with 0.1% formic acid; see detail in ESM Table S2). A heated electrospray ionization (HESI) in positive ionization mode (3.5 kV) and nitrogen as the sheath gas (40 arbitrary units), auxiliary gas (10 arbitrary units), and collision gas was used. The high-resolution product scan mode setting is described above.

Sample description and preparation

The analyte free matrix used for method development and validation was obtained from adult rainbow trout (*Oncorhynchus mykiss*) from a local commercial hatchery (Vodnany, Czech Republic). Fish brain tissue was collected immediately after the fish were sacrificed according to the Czech National Directive (the Law against Animal Cruelty, No. 246/1992), and then stored at − 20 °C until sample preparation. Sample preparation for LDTD-APCI method development and evaluation was performed according to Grabicova et al. [30]. Briefly, all brain tissue samples were weighted and extracted by TissueLyser II (Quiagen, Germany) at 30 Hz for 10 min in Eppendorf tubes with the addition of a mixture of solvents (acetonitrile + isopropanol (3:1), acidified with 0.1% formic acid), internal standards, and a stainless steel ball. These samples were then centrifuged in a Micro 200 R centrifuge (Hettich Zentrifugen, Germany) at 9500×*g* for 10 min. The supernatant was left in the freezer for 24 h to allow protein precipitation. The only difference between sample preparations for LC and LDTD-APCI was a filtration step (0.45-μm regenerated cellulose filter), which is not necessarily required for direct sample introduction. Samples prepared for analysis were spotted (5 μL) in cells of a LazWell 96-well plate and allowed to evaporate to dryness at room temperature (25 °C ≈ 15–20 min).

Comparison of the conventional LC-HESI-HRPS method with these newly developed LDTD-APCI-HRPS and LDTD-APCI-QqQ-MS/MS methods was performed with analysis of fish brain samples from an in vivo behavioral experiment. Juvenile chubs (*Squalius cephalus*) were exposed to a concentration of 1 μg L^{-1} of CIT (for further details, see the ESM). These samples were prepared in the same way as described above. The extraction solvent volume was adopted to a lower amount of juvenile brain tissue (approx. 0.05 g of tissue was extracted with 200 μL of solvent). Due to LC-HESI-HRPS analysis, the filtration step was involved to prevent LC clogging. Potential effects of this sample filtration step on the response factor of CIT were tested and evaluated on different sample sets.

Method performance evaluation

The same performance parameters obtained from analyses of identical samples with LDTD-APCI-QqQ-MS/MS and LDTD-APCI-HRPS were used for evaluation of methods. The analyte concentrations were calculated by the following equations:

$$\mathrm{RF} = \left(\frac{\text{Peak area}_{\mathrm{cal}}}{C_{\mathrm{cal}}}\right) \times \left(\frac{\text{Peak area}_{\mathrm{IS}}}{C_{\mathrm{IS}}}\right) \quad (1)$$

$$C_{\mathrm{CIT}} = \left(\frac{\text{Peak area}_{\mathrm{CIT}}}{\text{Peak area}_{\mathrm{IS}}}\right) \times \left(\frac{1}{\mathrm{ARF}}\right) \times C_{\mathrm{IS}} \quad (2)$$

Equation (1) presents the calculation of response factor (RF), where Peak $\text{area}_{\mathrm{cal}}$ is the peak area corresponding to the analyte, C_{cal} is the concentration of the native analyte at corresponding calibration point, Peak $\text{area}_{\mathrm{IS}}$ is the peak area of isotopically labeled standard, and C_{IS} is the concentration of isotopically labeled standard. CIT concentration in an individual sample was calculated according to Eq. (2), where Peak $\text{area}_{\mathrm{CIT}}$ is the analyte area in the sample, Peak $\text{area}_{\mathrm{IS}}$ is the corresponding signal of internal standard, and ARF is the mean RF obtained from a RF calibration curve. This calibration curve was prepared in clean brain sample extract at concentrations 0.02 ng g^{-1}, 0.05 ng g^{-1}, 0.1 ng g^{-1}, 0.5 ng g^{-1}, 1 ng g^{-1}, 5 ng g^{-1}, 10 ng g^{-1}, 25 ng g^{-1}, 50 ng g^{-1}, and 100 ng g^{-1} and used for method linearity evaluation. Limit of detection (LOD) and limit of quantification (LOQ) were calculated as 3.3 and 10 times, respectively, of the calibration curve intercept SD divided by the slope [31].

Accuracy was calculated as recovery (RE) of nominal spiked concentration, and precision was expressed as relative standard deviation (RSD) by evaluating multiple concentration levels (1 ng g^{-1}, 2 ng g^{-1}, 5 ng g^{-1}, 25 ng g^{-1}, 100 ng g^{-1}) in fish brain tissue ($n = 6$), according to Eq. (3):

$$\mathrm{RE} = \frac{C_{\mathrm{calc}}}{C_{\mathrm{spiked}}} \times 100\% \quad (3)$$

where C_{calc} is the concentration obtained by an instrument method and C_{spiked} is the nominal concentration added into the sample [32].

Matrix effects (tested at the concentration level 25 ng g^{-1}) were estimated according to Matuszewski et al. [33]. The statistical method comparison and data visualization were performed with Passing-Bablok regression [34] and Bland-Altman difference plot [35]. This experiment was performed

in accordance with the EU-harmonized Animal Welfare Act of the Czech Republic. The research facility is authorized under (No. 53100/2013-MZE-17214) the framework of the law against Animal Cruelty of the Czech Republic (No. 246/1992), with the ethical approval committee number MSMT-6744/2018-4.

Optimization of LDTD-APCI methods

In this study, two laser patterns were tested as the most critical parameter in LDTD-APCI method development. The first one included a fixed ramping time (time from zero to maximum laser energy setup) and a fixed holding time (time of maxim laser energy application), and the second pattern included a fixed holding time, but the ramping time was proportionally increased with increasing laser energy. The four applied laser energies (15%, 35%, 55%, and 75%) were investigated for both laser patterns. Subsequently, analyte peak area, signal-to-noise ratio, peak symmetry, and total desorption time were used as evaluation parameters of tested laser patterns.

The Q Exactive mass spectrometer required optimization of ion trap (C-trap) setup, which defines ion population injected into the orbital ion trap. Injection is regulated by automatic gain control (AGC, maximal ion count) and ion time (IT, defining maximal filing time period). All tests were performed in hexaplicates at the concentration level 25 ng g^{-1} of CIT in the fish brain matrix, and results were expressed as mean (± SD).

Results and discussion

Development of LDTD-APCI-QqQ-MS/MS and LDTD-APCI-HRPS methods

Power and time distribution of applied laser energy determines the efficiency and reproducibility of obtained results. The power of the laser should be carefully optimized to obtain the highest signal of a target analyte and maintain the lowest possible background signal caused by interfering compounds desorbed from the matrix. Consequently, laser radiation energy should not to be set too high to prevent thermal degradation of target compounds [31].

In the present study, two types of laser patterns (LP 1 and LP 2) were investigated (see details in "Optimization of LDTD-APCI methods" section). The results (Table 1) showed that LP 1 with 35% laser energy and LP 2 with 55% laser energy exhibited a similar peak area and signal-to-noise ratio. Better peak symmetry and higher number of data points (approx. 12) were obtained for LP 1 (35%) than for other patterns. Consequently, LP 1 with 35% laser energy was chosen as desorption setup for LDTD-APCI-QqQ-MS/MS analysis. The laser pattern and analyte signal are illustrated in Fig. 1. With LP 1, the optimal sample volume was evaluated with 3 μL, 5 μL, 8 μL, and 12 μL of spiked brain tissue extracts. As displayed in Fig. 2, the highest peak area was obtained for 5 μL of spotted sample volume.

LDTD-APCI-HRPS method development was based on the results described above, which used a low-resolution instrument. The initial setup of LP 1 with 35% laser energy was applied, but the response of CIT (shown in Fig. 3a) did not exceed eight data points, because the acquisition rate of Q Exactive is limited to approx. 12 Hz at a resolving power of 17,500 FWHM [36]. In the next step, LP 2 with 55% laser energy was investigated, and the continuously increasing laser energy over 30% led to the prolonged desorption time of CIT and the corresponding increase of the number of data points. A sufficient number of data points (minimum 12) was obtained (Fig. 3b). An effect of different C-trap settings on instrument response was investigated. First, IT was tested in the range of 10–70 ms and the highest possible C-trap filling time was investigated without slowing data acquisition frequency. All tested IT values were examined with AGC set to 5e5 ion count, which represents the middle value, and the acquisition frequency should not be affected by AGC. Application of IT in the range of 10–50 ms did not show a decrease of acquisition frequency, and the observed data points never dropped under 12. Higher IT (60–70 ms) resulted in the decrease of the number of data points from 10 to 8, respectively (ESM Fig. S1). An optimized IT value was thus used for evaluation of AGC. The results showed a negligible effect on CIT response across the tested injection ion population across the range from 2e5 to 3e6 counts (Fig. 4). Based on the abovementioned optimization, LP 2 with a maximal laser energy level of 55% and conditions for ion accumulation in C-trap (50 ms IT and 1e6 AGC) can be considered as optimal parameters for this LDTD-APCI-HRPS method.

Evaluation of LDTD-APCI-QqQ-MS/MS and LDTD-APCI-HRPS methods

As noted above, both developed methods were validated with the same parameters (accuracy, precision, linearity, intraday precision, LOQ, LOD) and under the same conditions. Linearity was calculated as the response ratio of native compound peak area and IS peak area. The LDTD-APCI-QqQ-MS/MS method exhibited sufficient linearity across the working range of 0.02–100 ng g^{-1} in the brain tissue matrix with $R^2 > 0.9997$, and a similar result was obtained for LDTD-APCI-HRPS as well ($R^2 > 0.9998$).

Calculations of LODs and LOQs were derived from a calibration curve (see the section "Method performance evaluation") with obtained LODs of 1.2 ng g^{-1} and 0.39 ng g^{-1} for the LDTD-APCI-QqQ-MS/MS and the LDTD-APCI-HRPS methods, respectively. Corresponding LOQs were 3.7 ng g^{-1}

Table 1 Summary of the optimization of the laser pattern setup ($n = 6$)

	Laser energy (%)	Ramping time (s)	Holding time (s)	Peak area (arbitrary units)		Signal-to-noise ratio		Peak symmetry (%)		Desorption time (s)	
				Ave	SD	Ave	SD	Ave	SD	Ave	SD
Laser pattern 1	15	2.4	2	26,000	11,000	63,000	49,000	64	28	3.0	1.3
	35	2.4	2	22,000	4500	130,000	67,000	74	15	1.5	0.11
	55	2.4	2	13,000	1200	100,000	64,000	60	18	1.1	0.15
	75	2.4	2	11,000	2400	130,000	32,000	76	11	0.85	0.13
Laser pattern 2	15	1	2	32,000	3300	91,000	63,000	59	23	2.6	0.30
	35	2.4	2	22,000	4500	130,000	67,000	74	15	1.5	0.11
	55	3.6	2	27,000	6500	160,000	78,000	52	22	1.8	0.34
	75	4.8	2	22,000	5700	65,000	40,000	56	12	1.6	0.12

for the LDTD-APCI-QqQ-MS/MS method and 1.2 ng g^{-1} for the LDTD-APCI-HRPS method.

Recovery was evaluated at concentration levels 1 ng g^{-1}, 2 ng g^{-1}, 5 ng g^{-1}, 25 ng g^{-1}, and 100 ng g^{-1}. The LDTD-APCI-HRPS method exhibited excellent results, where all tested concentrations showed recovery between 97 and 108%. Further, the highest RSD was observed for the lowest concentration (37%) and RSD did not exceed 17% for other concentrations (Fig. 5). Worse results were obtained with a low-resolution mass spectrometer, where the highest differences observed at the two lowest concentrations with recoveries of 230% and 138% for LDTD-APCI-QqQ-MS/MS. Despite obvious interferences at the lowest concentration level, RSDs never exceeded 20%.

MS/MS fragmentation of CIT (325.1711 m/z) led to two major products (262.1027 and 109.0448 m/z), where the fragment 109.0448 was the most intensive. LDTD-APCI-HRPS did not exhibit a signal of both mass transitions in the juvenile fish brain blank matrix, which confirmed the selectivity of this LDTD-APCI-HRPS method (ESM Fig. S2a). Unfortunately, obtained MS/MS spectra showed a potentially interfering mass transition (109.0768 m/z) from sample background (ESM Fig. S2a), which could not be resolved by a quadrupole analyzer even with a minimum isolation window setup of 0.1 FWHM. The expected interference-induced response is shown in ESM Fig. S2d (gray color). Higher selectivity of transition 262.1027 was confirmed, expressing only low baseline noise (red color) in the blank sample (ESM Fig. S2d). It can thus be concluded that HRPS detection, not QqQ-MS/MS, showed enough high selectivity, allowing detection of CIT at a low concentration level.

Intraday precision was tested at a concentration level of 5 ng g^{-1} set according to the lowest concentration level with comparable method accuracy. Intraday precision was calculated as RSD of pentaplicates obtained by both methods. The LDTD-APCI-HRPS method exhibited 5% RSD, compared to 24% obtained with LDTD-APCI-QqQ-MS/MS. We also evaluated matrix effects, which showed CIT signal suppression of − 80% and − 67% for LDTD-APCI-QqQ-MS/MS and LDTD-APCI-HRPS, respectively. These results corresponded to

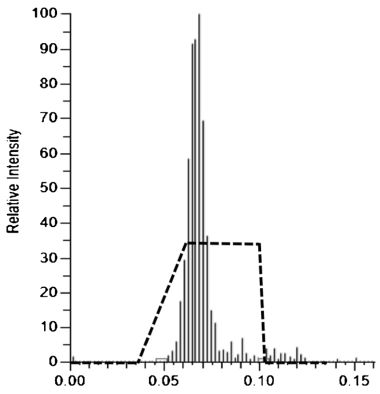


Fig. 1 Visualization of laser pattern (LP) 1 with a laser energy level of 35% (dash lines) selected for the LDTD-QqQ-MS/MS method and resulting analyte signal

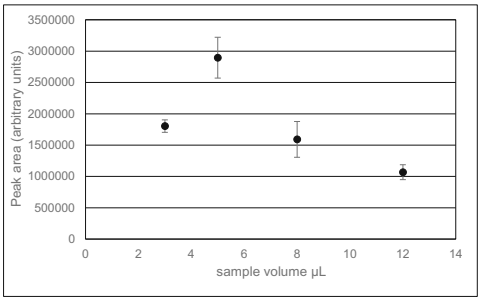


Fig. 2 Optimization of the spotted sample volume. Results are displayed as a mean value of peak area with error bars representing SD ($n = 6$)

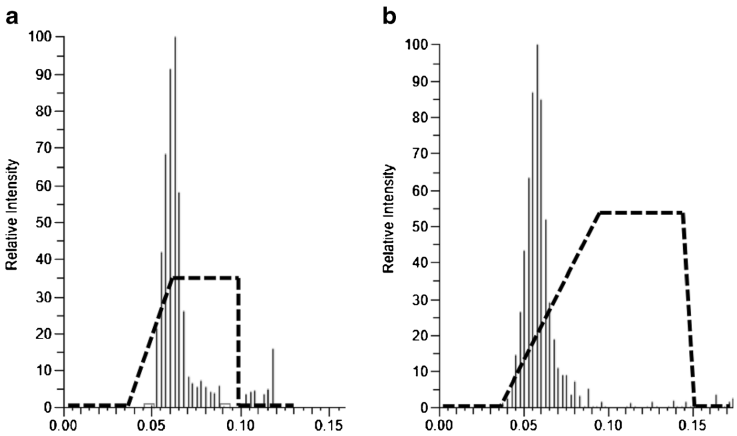


Fig. 3 Visualization of laser patterns (dash lines) tested for the LDTD-APCI-HRPS method and resulting analyte signals. **a** Laser pattern (LP) 1 with a laser energy level of 35%. **b** LP 2 with a laser energy level of 55%

direct sample introduction, when the target analyte was potentially co-desorbed with the sample matrix. Subsequently, simultaneously desorbed matrix compounds competed in ionization.

Direct sample introduction by LDTD-APCI can be beneficial for simplifying sample preparation because a filtration step can be avoided. To evaluate this potential effect, the differences between CIT response factors in filtered and non-filtered samples for both LDTD-APCI methods were evaluated. As displayed in ESM Fig. S3, there was no appreciable effect of this step on LDTD-APCI-QqQ-MS/MS detection and only a 10% increase of response factor for LDTD-APCI-HRPS when filtration was not performed. Such a negligible effect of this filtration step enhances analytical application of LDTD-APCI when extract volumes are only microliters. This methodological benefit can be crucial in cases where limited sample masses (small organs; individual fish in early ontogenesis stage; individual invertebrates) are available for analysis.

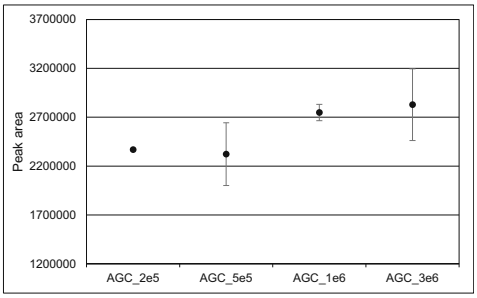


Fig. 4 Effect of C-trap AGC with IT of 50 ms on the CIT peak area. Experimental condition: LP 2 with 55% laser energy. The results are represented as the mean ± SD ($n = 6$)

LC-HESI-HRPS method in comparison with LDTD-APCI-QqQ-MS/MS and LDTD-APCI-HRPS

Concordance of the newly developed LDTD-APCI-QqQ-MS/MS and LDTD-APCI-HRPS methods with a state-of-the-art LC-HESI-HRPS method [34, 37, 38] was observed using individual fish brain samples from in vivo experiments, where juvenile chubs were exposed to CIT at an environmental relevant concentration (1 μg L^{-1}).

The non-parametric method Passing-Bablok regression was used to compare the abovementioned methods. This approach allows the identification of systematic and/or proportional differences. Comparability of these two methods was identified when 95% confidence intervals (CIs) did not include a value of 0 for intercept and the value of 1 for slope (both types of difference are rejected) [34, 37, 38]. Figure 6 a shows a biplot of LC-HESI-HRPS (*y*-axis) with LDTD-APCI-HRPS (*x*-axis). These results are plotted consistently close to the identity line indicating optimal fitting of results (gray-colored line), across the entire concentration range. Calculated

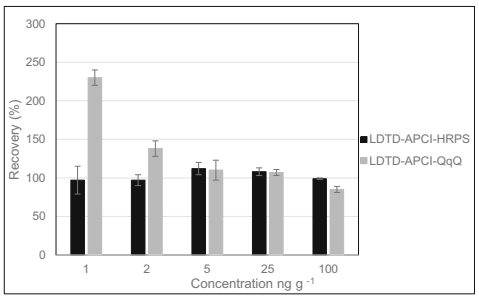


Fig. 5 Recovery of LDTD-APCI-QqQ-MS/MS and LDTD-APCI-HRPS methods for five different concentration levels ($n = 6$)

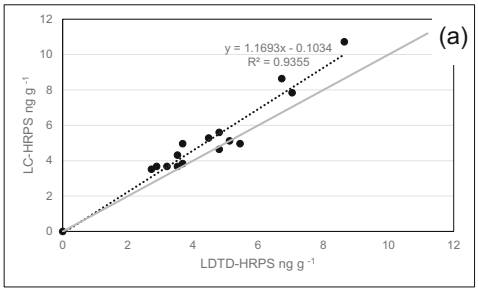


Fig. 6 Passing-Bablok regression plot. **a** Comparison of LC-HESI-HRPS and LDTD-APCI-HRPS methods. **b** Comparison of LC-HESI-HRPS and LDTD-APCI-QqQ-MS/MS methods ($n = 15$)

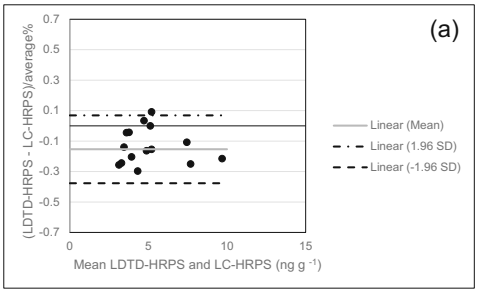


Fig. 7 Bland-Altman plots. Bias means and 95% confidence interval. **a** Bias of LC-HESI-HRPS and LDTD-APCI-HRPS. **b** Bias of LC-HESI-HRPS and LDTD-APCI-QqQ-MS/MS ($n = 15$)

CIs for slope and intercept confirmed that LDTD-APCI-HRPS results are comparable with the reference method (slope CI = 0.99–1.35; intercept CI = − 0.06 to 0.05). Figure 6 b specifically presents a comparison of data from the LC-HESI-HRPS (*y*-axis) and LDTD-APCI-QqQ-MS/MS (*x*-axis) methods. Despite an obviously higher deviation of the results from optimal fit, calculated CIs were in the range of method agreement (slope CI = 0.57–1.06; intercept CI = − 0.01 to 0.16). The relative biases of methods are expressed in Fig. 7 a and b by Bland-Altman difference plots, where LC-HESI-HRPS and LDTD-APCI-HRPS (Fig. 7a) exhibited a mean bias of 15.4% (CI = − 37 to 7%). LC-HESI-HRPS and LDTD-APCI-QqQ-MS/MS showed a similar mean bias (− 16.4%), but this CI showed a higher range, especially for samples at low analyte concentration levels. This observation corresponds to interfering matrix compounds identified by HRPS. Such findings collectively result in higher LOQ and LOD levels of the LDTD-APCI-QqQ-MS/MS method, and its false-positive results are identified at the lowest validated level (recovery values up to 230%) as reported in Fig. 5.

The direct sample introduction brings particular limiting aspects, which have to be considered before LDTD method application. One of the critical considerations is the required number of target analytes in a complex sample matrix. The presented in vivo experiment represents a common case of toxicological studies, which aims to understand relationships between chemical exposure (from single to few) and associated impacts on investigated organism under controlled conditions. This single compound analysis has good suitability for the LDTD-APCI-HRPS method according to relatively limited acquisition speed of the high-resolution mass spectrometer and short desorption time of the chemical by LDTD. Consequently, eventual application of LDTD-APCI-HRPS for multi-residue analysis requires multiple runs of a sample with individual parallel reaction monitoring setups or choice of full scan acquisition mode. Obtained high-resolution full scan spectra can provide detailed information about molecule formulas of desorbed compounds, but lack of structural information from product scan can present false-positive results [39]. These special circumstances could be analytes with identical m/z of precursors and major fragmentation products, such as tramadol and *o*-desmethylvenlafaxine [40]. These analytical issues can be solved by selection of minor but unique mass transition (if presented) or with separation techniques instead of LDTD. For similar reasons, application of LDTD-APCI-HRPS for non-target metabolome investigations, which represents an increasingly important pursuit (even in environmental science) [41–43], can be challenging. However, other ambient ionization techniques have been successfully used for such purposes [44], but to our knowledge, LDTD

instrumentation has not been applied for metabolomic or any non-targeted investigation.

Conclusion

Our study utilized two mass spectrometer types to examine strengths and weaknesses of high- and low-resolution mass spectrometry in combination with LDTD-APCI when a complex biological matrix was investigated. It can be concluded that both LDTD-APCI methods fulfilled conditions for agreement with reference LC-HESI-HRPS but LDTD-APCI-HRPS exhibited lower quantification limits. Further, no interferences were observed at the concentration levels examined, which is particularly relevant for environmental research and biomedical studies of alternative fish models (units of ng per g). The LDTD-APCI-HRPS method reported here represents a comparable and beneficial alternative to a more traditional reference LC-HESI-HRPS method.

LDTD-APCI, especially with highly sensitive and selective HRPS detection, represents a useful alternative approach for sample introduction to mass spectrometer and analyses of complex biological matrices. Avoiding compound separation provides important benefits, including rapid reduction of analysis time and reduction sample treatment requirements, both which result in increased sample analysis throughput. In addition, avoiding sample filtration presents additional potential for application of LDTD-APCI during analyses with critically small sample amounts.

Funding information The study was financially supported by the Ministry of Education, Youth and Sports of the Czech Republic projects "CENAKVA" (LM2018099) and "CENAKVA Center Development (No. CZ.1.05/2.1.00/19.0380)" and by the Czech Science Foundation (No. 18-15802S).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Research involving animals This experiment was performed in accordance with the EU-harmonized Animal Welfare Act of the Czech Republic. The research facility is authorized under (No. 53100/2013-MZE-17214) the framework of the law against Animal Cruelty of the Czech Republic (No. 246/1992), with the ethical approval committee number MSMT-6744/2018-4.

References

1. Carter LJ, Harris E, Williams M, Ryan JJ, Kookana RS, Boxall ABA. Fate and uptake of pharmaceuticals in soil–plant systems. J Agric Food Chem. 2014;62:816–25. https://doi.org/10.1021/jf404282y.
2. Agunbiade FO, Moodley B. Occurrence and distribution pattern of acidic pharmaceuticals in surface water, wastewater, and sediment of the Msunduzi River, Kwazulu-Natal, South Africa. Environ Toxicol Chem. 2016;35:36–46. https://doi.org/10.1002/etc.3144.
3. Gualano MR, Bert F, Mannocci A, La Torre G, Zeppegno P, Siliquini R. Consumption of antidepressants in Italy: recent trends and their significance for public health. Psychiatr Serv. 2014;65:1226–31. https://doi.org/10.1176/appi.ps.201300510.
4. Ilyas S, Moncrieff J. Trends in prescriptions and costs of drugs for mental disorders in England, 1998-2010. Br J Psychiatry. 2012;200:393–8. https://doi.org/10.1192/bjp.bp.111.104257.
5. Státní ústav pro kontrolu léčiv. http://www.sukl.cz/. Accessed 22 Oct 2019.
6. Mackulak T, Mosny M, Skubak J, Grabic R, Birosova L. Fate of psychoactive compounds in wastewater treatment plant and the possibility of their degradation using aquatic plants. Environ Toxicol Pharmacol. 2015;39:969–73. https://doi.org/10.1016/j.etap.2015.02.018.
7. Golovko O, Kumar V, Fedorova G, Randak T, Grabic R. Seasonal changes in antibiotics, antidepressants/psychiatric drugs, antihistamines and lipid regulators in a wastewater treatment plant. Chemosphere. 2014;111:418–26. https://doi.org/10.1016/J.CHEMOSPHERE.2014.03.132.
8. Gurke R, Rößler M, Marx C, Diamond S, Schubert S, Oertel R, et al. Occurrence and removal of frequently prescribed pharmaceuticals and corresponding metabolites in wastewater of a sewage treatment plant. Sci Total Environ. 2015;532:762–70. https://doi.org/10.1016/j.scitotenv.2015.06.067.
9. Bradley PM, Barber LB, Clark JM, Duris JW, Foreman WT, Furlong ET, et al. Pre/post-closure assessment of groundwater pharmaceutical fate in a wastewater-facility-impacted stream reach. Sci Total Environ. 2016;568:916–25. https://doi.org/10.1016/j.scitotenv.2016.06.104.
10. González Alonso S, Catalá M, Maroto RR, Gil JLR, de Miguel ÁG, Valcárcel Y. Pollution by psychoactive pharmaceuticals in the rivers of Madrid metropolitan area (Spain). Environ Int. 2010;36:195–201. https://doi.org/10.1016/j.envint.2009.11.004.
11. Mackuľak T, Bodík I, Hasan J, Grabic R, Golovko O, Vojs-Staňová A, et al. Dominant psychoactive drugs in the Central European region: a wastewater study. Forensic Sci Int. 2016;267:42–51. https://doi.org/10.1016/J.FORSCIINT.2016.08.016.
12. Mole RA, Brooks BW. Global scanning of selective serotonin reuptake inhibitors: occurrence, wastewater treatment and hazards in aquatic systems. Environ Pollut. 2019:1019–31.
13. Grabicova K, Lindberg RH, Östman M, Grabic R, Randak T, Joakim Larsson DG, et al. Tissue-specific bioconcentration of antidepressants in fish exposed to effluent from a municipal sewage treatment plant. Sci Total Environ. 2014;488:46–50. https://doi.org/10.1016/j.scitotenv.2014.04.052.
14. Grabicova K, Grabic R, Fedorova G, Fick J, Cerveny D, Kolarova J, et al. Bioaccumulation of psychoactive pharmaceuticals in fish in an effluent dominated stream. Water Res. 2017;124:654–62. https://doi.org/10.1016/j.watres.2017.08.018.
15. Grabicova K, Grabic R, Blaha M, Kumar V, Cerveny D, Fedorova G, et al. Presence of pharmaceuticals in benthic fauna living in a small stream affected by effluent from a municipal sewage treatment plant. Water Res. 2015;72:145–53. https://doi.org/10.1016/j.watres.2014.09.018.
16. Cunha DL, Mendes MP, Marques M. Environmental risk assessment of psychoactive drugs in the aquatic environment. Environ Sci Pollut Res. 2019;26:78–90.
17. Elliott S, Evans J. A 3-year review of new psychoactive substances in casework. Forensic Sci Int. 2014;243:55–60. https://doi.org/10.1016/J.FORSCIINT.2014.04.017.
18. Marchei E, Escuder D, Pallas CR, Garcia-Algar O, Gómez A, Friguls B, et al. Simultaneous analysis of frequently used licit and

illicit psychoactive drugs in breast milk by liquid chromatography tandem mass spectrometry. J Pharm Biomed Anal. 2011;55:309–16. https://doi.org/10.1016/J.JPBA.2011.01.028.

19. Mallett DN, Ramírez-Molina C. The use of partially porous particle columns for the routine, generic analysis of biological samples for pharmacokinetic studies in drug discovery by reversed-phase ultra-high performance liquid chromatography–tandem mass spectrometry. J Pharm Biomed Anal. 2009;49:100–7. https://doi.org/10.1016/J.JPBA.2008.09.041.
20. Ali AM, Rønning HT, Sydnes LK, Alarif WM, Kallenborn R, Al-Lihaibi SS. Detection of PPCPs in marine organisms from contaminated coastal waters of the Saudi Red Sea. Sci Total Environ. 2018;621:654–62. https://doi.org/10.1016/j.scitotenv.2017.11.298.
21. Poliakoff M, Fitzpatrick JM, Farren TR, Anastas PT (2002) Green chemistry: science and politics of change. Science (80-.)
22. Erythropel HC, Zimmerman JB, De Winter TM, Petitjean L, Melnikov F, Lam CH, et al. The Green ChemisTREE: 20 years after taking root with the 12 principles. Green Chem. 2018;20:1929–61.
23. Roy-Lachapelle A, Solliec M, Sinotte M, Deblois C, Sauvé S. Total analysis of microcystins in fish tissue using laser thermal desorption–atmospheric pressure chemical ionization–high-resolution mass spectrometry (LDTD-APCI-HRMS). J Agric Food Chem. 2015;63:7440–9. https://doi.org/10.1021/acs.jafc.5b02318.
24. Munoz G, Vo Duy S, Budzinski H, Labadie P, Liu J, Sauvé S. Quantitative analysis of poly- and perfluoroalkyl compounds in water matrices using high resolution mass spectrometry: optimization for a laser diode thermal desorption method. Anal Chim Acta. 2015;881:98–106. https://doi.org/10.1016/j.aca.2015.04.015.
25. Lonappan L, Pulicharla R, Rouissi T, Brar SK, Verma M, Surampalli RY, et al. Diclofenac in municipal wastewater treatment plant: quantification using laser diode thermal desorption—atmospheric pressure chemical ionization—tandem mass spectrometry approach in comparison with an established liquid chromatography-electrospray ionization–tandem mass spectrometry method. J Chromatogr A. 2016;1433:106–13. https://doi.org/10.1016/J.CHROMA.2016.01.030
26. Mohapatra DP, Brar SK, Tyagi RD, Picard P, Surampalli RY. Carbamazepine in municipal wastewater and wastewater sludge: ultrafast quantification by laser diode thermal desorption-atmospheric pressure chemical ionization coupled with tandem mass spectrometry. Talanta. 2012;99:247–55. https://doi.org/10.1016/J.TALANTA.2012.05.047.
27. Swales JG, Gallagher R, Peter RM. Determination of metformin in mouse, rat, dog and human plasma samples by laser diode thermal desorption/atmospheric pressure chemical ionization tandem mass spectrometry. J Pharm Biomed Anal. 2010;53:740–4. https://doi.org/10.1016/J.JPBA.2010.04.033.
28. Heudi O, Barteau S, Picard P, Tremblay P, Picard F, Kretz O. Laser diode thermal desorption–positive mode atmospheric pressure chemical ionization tandem mass spectrometry for the ultra-fast quantification of a pharmaceutical compound in human plasma. J Pharm Biomed Anal. 2011;54:1088–95. https://doi.org/10.1016/J.JPBA.2010.11.025.
29. Borik A, Vojs Stanova A, Kodesova R, Brooks BW, Grabicova K, Novakova P, et al. Ultrafast laser diode thermal desorption method for analysis of representative pharmaceuticals in soil leachate samples. Talanta. 2020;208:120382. https://doi.org/10.1016/j.talanta.2019.120382.
30. Grabicova K, Staňová AV, Koba O, Borik A, Randak T, Grabic R. Development of a robust extraction procedure for the HPLC-ESI-HRPS determination of multi-residual pharmaceuticals in biota samples. Anal Chim Acta. 2018. https://doi.org/10.1016/j.aca.2018.04.011.
31. Solliec M, Massé D, Sauvé S. Analysis of trimethoprim, lincomycin, sulfadoxin and tylosin in swine manure using laser diode thermal desorption-atmospheric pressure chemical ionization-tandem mass spectrometry. Talanta. 2014;128:23–30. https://doi.org/10.1016/J.TALANTA.2014.04.023.
32. Kruve A, Rebane R, Kipper K, Oldekop M-L, Evard H, Herodes K, et al. Tutorial review on validation of liquid chromatography–mass spectrometry methods: part II. Anal Chim Acta. 2015;870:8–28. https://doi.org/10.1016/J.ACA.2015.02.016.
33. Matuszewski BK, Constanzer ML, Chavez-Eng CM. Strategies for the assessment of matrix effect in quantitative bioanalytical methods based on HPLC-MS/MS. Anal Chem. 2003;75:3019–30. https://doi.org/10.1021/ac020361s.
34. Passing H, Bablok W. Application of statistical procedures in analytical instrument testing. J Automat Chem. 1985;7:74–9.
35. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet (London, England). 1986;1:307–10.
36. Peterson AC, Russell JD, Bailey DJ, Westphall MS, Coon JJ. Parallel reaction monitoring for high resolution and high mass accuracy quantitative, targeted proteomics. Mol Cell Proteomics. 2012;11:1475–88. https://doi.org/10.1074/mcp.O112.020131.
37. Giavarina D. Understanding Bland Altman analysis. Biochem Medica. 2015;25:141–51. https://doi.org/10.11613/BM.2015.015.
38. Lidija B-Z. Comparison of methods: Passing and Bablok regression. Biochem Medica. 2011;21:49–52.
39. Roy-Lachapelle A, Solliec M, Sinotte M, Deblois C, Sauvé S. High resolution/accurate mass (HRMS) detection of anatoxin-a in lake water using LDTD–APCI coupled to a Q-Exactive mass spectrometer. Talanta. 2015;132:836–44. https://doi.org/10.1016/J.TALANTA.2014.10.021.
40. Allen KR. Interference by venlafaxine ingestion in the detection of tramadol by liquid chromatography linked to tandem mass spectrometry for the screening of illicit drugs in human urine. Clin Toxicol. 2006;44:147–53. https://doi.org/10.1080/15563650500514434.
41. Viant MR, Davis JE, Duffy C, Engel J, Stenton C, Sebire M, et al. Application of passive sampling to characterise the fish exometabolome. Metabolites. 2017;7. https://doi.org/10.3390/metabo7010008.
42. Young T, Walker SP, Alfaro AC, Fletcher LM, Murray JS, Lulijwa R, et al. Impact of acute handling stress, anaesthesia, and euthanasia on fish plasma biochemistry: implications for veterinary screening and metabolomic sampling. Fish Physiol Biochem. 2019;45:1485–94. https://doi.org/10.1007/s10695-019-00669-8.
43. Sogin EM, Puskás E, Dubilier N, Liebeke M. Marine metabolomics: a method for nontargeted measurement of metabolites in seawater by gas chromatography–mass spectrometry. mSystems. 2019:4. https://doi.org/10.1128/msystems.00638-19.
44. Clendinen CS, Monge ME, Fernández FM. Ambient mass spectrometry in metabolomics. Analyst. 2017;142:3101–17.

CHAPTER 4

ULTRAFAST LASER DIODE THERMAL DESORPTION METHOD FOR ANALYSIS OF REPRESENTATIVE PHARMACEUTICALS IN SOIL LEACHATE SAMPLES

Borik, A., Vojs Stanova, A., Kodesova, R., Brooks, B.W., Grabicova, K., Novakova, P., Grabic, R., 2020. Ultrafast laser diode thermal desorption method for analysis of representative pharmaceuticals in soil leachate samples. Talanta 208. https://doi.org/10.1016/j.talanta.2019.120382

My share on this work was about 50%

Talanta 208 (2020) 120382

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Ultrafast laser diode thermal desorption method for analysis of representative pharmaceuticals in soil leachate samples

A. Borik[a,*], A. Vojs Stanova[a,b], R. Kodesova[c], B.W. Brooks[a,d], K. Grabicova[a], P. Novakova[a], R. Grabic[a]

[a] University of South Bohemia in Ceske Budejovice, Faculty of Fisheries and Protection of Waters, South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses, Zatisi 728/II, 389 25, Vodnany, Czech Republic
[b] Comenius University in Bratislava, Faculty of Natural Sciences, Department of Analytical Chemistry, Ilkovicova 6, SK-842 15, Bratislava, Slovak Republic
[c] Czech University of Life Sciences Prague, Faculty of Agrobiology, Food and Natural Resources, Dept. of Soil Science and Soil Protection, Kamycka 129, Prague 6, 16500, Czech Republic
[d] Department of Environmental Science, Institute of Biomedical Studies, Center for Reservoir and Aquatic Systems Research, Baylor University, Waco, TX, USA

ARTICLE INFO

Keywords:
Green chemistry
Soil sorption
Laser diode thermal desorption
Pharmaceuticals
Ambient ionization
Analytical method comparison

ABSTRACT

We developed and evaluated a novel analytical method combining ambient ionization technique - laser diode thermal desorption with chemical ionization (LDTD-APCI) and tandem mass spectrometry detection. The LDTD/APCI-MS/MS method was developed for determination of representative pharmaceuticals from different classes (carbamazepine, sulfamethoxazole, irbesartan, fexofenadine) in leachate samples from soil sorption experimentation. We then optimized laser pattern, laser energy and spiked sample volume, which are crucial parameters for this LDTD/APCI-MS/MS method. We further identified utility of a chelating agent (Na_2-EDTA) to obtain the highest achievable and reproducible signal of target analytes. Achieved method performance parameters (LODs, LOQs, trueness and precision) were comparable with those obtained from LC-MS/MS. However, application of this novel LDTD/APCI-MS/MS method reduced analysis time by two orders of magnitude (to 12 s), compared to more conventional LC-MS/MS approaches, without use of organic solvents. We expect this novel method will reduce costs and increase throughput for future analyses of pharmaceuticals in the environment while advancing a timely principle of green chemistry.

1. Introduction

Pharmaceutical contaminants in the environment are receiving extensive study, and present unique opportunities for green chemistry [1]. Over the last decade, hundreds of papers have been published each year examining environmental fate, transport, bioaccumulation, effects, hazards and risks of active pharmaceutical ingredients (APIs) to public health and the environment [2–4]. In fact, pharmaceutical pollutants have been identified as an emerging policy issue by the International Conference on Chemicals Management of the Strategic Approach for International Chemicals Management (SAICM) through the United Nations Environment Program (UNEP) [5]. Therefore, an advanced understanding of environmental exposure and risks of APIs thus represents a pressing need. Herein, robust analytical methods are necessary to understand environmental exposures to APIs.

Pharmacokinetics in general and metabolism in particular primarily influence API and associated metabolites excreted from patients. Following sewage collection in countries with environmental management capacity, wastewater treatment plant technologies can reduce and transform parent APIs and metabolites [6], which are then introduced to the environment by effluent discharge or sludge application to soils [7]. Subsequently, agricultural lands can be exposed to pharmaceuticals and other wastewater contaminants of emerging concern (CEC) when sludge or reclaimed wastewater are applied for agriculture fertilization [8] and irrigation [9] during water reuse projects. A number of pharmaceuticals have been reported from multiple environmental matrices (e.g., wastewater, surface water, sludge, soil) [10–12]. Consequently, pharmaceuticals and other CECs can be taken up by plants [13,14], or contaminate groundwater [15] and associated source waters for potable consumption. Risks to public health and the environment from contaminated groundwater and terrestrial agricultural products are influenced by sorption of environmental contaminants to soils [16–18].

Environmental monitoring and surveillance, which represents an essential service of environmental public health of APIs relies on liquid

* Corresponding author. Faculty of Fisheries and Protection of Waters, Zatisi 728/II, 389 25, Vodnany, Czech Republic.
E-mail address: aborik@frov.jcu.cz (A. Borik).

https://doi.org/10.1016/j.talanta.2019.120382
Received 23 June 2019; Received in revised form 18 September 2019; Accepted 19 September 2019
Available online 20 September 2019
0039-9140/

chromatography (LC) with mass spectrometry (MS) using atmospheric pressure ionization techniques. These gold standard approaches are robust, but sample processing, extraction and analysis can be extensive, which thus reduces sample throughput, elevates costs and limits delivery of essential services. Solvent usage during analysis adds to these costs, particularly when large sample numbers must be analyzed from field monitoring campaigns or laboratory experiments. For example, determination of sorption dynamics and environmental exposure inherently depends on robust quantification of contaminants in leachates from sewage sludge, soils and sediments, but large numbers of samples are generated from these efforts, requiring extensive analysis.

Ambient ionization (AI) represents a relatively new group of techniques comprising all of these resource saving attributes. Further, AI is advantaged because it includes direct ionization of analytes from an untreated sample, and chromatographic separation is not required [19]. Consequently, AI techniques can be significantly less time and resource consuming compared to conventional liquid chromatography techniques. Laser diode thermal desorption (LDTD) has been classified as two step AI [20], where compounds are firstly thermally desorbed from the LDTD plate and then reach the corona discharge region of an atmospheric pressure chemical ionization ion source (APCI). LDTD-APCI are considered a soft-ionization technique. A similar ionization mechanism includes a conventional APCI technique combined with LC. One important LDTD attribute that is consistent with any AI approach is the absence of a mobile phase, which results in reaction of uncharged molecules in the corona discharge region. Consequently, a synthetic carrier gas with defined water content is responsible for proton transfer from produced hydronium species to desorbed molecules [21].

Up to 10 µL of sample can be placed in a single well of the proprietary plate; the sample is then completely evaporated prior to analysis and before a laser beam with predefined energy impacts with the bottom of the well. The resulting heat converts sample constituents into a gas phase that is then transported by the carrier gas. These steps collectively only take tens of seconds [22]. Subsequently, the LDTD system has already been applied for analysis of representative pharmaceuticals in diverse environmental matrices such as wastewater and sewage sludge [23]. However, broader application of this approach has not yet been employed with complex aquatic leachates, including soils or to examine soil partitioning. Therefore, in the present study, we developed a novel fast and reliable analytical method suitable for large numbers of water samples generated by soil sorption experiments with representative pharmaceuticals. We specifically aimed to rapidly reduce analysis time and eliminate use of organic solvents, compared to traditional LC methods. We then compared performance of this novel LDTD-APCI-MS/MS method to more conventional LC-HESI-MS/MS approaches.

2. Materials and methods

2.1. Chemicals

We selected pharmaceuticals representative of other classes in order to maximize utility of our efforts. The antibiotic sulfamethoxazole and the antiepileptic carbamazepine were acquired from Merck (Germany), while fexofenadine and irbesartan were purchased from Toronto Research Chemicals. Standards stock solutions (1 mg mL $^{-1}$) were prepared in methanol for individual compounds. For each target analyte, we also included isotopically labelled standards obtained from Toronto Research Chemicals (sulfamethoxazole-d4, carbamazepine-d10, fexofenadine-d6, irbesartan-d4). For preparation of mobile phases, acetonitrile (LC-MS grade, Merck, Germany) and ultra-pure water (prepared with AquaMax Basic 360 Series and Ultra 370 Series instruments, Young Lin Instruments, purchased from Labicom, Czech Republic) and formic acid (LC-MS grade, Merck, Germany) were used. The chelating agent ethylene diamine tetraacetic acid disodium hydrate (Na_2-EDTA, Merck, Germany) was used for response improvement in the LDTD-APCI approach. Synthetic air for LDTD (21% of O_2 in nitrogen with 35 ppm of water), liquid nitrogen and argon (5.0 purity) were purchased from SIAD (SIAD, Czech Republic).

2.2. Instrumentation and instrumental conditions

This instrument method was based on an AI technique, which combined thermal sample desorption by laser beam (980 nm, 20 W) and APCI. The instrument for sample ionization and direct introduction was a T-960-LDTD APCI (Phytronix Technologies, Canada). Thermally desorbed compounds were transported through a transfer tube and by the ion source corona discharge region by heated carrier gas (synthetic air, flow 3 L min^{-1}, 50 °C). All other LDTD-APCI parameters such as sweep gas pressure (0.3 arbitrary units), discharge current (3 µA, positive polarity), capillary (270 °C) and vaporizer (31 °C) temperatures followed LDTD manufacturer recommendations. Laser pattern setup is an essential part of LDTD-APCI-MS/MS method development, and its parameters are discussed in detail below in the section, *LDTD-MS/MS method development and evaluation*. Total run time of this LDTD-APCI-MS/MS methods was 12 s.

The reference LC method was performed with a Dionex Ultimate 3000SD system (Dionex Softron GmbH, Germany). Our previous LC-MS/MS method [24] was adopted for analysis of soil leachates. A Hypersil Gold phenyl LC column (50 × 2.1 mm, 3 µm particles, Thermo Fisher Scientific) was used for analytes separation because the shortest elution window of target compounds were previously reported for this column. A gradient of acetonitrile and water, acidified with formic acid to 0.1%, was used for elution (SM. 1). The total run time of LC-HESI-MS/MS method was shorted to 6.5 min. In addition, the following source parameters were applied: vaporizer temperature was 250 °C, aux gas pressure was 10 au (arbitrary units), sheath gas was 40 au, spray voltage was 3500 V (positive polarity mode) and capillary temperature was 350 °C.

A triple quadrupole mass spectrometer TSQ Quantum Ultra (Thermo Fisher Scientific, USA) was operated in selection reaction monitoring (SRM) mode. The isolation window of the first and third quadrupoles were set to 0.7 FWHM, cycle time to 0.05 (LDTD-APCI-MS/MS) and 0.3 s (LC-HESI-MS/MS) and collision gas (argon, SIAD, Czech Republic) pressure to 1.5 mTorr. Two selected mass transitions from precursor to product ion were chosen for each analyte (SM. 2). Both analytical system configurations were controlled via Xcalibur 3.0 (Thermo Fisher Scientific, USA). Data post-processing was performed in TraceFinder 4.1 (Thermo Fisher Scientific, USA) software.

2.3. Sample description and method evaluation

LDTD-APCI laser pattern was optimized in ultra-pure water at analyte concentration levels of 100 ng mL^{-1} and testing of the effect of Na_2-EDTA on target analytes responses was performed. Na_2-EDTA was tested as a pre-coating LazWell plate cell step when 10 µL of chelating agent at the concentration levels 0.01, 0.1, 1 and 10 mM were spotted and then allowed to completely evaporate. LDTD-APCI laser pattern setup for Na_2-EDTA effect evaluation was selected based on previously obtained results when applied laser energy was tested across a scale of 15–80%. We included this step to identify suitable analytical conditions for all target compounds in one method. The results of laser pattern and Na_2-EDTA evaluation were compared as peak areas of analytes, their signal to noise ratio and peak symmetry of selected quantification mass transition. The peak symmetry parameter was calculated according to Eq. (1):

$$\text{Peak symmetry} = abs(1 - (LHS - RHS)) \quad (1)$$

where LHS is left hand side and RHS is right hand peak side width measured in 30% of a peak height. Zero value represents total peak symmetry. All parameters mentioned above were evaluated in hexaplicate analyses. Sample deposition volume were tested in set of

triplicates. Mean peak areas of sample volumes 2, 4, 7 and 10 μL were normalized to the highest obtained response. Standard deviations (SD) and relative standard deviation (RSD) were calculated from obtained data for all method development parameters.

Stagnic Chernozem Siltic developed on marlite (X) and Dystric Cambisol on orthognesis (W) are two soil types with different physical and chemical properties (described in Kodešová et al., 2015 and breafly summarized in SM. 3). These soils were selected as sample matrices for analytes sorption experiments; their influences on LDTD-APCI-MS/MS and LC-HESI-MS/MS methods performance parameters were also evaluated. For this purpose both soils were prepared according to standard sorption experiment protocol for sorption isotherm measurement [25] with one exception, which was addition of analytes at required concentrations into obtained soil leachates. Both soil leachates were used for calibration model construction in concentrations 1, 2, 5, 10, 50, 100, 500, 1000 ng mL $^{-1}$ of native standards and 20 ng mL $^{-1}$ of isotopically labelled standards. Calibration curves results were used for evaluation of linearity (r-square coefficient) and calculation of quantification limits (LOQ), detection limits (LOD), matrix effect and analyte carry over for both LDTD-APCI-MS/MS and LC-HESI-MS/MS methods. The quantification and detection limits were estimated by following Eqs (2) and (3), respectively [26].

$$LOQ = \left(\frac{SDintercept}{b}\right)x\ 10 \tag{2}$$

$$LOD = \left(\frac{SDintercept}{b}\right)x\ 3.3 \tag{3}$$

where $SD_{intercept}$ is SD of intercept and b is slope.

Estimation of analytes signal suppression/enhancement caused by W and X soil matrix were investigated by comparison of response intensity of each analyte (100 ng mL $^{-1}$) in soil matrices and in matrix free ultrapure water. Na_2-EDTA addition effects on LDTD-APCI-MS/MS were also evaluated among matrices for each study compound. Here again, all experiments were performed in hexaplicates. Calculation of matric effect is described in Eq. (4)[41] [].

$$Matrix\ Effect = \left(\frac{A}{B}\right)x\ 100\% \tag{4}$$

where A represents analyte peak area measured in soil matrix and B in matrix free sample, both spiked with native compounds. A similar equation can be used for description of carryover effect evaluation where B represents peak area of the highest point of calibration curve and A is analyte signal of blank sample measured afterwards.

Accuracy of LDTD-APCI-MS/MS and LC-HESI-MS/MS methods was evaluated at concentration levels 5, 50 and 500 ng mL $^{-1}$ in hexaplicates. The trueness parameter was evaluated as relative agreement of measured sample concentration with nominal value. Method precision was expressed as calculated RSD. The inter day precision followed the same parameters as method accuracy testing and samples were measured in pairs across three days.

All results were calculated with internal standard calibration, described by Eqs. (5) and (6)

$$RF = \left(\frac{Peak\ area_{cal}}{C_{cal}}\right)x\left(\frac{Peak\ area_{IS}}{C_{IS}}\right) \tag{5}$$

$$C_1 = \left(\frac{Peak\ area_1}{Peak\ area_{IS}}\right)x\left(\frac{1}{ARF}\right)x\ C_{IS} \tag{6}$$

where Peak $area_{cal}$ is analyte measured peak area, C_{cal} is known concentration of calibration point, Peak $area_{IS}$ is peak area of isotopically labelled standard, C_{is} is concentration of isotopically labelled standard, C_1 is unknown concentration of an analyte and Peak $area_1$ refers to measured analyte area of unknown concentration [28]. Average response factor (ARF) was obtained from response factor (RF) calculated from calibration curve points in sample matrix.

Final method evaluation was performed on sorption experiments with all analytes of interest with nominal concentrations up to 1000 ng mL $^{-1}$ and seven different soil matrices: Haplic Chernozem on loess (D), Arenosol Epieutric on sand (E), Haplic Cambisol on paragneiss (H), Haplic Luvisol on loess (S), Greyic Phaeozem on loess (C) (described in Kodešová et al., 2015 and breafly summarized in SM. 3) including both soil types (X,W) used for methods evaluation. The additional soil types were selected to evaluate methods robustness, comparison of the previous method and the newly developed analytical method. Both methods agreement was quantified with Lin's concordance correlation coefficient [29], visualized by and Bland-Altman plot [30] and scatter plot. All method agreement tests were performed with MedCalc v 19.0.3. (MedCalc Software, Belgium).

3. Results and discussion

3.1. LDTD-method development and evaluation

The laser source of LDTD can provide maximal heat transfer of 3000 °C s $^{-1}$ [31], which allows fast sample desorption after a laser beam with predefined relative intensity impacts the back part of the LDTD sample plate. This applied laser power and its time distribution are defined by a laser pattern setup. However, intensity of applied laser energy should be optimized to maximize desorption of crystallized analytes of interest and to minimize desorption and thermal degradation of sample matrix components. Besides optimizing maximal response, signal reproducibility and easy automated integration (signal symmetry) are other important parameters of the instrumental method reported here. The most important indicator for evaluation of tested laser patterns were considered peak area normalized to the highest obtained value. Results of this optimization are summarized in Fig. 1 as mean of hexaplicates with RSD. Whereas Fig. 1b shows signal to noise ratio in decadic logarithm scale, quality of peak shape as relative deviation from symmetry is illustrated in Fig. 1c, which is important because this parameter can affect detection sensitivity and appropriate peak integration [32].

In the present study, LDTD method optimization was started with laser pattern with maximum applied power 15% reached in 1 s, then held for the same time interval and finally decreased to 0% in 0.1 s. Results obtained with the initial experiment exhibited absence of signals for irbesartan and fexofenadine, though sulfamethoxazole demonstrated relatively low and insufficient peak area stability with RSD 67%. Carbamazepine was the only target analyte with acceptable signal stability and intensity even for our initial laser pattern energy setup. Subsequently, we tested laser patterns with the same time distribution as the initial experiment but increased maximum laser power up to 80% by 10 and 15% respectively in five steps. Sulfamethoxazole signal intensity and peak area was gradually increased with laser energy set up to 55%. The two laser patterns with the highest laser energy exhibit thermal decomposition of this compound. The most effective dissociation of intermolecular bond of carbamazepine was observed when 40% laser energy was applied; all other higher laser energies caused compound decomposition and signal suppression. However, signal intensities of fexofenadine and irbesartan show slightly increasing tendencies with growing laser energy. The laser energy parameter had a negligible effect on signal to noise ratio (Fig. 1b) and negatively affected obtained peak shape at higher levels (Fig. 1c).

Optimization of laser energy and laser pattern was not sufficient for fexofenadine and irbesartan, respectively. Previously published studies identified the possibility to improve response and stability of desorbed small organic molecules through the use chelating agents such as Na_2-EDTA [33,34]; however, relevant mechanisms affecting sample desorption improvement were not investigated and described prior to the current study. Dion-Fortier et al. (2019) recently considered formation of a homogenous layer of analyte nanocrystals after chelation addition as the relevant mechanism rather than prevention of chemisorption

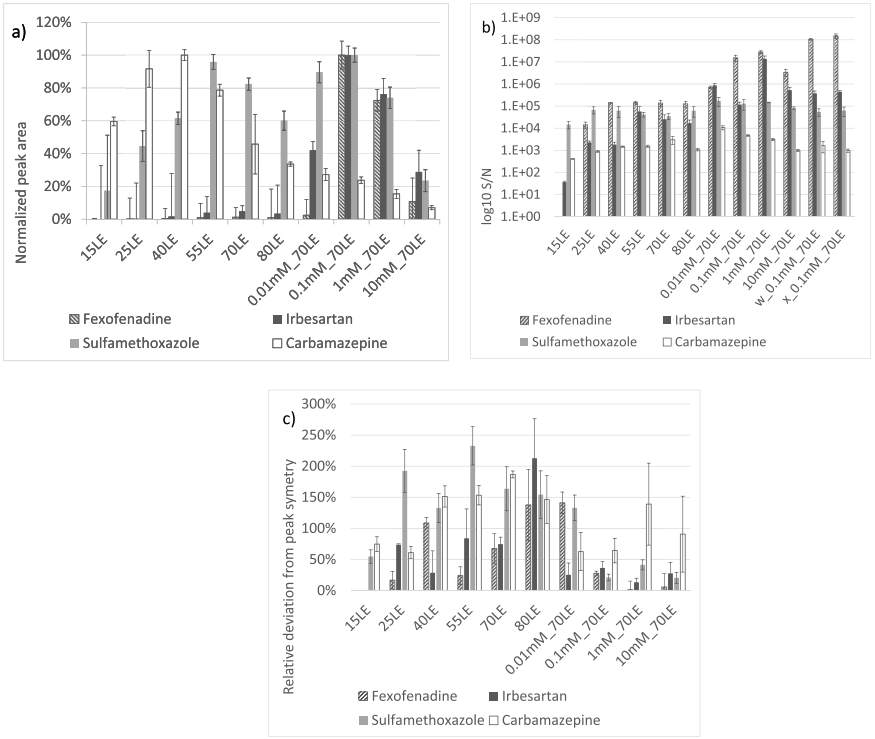


Fig. 1. a) Evaluation of laser patter and EDTA concentration. Results displayed as peak areas normalized to the highest obtained response. Error bars represent RSD of peak area. b) Evaluation of laser patter and Na_2-EDTA concentration. Results show effect of laser pattern and EDTA on signal to noise ration. W and X represent samples with soil matrix. S/N in log scale and error bars represent its SD. c) Evaluation of laser patter and Na_2-EDTA concentration. Results show deviation from 100% peak symmetry measured in 30% peak high. Error bars represent RSD. n = 6.

[35]. For our study purpose, we specifically pre-coated LDTD LazWell plates with Na_2-EDTA at four concentration levels (0.01, 0.1, 1, 10 mM), and then performed experiments with laser energy set to 70%. We subsequently observed relatively good response in total scale for carbamazepine and sulfamethoxazole with improved peak symmetry compare to that obtained for 55% laser energy. The lowest RSDs for analytes were also obtained under these conditions except carbamazepine (the highest RSD exhibit fexofenadine-12% and then carbamazepine 36%). Application of Na_2-EDTA resulted in decrease of thermal energy required for fexofenadine and irbesartan desorption and consequently rapidly improved signal intensities. At 0.1 mM, Na_2-EDTA exhibited the best responses for fexofenadine and irbesartan, and the signal of sulfamethoxazole was also slightly improved. This method setup showed the best peak shape results and improvement of signal to noise ratios. In fact, the RSD of carbamazepine peak area was improved from 36% to 4%, even though RSD values of other analytes did not exceed 17%. Application of higher Na_2-EDTA concentrations (1 and 10 mM) caused response suppression of all analytes (Fig. 1a).

The last step in method development was optimization of deposited sample volume. Analytes responses increased proportionally to sample volume (Fig. 2). Consequently, the highest responses for all analytes were observed when maximum applicable volume (10 μL) of samples were spotted.

3.2. LC-HESI-MS/MS and LDTD-APCI-MS/MS method comparison

The combination of LC as separation technique with atmospheric pressure ionization techniques represents a standard approach for introduction of separated molecular ions to a triple quadrupole MS/MS such that this approach is considered to provide selective and sensitive detection [36]. The selectivity of LC by itself is defined as selectivity factor, which can be altered by individual adjustable parameters such as type of stationary phase, column temperature, composition of mobile phase, etc. [37]. Compare to LC, the variables of LDTD, which can affect method selectivity are limited to laser pattern setup. The laser intensity correlated with energy applied on samples and its level should be evaluated to find optimal value provides sufficient dissociation of intermolecular bonds without thermal decomposition of the analyte. Consequently, the laser intensity can be setup to level provides energy for desorption of target analyte but not for interfering compound or higher resulting in thermal decomposition of interfering compound [21]. The example can be seen in Fig. 1c where 15% of laser energy

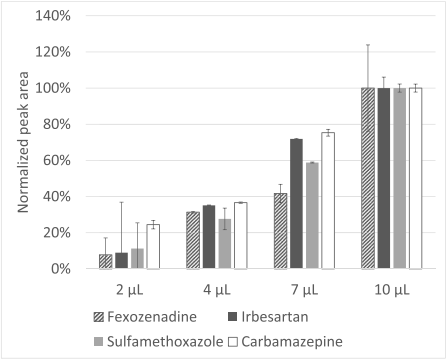


Fig. 2. Effect of spotted volume into LDTD LazWell plate on total response. Results show effect of spiked sample volume on obtained analyte signal in relative scale, nominal to the highest obtained average value. Error bars represent RSD (n = 3).

provides acceptable signal intensity and stability for carbamazepine, but all other compounds of interest cannot be quantified. Despite of limited selectivity, LDTD has shown potential to be a beneficial alternative to established LC-ESI-MS/MS methods with equal performance parameters for several analytes in complex matrices [38]. It follows from the above that limited LDTD selectivity require circumspect evaluation of eventual mass interferences for individual sample matrix and target compound. For this purpose, blank matrix samples of each soil type with negative result for all no significant matrix effect observed for target analytes in studied the matrices were employed.

The basic methods performance parameters are summarized in Table 1. Linearity of an eight point calibration curve was tested across a concentration range of 1–1000 ng mL^{-1} of analytes in two matrices (water phase from two different soil types). For the LC-HESI-MS/MS method, we observed equal linearity results across all analytes and both matrices (R^2 = 0.999). The new LDTD-APCI-MS/MS method reported here exhibited generally good linearity and comparable linear curves. The lowest determination coefficient was obtained for irbesartan in matrix W (R^2 = 0.996). Limit of detection (LOD) and limit of quantification (LOQ) are the lowest concentrations where the analyte is detected and quantified with acceptable uncertainty (max. 20% RSD of response factors in calibration curve). Both methods exhibited LOQs at low nanogram per milliliter levels, though limits for LC-HESI-MS/MS method were slightly lower. In soil leachates X and W, the highest LOQs for LDTD-APCI-MS/MS were found for irbesartan (3.9 and 3.6 ng mL^{-1}) and carbamazepine (3.4 and 4.0 ng mL^{-1}), respectively. The highest matrix effect using LC-HESI-MS/MS method was observed for sulfamethoxazole (11%). Optimized LDTD-APCI-MS/MS showed signal suppression in both soil sample types for carbamazepine (29% in both matrices). Negligible signal improvement was found for sulfamethoxazole in both soil sample types (X soil 9% and W soil 4%), significant enhancement was observed for fexofenadine (X soil 71% and W soil 87%) and irbesartan (X soil 71% and W soil 76%). In addition, signal to noise ratios in both soil matrices were improved for fexofenadine; no relevant impairment for other analytes were observed (Fig. 1b). Such results may suggest a positive effect of these soil leachates on LDTD-APCI signal.

Trueness and precision of both methods were evaluated at three concentrations (5, 50, 500 ng mL^{-1}) from hexaplicate analysis of water leachate fortified with corresponding native compounds. Results from experiments with LDTD-APCI-MS/MS and LC-HESI-MS/MS are shown in Figs. 3 and 4(a and b), respectively. As noted above, the trueness was calculated as a process efficiency value, which refers to effects of sample preparation and ionization [39]. LDTD-APCI-MS/MS trueness for all analytes at all evaluated nominal concentrations and soil matrices were within the range of 88–111% with median 104%. The lowest trueness value was observed in the W soil type at concentration level 500 ng mL^{-1}, while the highest were observed in X soil type at concentration level 5 ng mL^{-1}, both for sulfamethoxazole. The highest uncertainty of analysis (20% RSD) was obtained for sulfamethoxazole in W soil type at the lowest concertation level examined. All other uncertainty results were below this value with median 9%. Fig. 4a and b shows results for the previously reported LC-HESI-MS/MS method, for which method trueness for analytes at all evaluated nominal concentrations and soil matrices were within 90–105% with median 98%. Both extreme values were observed for irbesartan in soil type W at the middle concentration tested and the X soil type at the lowest concentration level, respectively.

Uncertainty of LC-HESI-MS/MS was noticeably lower with median 2% RSD and the highest RSD value of 6%. Further, precision of the LDTD-APCI-MS/MS method over three consecutive days never exceeded 15%, while the RSD of the HESI-LC-MS/MS method was always lower than 10% (Table 2). We also estimated the carry over effect as analyte signal in blank after the highest calibration point. This effect was negligible for both methods. Fexofenadine in the LDTD-APCI-MS/MS method and fexofenadine and irbesartan in the LC-HESI-MS/MS method showed low signals close to the LOQ, reaching 0.1% of signals from results presented above.

Results of our comparison of these two methods showed the best method agreement for carbamazepine and fexofenadine. These two analytes exhibited nearly identical correlation coefficients 0.993 (95% confidence interval (CI) 0.989–0.996) and 0.997 (95% CI 0.994–0.999),

Table 1
The basic LDTD-APCI-MS/MS and LC-HESI-MS/MS method performance parameters.

	X				W			
	Fexofenadine	Carbamazepine	Irbesartan	Sulfamethoxazole	Fexofenadine	Carbamazepine	Irbesartan	Sulfamethoxazole
LDTD-APCI-MS/MS								
Linearity (R^2)	0.999	0.999	0.999	0.998	0.999	0.999	0.996	0.999
LOQ (ng mL^{-1})	2.6	3.4	3.9	1.5	2.5	4.0	3.6	1.1
LOD (ng mL^{-1})	0.86	1.1	1.3	0.48	0.83	1.3	1.2	0.36
Matrix effect (%)	71	−29	71	9.0	87	−29	76	4.0
Carryover (%)	0.08	0	0	0	0.07	0	0	0
LC-HESI-MS/MS								
Linearity (R^2)	0.999	0.999	0.999	0.999	0.999	0.999	0.999	0.999
LOQ (ng mL^{-1})	2.2	2.6	2.5	2.0	2.0	1.3	1.9	1.8
LOD (ng mL^{-1})	0.70	0.85	0.81	0.65	0.72	0.42	0.62	0.60
Matrix effect (%)	−1.0	0.5	1.0	5.0	1.2	5.8	9.0	11
Carryover (%)	0.08	0	0.08	0	0.07	0	0.1	0

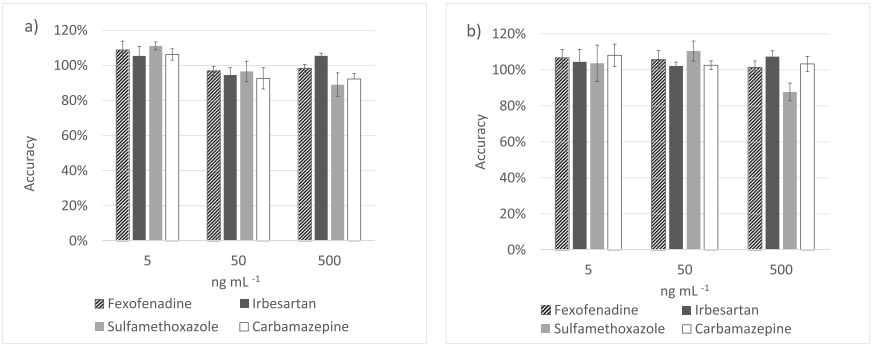


Fig. 3. LDTD-APCI-MS/MS method accuracy evaluation. a) represents soil type X and b) show results for soil type W. Results are displayed as relative trueness of nominal concentration in two soil matrix. Error bars represent RSD. n = 6.

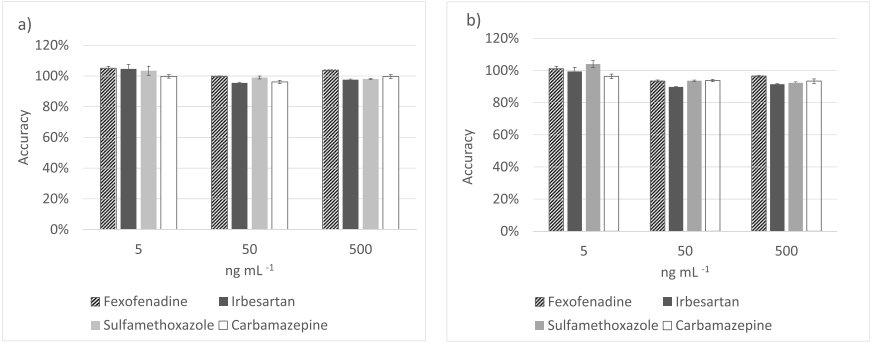


Fig. 4. LC-HESI-MS/MS method accuracy evaluation. a) soil type X and b) shown results for soil type W. Results are displayed as trueness in two soil matrices. Error bars represent RSD. n = 6.

respectively, which were within strength-of-agreement on the top of method equality classification [40]. The other analytes had slightly lower but still robust method agreement. Specifically, a correlation coefficient of 0.98 (95% CI 0.967–0.988) was observed for sulfamethoxazole and 0.98 (95% CI 0.969–0.987) for irbesartan. The relative mean of method biases was visualized by Bland-Altman plots, where each soil type is presented (SM 4). Carbamazepine mean bias was 1.1% (95% CI -16.3–18.3%), fexofenadine was −1.3% (95% CI -26.6–24.1%), sulfamethoxazole was 5.1% (95% CI -14.6–25%) and irbesartan was −0.3% (95% CI -29.8–29.2%). Bland-Altman plots show relatively comparable bias distribution for all investigated soil matrices. This balanced result confirms applicability and robustness of the novel

Table 2
LDTD-APCI-MS/MS and LC-HESI-MS/MS method inter day precision. Values are figured as RSDs of analytes concentrations in X and W soil leachate.

	X			W		
	5 ng mL $^{-1}$	50 ng mL $^{-1}$	500 ng mL $^{-1}$	5 ng mL $^{-1}$	50 ng mL $^{-1}$	500 ng mL $^{-1}$
LDTD-APCI-MS/MS						
Fexofenadine	12	4.7	6.7	5.4	8.6	3.7
Carbamazepine	7.0	3.9	4.3	7.2	3.6	3.4
Irbesartan	15	0.30	3.0	11	0.60	5.5
Sulfamethoxazole	8.2	7.6	4.2	3.9	6.1	9.4
LC-HESI-MS/MS						
Fexofenadine	9.0	3.4	1.8	2.6	6.1	1.9
Carbamazepine	5.0	10	7.0	2.7	5.8	10
Irbesartan	9.4	6.6	2.7	4.3	10	6.0
Sulfamethoxazole	7.1	6.2	3.9	4.5	8.3	6.7

LDTD-APCI-MS/MS method reported here. Further, SM 5 shows an equality line allowing visual inspection of data fitting and linearity; data sets of each scatter diagram consists of all investigated soil types.

4. Conclusion

Advanced approaches are needed to expand environmental monitoring and surveillance of pharmaceuticals in the environment. Though LC-HESI-MS/MS represents a standard approach for environment and pharmaceuticals research, this includes a relatively time-consuming separation (approximately 7 samples per hour) requiring about 3.5 mL of organic solvents. In the present study, we developed a novel LDTD-APCI-MS/MS analytical method, and achieved an accurate, time saving approach for determination of representative pharmaceuticals in complex matrices. Considering accuracy parameters, intermediate precision and mutual method comparison (Bland Altman plots and Lin's concordance correlation coefficient) performed on sorption samples, we conclude that results obtained with both methods show uncertainty acceptable for residual analysis in soil leachates. However, the LDTD-APCI approach reported here represents a two order of magnitude faster method than conventional LC-HESI-MS/MS that advances an important green chemistry principle (e.g., no organic solvents were required) with the ability to analyze a large series of samples in a timely manner (i.e. 180 samples per hour).

Acknowledgements

The study was financially supported by the Czech Science Foundation (project No. 17-08937S), Ministry of Education, Youth and Sports of the Czech Republic - projects „CENAKVA" (No. LM2018099), "CENAKVA Center Development (No. CZ.1.05/2.1.00/19.0380)" and the Grant Agency of the University of South Bohemia (061/2019/Z).

Bryan W. Brooks acknowledges visiting professorship support from the University of South Bohemia.

References

[1] B.W. Brooks, Greening chemistry and ecotoxicology towards sustainable environmental quality, Green Chem. 21 (2019) 2575–2582, https://doi.org/10.1039/c8gc03893g.

[2] D.J. Caldwell, B. Mertens, K. Kappler, T. Senac, R. Journel, P. Wilson, R.D. Meyerhoff, N.J. Parke, F. Mastrocco, B. Mattson, R. Murray-Smith, D.G. Dolan, J.O. Straub, M. Wiedemann, A. Hartmann, D.S. Finan, A risk-based approach to managing active pharmaceutical ingredients in manufacturing effluent, Environ. Toxicol. Chem. 35 (2016) 813–822, https://doi.org/10.1002/etc.3163.

[3] B. Du, S.P. Haddad, A. Luek, W.C. Scott, G.N. Saari, L.A. Kristofco, K.A. Connors, C. Rash, J.B. Rasmussen, C.K. Chambliss, B.W. Brooks, Bioaccumulation and trophic dilution of human pharmaceuticals across trophic positions of an effluent-dependent wadeable stream, Philos. Trans. R. Soc. Biol. Sci. 369 (2014), https://doi.org/10.1098/rstb.2014.0058 20140058–20140058.

[4] R.S. Kookana, M. Williams, A.B.A. Boxall, D.G.J. Larsson, S. Gaw, K. Choi, H. Yamamoto, S. Thatikonda, Y.-G. Zhu, P. Carriquiriborde, Potential ecological footprints of active pharmaceutical ingredients: an examination of risk factors in low-, middle- and high-income countries, Philos. Trans. R. Soc. Lond. B Biol. Sci. 369 (2014), https://doi.org/10.1098/rstb.2013.0586.

[5] (n.d. SAICM, (accessed June 18, 2019), http://www.saicm.org/Home/tabid/5410/language/en-US/Default.aspx.

[6] M.D. Celiz, J. Tso, D.S. Aga, Pharmaceutical metabolites in the environment: analytical challenges and ecological risks, Environ. Toxicol. Chem. 28 (2009) 2473–2484, https://doi.org/10.1897/09-173.1.

[7] A. Ghirardini, P. Verlicchi, A review of selected microcontaminants and microorganisms in land runoff and tile drainage in treated sludge-amended soils, Sci. Total Environ. 655 (2019) 939–957, https://doi.org/10.1016/J.SCITOTENV.2018.11.249.

[8] M. Bourdat-Deschamps, S. Ferhi, N. Bernet, F. Feder, O. Crouzet, D. Patureau, D. Montenach, G.D. Moussard, V. Mercier, P. Benoit, S. Houot, Fate and impacts of pharmaceuticals and personal care products after repeated applications of organic waste products in long-term field experiments, Sci. Total Environ. 607–608 (2017) 271–280, https://doi.org/10.1016/J.SCITOTENV.2017.06.240.

[9] B. Chefetz, T. Mualem, J. Ben-Ari, Sorption and mobility of pharmaceutical compounds in soil irrigated with reclaimed wastewater, Chemosphere 73 (2008) 1335–1343, https://doi.org/10.1016/J.CHEMOSPHERE.2008.06.070.

[10] L. Ivanová, T. Mackuľak, R. Grabic, O. Golovko, O. Koba, A.V. Staňová, P. Szabová, A. Grenčíková, I. Bodík, Pharmaceuticals and illicit drugs – a new threat to the application of sewage sludge in agriculture, Sci. Total Environ. 634 (2018) 606–615, https://doi.org/10.1016/J.SCITOTENV.2018.04.001.

[11] M. Biel-Maeso, C. Corada-Fernández, P.A. Lara-Martín, Monitoring the occurrence of pharmaceuticals in soils irrigated with reclaimed wastewater, Environ. Pollut. 235 (2018) 312–321, https://doi.org/10.1016/J.ENVPOL.2017.12.085.

[12] O. Golovko, V. Kumar, G. Fedorova, T. Randak, R. Grabic, Removal and seasonal variability of selected analgesics/anti-inflammatory, anti-hypertensive/cardiovascular pharmaceuticals and UV filters in wastewater treatment plant, Environ. Sci. Pollut. Res. 21 (2014) 7578–7585, https://doi.org/10.1007/s11356-014-2654-9.

[13] R. Kodešová, A. Klement, O. Golovko, M. Fér, A. Nikodem, M. Kočárek, R. Grabic, Root uptake of atenolol, sulfamethoxazole and carbamazepine, and their transformation in three soils and four plants, Environ. Sci. Pollut. Res. 26 (2019) 9876–9891, https://doi.org/10.1007/s11356-019-04333-9.

[14] L.J. Carter, E. Harris, M. Williams, J.J. Ryan, R.S. Kookana, A.B.A. Boxall, Fate and uptake of pharmaceuticals in soil–plant systems, J. Agric. Food Chem. 62 (2014) 816–825, https://doi.org/10.1021/jf404282y.

[15] A.D. McEachran, D. Shea, W. Bodnar, E.G. Nichols, Pharmaceutical occurrence in groundwater and surface waters in forests land-applied with municipal wastewater, Environ. Toxicol. Chem. 35 (2016) 898–905, https://doi.org/10.1002/etc.3216.

[16] X. Wu, L.K. Dodgen, J.L. Conkle, J. Gan, Plant uptake of pharmaceutical and personal care products from recycled water and biosolids: a review, Sci. Total Environ. 536 (2015) 655–666, https://doi.org/10.1016/J.SCITOTENV.2015.07.129.

[17] M. Boisvert, P.B. Fayad, S. Sauvé, Development of a new multi-residue laser diode thermal desorption atmospheric pressure chemical ionization tandem mass spectrometry method for the detection and quantification of pesticides and pharmaceuticals in wastewater samples, Anal. Chim. Acta 754 (2012) 75–82, https://doi.org/10.1016/J.ACA.2012.09.046.

[18] M. Kočárek, R. Kodešová, L. Vondráčková, O. Golovko, M. Fér, A. Klement, A. Nikodem, O. Jakšík, R. Grabic, Simultaneous sorption of four ionizable pharmaceuticals in different horizons of three soil types, Environ. Pollut. 218 (2016) 563–573, https://doi.org/10.1016/J.ENVPOL.2016.07.039.

[19] R. Chen, J. Deng, L. Fang, Y. Yao, B. Chen, X. Wang, T. Luan, Recent applications of ambient ionization mass spectrometry in environmental analysis, Trends Environ. Anal. Chem. 15 (2017) 1–11, https://doi.org/10.1016/J.TEAC.2017.07.001.

[20] M.Z. Huang, S.C. Cheng, Y.T. Cho, J. Shiea, Ambient ionization mass spectrometry: a tutorial, Anal. Chim. Acta 702 (2011) 1–15, https://doi.org/10.1016/j.aca.2011.06.017.

[21] P.B. Fayad, M. Prévost, S. Sauvé, Laser diode thermal desorption/atmospheric pressure chemical ionization tandem mass spectrometry analysis of selected steroid hormones in wastewater: method optimization and application, Anal. Chem. 82 (2010) 639–645, https://doi.org/10.1021/ac902074x.

[22] M. Boisvert, P.B. Fayad, S. Sauvé, Development of a new multi-residue laser diode thermal desorption atmospheric pressure chemical ionization tandem mass spectrometry method for the detection and quantification of pesticides and pharmaceuticals in wastewater samples, Anal. Chim. Acta 754 (2012) 75–82, https://doi.org/10.1016/J.ACA.2012.09.046.

[23] D.P. Mohapatra, S.K. Brar, R.D. Tyagi, P. Picard, R.Y. Surampalli, Carbamazepine in municipal wastewater and wastewater sludge: ultrafast quantification by laser diode thermal desorption-atmospheric pressure chemical ionization coupled with tandem mass spectrometry, Talanta 99 (2012) 247–255, https://doi.org/10.1016/J.TALANTA.2012.05.047.

[24] R. Grabic, J. Fick, R.H. Lindberg, G. Fedorova, M. Tysklind, Multi-residue method for trace level determination of pharmaceuticals in environmental samples using liquid chromatography coupled to triple quadrupole mass spectrometry, Talanta 100 (2012) 183–195, https://doi.org/10.1016/J.TALANTA.2012.08.032.

[25] R. Kodešová, R. Grabic, M. Kočárek, A. Klement, O. Golovko, M. Fér, A. Nikodem, O. Jakšík, Pharmaceuticals' sorptions relative to properties of thirteen different soils, Sci. Total Environ. 511 (2015) 435–443, https://doi.org/10.1016/J.SCITOTENV.2014.12.088.

[26] M. Solliec, D. Massé, S. Sauvé, Analysis of trimethoprim, lincomycin, sulfadoxin and tylosin in swine manure using laser diode thermal desorption-atmospheric pressure chemical ionization-tandem mass spectrometry, Talanta 128 (2014) 23–30, https://doi.org/10.1016/j.talanta.2014.04.023.

[28] K. Rome, A. Mcintyre, Intelligent use of relative response factors in gas chromatography-flame ionisation detection, Chromatogr. Today. (2012) 52–56.

[29] L.I.-K. Lin, A concordance correlation coefficient to evaluate reproducibility, Biometrics 45 (1989) 255, https://doi.org/10.2307/2532051.

[30] J.M. Bland, D.G. Altman, Statistical methods for assessing agreement between two methods of clinical measurement, Lancet 1 (1986) 307–310 http://www.ncbi.nlm.nih.gov/pubmed/2868172 , Accessed date: 20 May 2019.

[31] P.B. Fayad, M. Prévost, S. Sauvé, Laser diode thermal desorption/atmospheric pressure chemical ionization tandem mass spectrometry analysis of selected steroid hormones in wastewater: method optimization and application, Anal. Chem. 82 (2010) 639–645, https://doi.org/10.1021/ac902074x.

[32] M. Armstrong, F.W. Darshan, C. Patel, D.W., Peak shapes and their measurements: the need and the concept behind total peak shape analysis, LCGC North Am. 35 (2017) 846–853 (accessed March 23, 2019), http://www.chromatographyonline.com/peak-shapes-and-their-measurements-need-and-concept-behind-total-peak-shape-analysis-0.

[33] J.J. Lohne, W.C. Andersen, S.B. Clark, S.B. Turnipseed, M.R. Madson, Laser diode thermal desorption mass spectrometry for the analysis of quinolone antibiotic residues in aquacultured seafood, Rapid Commun. Mass Spectrom. 26 (2012) 2854–2864, https://doi.org/10.1002/rcm.6414.

[34] I. Beattie, A. Smith, D.J. Weston, P. White, S. Szwandt, L. Sealey, Evaluation of laser diode thermal desorption (LDTD) coupled with tandem mass spectrometry (MS/MS) for support of in vitro drug discovery assays: increasing scope, robustness and

throughput of the LDTD technique for use with chemically diverse compound libraries, J. Pharm. Biomed. Anal. 59 (2012) 18–28, https://doi.org/10.1016/J.JPBA.2011.10.014.

[35] A. Dion-Fortier, A. Gravel, C. Guérette, F. Chevillot, S. Blais, S. Auger, P. Picard, P.A. Segura, Signal enhancement in laser diode thermal desorption-triple quadrupole mass spectrometry analysis using microwell surface coatings, J. Mass Spectrom. 54 (2019) 167–177, https://doi.org/10.1002/jms.4328.

[36] N. Barbarin, J.D. Henion, Y. Wu, Comparison between liquid chromatography–UV detection and liquid chromatography–mass spectrometry for the characterization of impurities and/or degradants present in trimethoprim tablets, J. Chromatogr., A 970 (2002) 141–154, https://doi.org/10.1016/S0021-9673(02)01035-X.

[37] V.F. Samanidou, Basic LC method development and optimization, Anal. Sep. Sci. Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany, 2015, pp. 25–42, , https://doi.org/10.1002/9783527678129.assep002.

[38] A. Roy-Lachapelle, P.B. Fayad, M. Sinotte, C. Deblois, S. Sauvé, Total microcystins analysis in water using laser diode thermal desorption-atmospheric pressure chemical ionization-tandem mass spectrometry, Anal. Chim. Acta 820 (2014) 76–83, https://doi.org/10.1016/J.ACA.2014.02.021.

[39] A. Kruve, R. Rebane, K. Kipper, M.-L. Oldekop, H. Evard, K. Herodes, P. Ravio, I. Leito, Tutorial review on validation of liquid chromatography–mass spectrometry methods: Part II, Anal. Chim. Acta 870 (2015) 8–28, https://doi.org/10.1016/J.ACA.2015.02.016.

[40] H. Akoglu, User's guide to correlation coefficients, Turkish J. Emerg. Med. 18 (2018) 91–93, https://doi.org/10.1016/J.TJEM.2018.08.001.

[41] B.K. Matuszewski, M.L. Constanzer, C.M. Chavez-Eng, Strategies for the assessment of matrix effect in quantitative bioanalytical methods based on HPLC-MS/MS, Anal. Chem. 75 (13) (2003) 3019–3030, https://doi.org/10.1021/ac020361s.

CHAPTER 5

GENERAL DISCUSSION
ENGLISH SUMMARY
CZECH SUMMARY
ACKNOWLEDGEMENTS
LIST OF PUBLICATIONS
TRAINING AND SUPERVISION PLAN DURING THE STUDY
CURRICULUM VITAE

General discussion

PPCPs impact on non-target aquatic organisms is an important point in environmental risk assessment. Investigation of compound fate in biological systems and determination of bioaccumulation/bioconcentration potential in whole organism or individual organs are essential for such purpose (Ding et al., 2015; Van der Oost, Beyer and Vermeulen, 2003). Therefore, development of analytical methodologies with good performance parameters is necessary for experimental evaluation of micropollutants distribution in aquatic environment.

Every sample preparation process follows basic concept of transformation sample matrix into form suitable for analysis. Consequently, complex matrices like animal tissue can be challenging and preparation usually involves several steps of extraction and purification.

Wide range of physical and chemical properties even within individual groups of PPCPs make optimization of extraction method difficult especially for multi-residue analysis. Although number of extraction protocols was evaluated and published in effort to find optimal procedure, multi-compound isolation from complex matrix makes always compromise between overall method efficiency and optimal extraction conditions for individual chemicals.

Study of Ramirez et al., evaluate extraction procedures with variable composition of solvents and its pH values, applied for 23 pharmaceuticals with individual hydrophobicity and pKa values. The best variant exhibited average recovery around 60%. Moreover, variable matrix effects across tested solvents illustrate problematic of co-eluting compounds from complex sample (Ramirez et al., 2007b). More sophisticated approach can be represented by hollow fiber solid-phase microextraction. Sampling consist with inserting fibers into tissue supernatant to adsorb analytes of interest without matrix compounds. Extraction procedure then includes microwave-assisted extraction, which as potential heat source requires additional method optimization, otherwise, it can cause compounds decomposition. This method was evaluated for 54 PPCPs in fish muscle samples with recoveries 56–120% (Zhang et al., 2017). Pressurized liquid extraction (PLE) is another relatively common technique involving organic solvents and compound isolation from sample matrix under high pressure and temperature. Therefore, analyte thermal stability should be evaluated as part of method development. PLE method reported recoveries 77–131% for some PPCPs in fish muscle samples (Subedi et al., 2011).

Sample purification represents cleanup step prior to instrument analysis. According to sample complexity respective matrix co-extracts, sample cleanup procedure is frequently required and most of them are based on some type of solid-phase extraction (Hashemi, Zohrabi and Shamsipur, 2018).

Sample preparation procedure reported in this thesis aimed at straight forward multi-residual methodology suitable for biological matrices. The whole process was reduced to mechanical sample homogenization with extraction solvent, centrifugation, biological macro-molecule precipitation in freezer and filtration through syringe cellulose filter. This approach omitting complex SPE extraction and purification is contrary to methods referred above. Our method was evaluated for 74 individual pharmaceuticals and their metabolites in five types of fish tissue. Regarding careful extraction solvent optimization, overall suitable recoveries 60–130% and low quantification limit (sub ng g^{-1}) have been reached.

The method was primally intended for application with extensive analyte number and consequently required liquid chromatography separation. This straightforward extraction was considered as potentially appropriate for direct analytical method where chromatography separation is avoided. The Laser Diode Thermal Desorption combined with atmospheric pressure chemical ionization (LDTD-APCI) is ambient ionization technique, which principle is based on rapid sample thermal desorption by infrared laser source when applied amount of energy have to be carefully optimized to enhance analyte response and minimize matrix

desorption. The main instrumentation advantages are fast analysis (usually below 20 seconds per sample) and low sample volume requirements (maximally 10 µl in case of use LazWell sample plate with 96 cells) (Borik et al., 2020).

The LDTD-APCI has several applications for investigations of pharmaceuticals in biological matrix. Some of them involved more complicated sample treatment like solid-phase microextraction, salting-out assisted liquid-liquid extraction, and solid-phase extraction (Bynum, Moore and Grabenauer, 2014; Heudi et al., 2011; Lanshoeft et al., 2014; Lohne et al., 2012). Moreover, all of referenced methods use only low-resolution mass spectrometers.

The second chapter refers LDTD-APCI method development for psychoactive compound analysis in fish brain tissue. The method purpose was established analytical approach including maximal reduction of sample treatment with ultrafast analysis. Direct sample introduction by LDTD-APCI causes increase risk of potentially interfering compound due to simultaneous desorption of target analyte along with tissue matrix. Also, overall sample treatment simplification without purification makes this disadvantage more relevant. Therefore, high-resolution mass spectrometers (LDTD-APCI-HRPS) operated in parallel reaction monitoring mode with resolving power 17500 FWHM was used to resolved and identified possible matrix interferences. LDTD-APCI-HRPS was evaluated along with low-resolution instrument (LDTD-APCI-QqQ-MS/MS) and then both methods were compared to established LC coupled with high-resolution mass spectrometer. Data sets for such purpose were obtained by analysis of *in vivo* experiment samples, specifically brain tissue of juvenile chubs exposed to citalopram at environmentally relevant concentration.

Although both mass spectrometers types show statistically comparable results with reference LC method analysis of high-resolution product scan mass spectra show matrix mass interferences that cannot be resolved with low-resolution instrument. LDTD-APCI with low-resolution mass spectrometer, therefore, exhibit decreasing accuracy on low concentration level. That's confirm importance of high-resolution mass spectrometry for rapid trace analysis in complex matrix when minimal sample treatment is applied. Moreover, LDTD-APCI method with high-resolution instrument exhibit overall similar performance parameter to reference LC-HRMS method. Also, possibility to skip sample filtration was confirmed with negligible effect on analyte response. The dramatic reduction analysis time, material for sample filtration and reduction organic solvent use is within agreement of green chemistry concept (Badami, 2008). Application field for such methods lays in fast and simple analysis of one or few target analytes in extensive sample sets e.g. toxicity or effect tests on small fish or invertebrates.

PPCPs are relevant soil pollutants introduced via sludge, treated wastewater (Huber et al., 2016) and manure (Prosser and Sibley, 2015). These matrices and potential PPCPs vectors are applied to agriculture fields as fertilizers or for irrigation purpose (Kibuye et al., 2019). Investigation of PPCPs fate in soil environment depends on their sorption potential, which can be experimentally determinate through post-experiment compound concentration in soil leachate. A common sorption experiment design involves single or few analytes and extensive total sample number (Klement et al., 2018). Those attributes make soil sorption research appropriate for LDTD-APCI instrumentation.

The third chapter describes LDTD-APCI method development for four pharmaceutical (carbamazepine, sulfamethoxazole, fexofenadine and irbesartan) and different soil type leachate. The study confirmed positive effect of chelating agents on analyte response previously observed by some authors (Beattie et al., 2012; Lonappan et al., 2016). The influence of different chelating agent concentrations was included as part of method evaluation, which has been previously rarely reported. Precoating LazWell plates with chelating agent Na2-EDTA rapidly improved response of fexofenadine and irbesartan. Relevant mechanism has not been clearly described, some authors expect chelating agent affection homogenous

formation of analyte nanocrystals and thus reduction amount of energy necessary for analyte desorption (Dion-Fortier et al., 2019). The use of chelation agent enabled simplify analysis of all compounds and reduced optimal instrument setup on single LDTD-APCI method. The LDTD-APCI performance parameters were compared with LC-MS and ultrafast approach was confirmed as sufficient for such analysis. Although the LDTD-APCI instrument have been previously successfully applied for pharmaceuticals investigation in wastewater sludge (Mohapatra et al., 2012), manure (Solliec, Massé and Sauvé, 2014) and wastewater (Fayad, Prévost and, Sauvé 2010), our instrument method was the first one applicable for direct investigation of pharmaceutical fate in soil environment.

Consequently to sorption behavior, we shifted our focus on PPCPs interaction with microbial organisms respectively their enzymatic system that causes compound complete degradation or formation byproducts with often unknown toxicological effects (Facey et al., 2018). Occurrence and identification of relevant PPCPs transformation products represent one of the most relevant knowledge gaps in environmental research.

Pilot experiment aiming at irbesartan degradation by soil bacteria and identification of transformation product was performed with individual bacteria taxons incubated in M9 minimal media with glucose and irbesartan 10 μg mL^{-1} (except blanks) for 10 days (Košinová, 2019). Figure 1. show results of degradation experiment. Irbesartan concentration in individual cultures was obtained with LDTD-APCI. The direct sample introduction method was used in purpose to reduce delay between the end of experiment and data acquisition and consequently minimize spontaneously degradation of analyte and consequent result distortion. The most efficient culture was then picked for non-target screening analysis of degradation products. In this case, it was culture with code 18AI. Screening method involves conventional reverse-phase liquid chromatography and heated electrospray ionization with high-resolution mass spectrometry. Initial data set was acquired with full scan positive acquisition mode, scan range 100–600 Da and high resolving power 70000 FWHM (LC-HRMS) necessary for identification compound elemental composition. Received data set was then processed with Compound Discoverer 3.0. This software tool allows analysis of high-resolution data with defined tasks like prediction of elemental composition, identification of up and downregulated compounds and predict elemental composition of transformation products via predefined transformation pathways (Brunner et al., 2019). Depending on analyte elemental composition and its response was chosen or excluded for further structural identification. More detailed structural information was then obtained with high-resolution product scan with resolving power 17500 FWHM. MS/MS spectra were then compared with in silico fragmentation product of predicted transformation structures. Figures 2a and 2b. Show examples of two identified most abundant irbesartan transformation products. Figure 2a. Dihydroxy irbesartan has two OH groups substituted on diazospirononen structure, which is confirmed with MS/MS product with mass 184.1332 m/z. Variable position of OH groups on diazospirononen structure is responsible for occurrence of structural isomers with different polarities represented in chromatograms by three peaks. Products 207.0916 and 235.0978 m/z are in common with native irbesartan (Harahap, Maysyarah and Suryadi, 2017) and it is proved origin of the structure. Figure 2b. Hydroxy irbesartan exhibit similar properties, exception is only one substitution on diazospirononen structure.

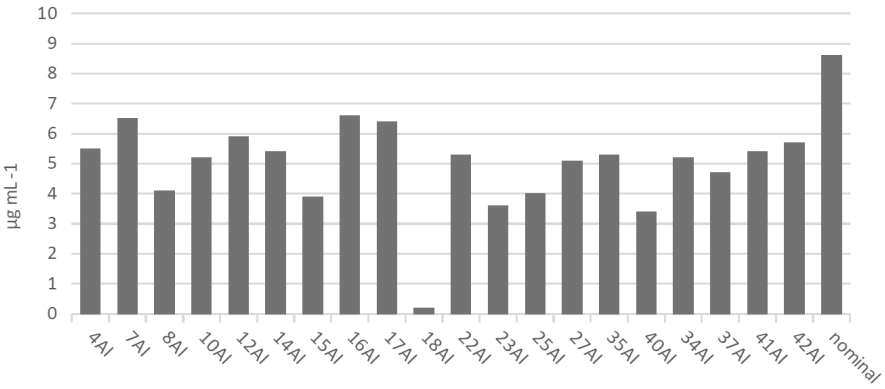


Figure 1. *Results of pilot bacterial degradation experiment of irbesartan. N=1.*

Similar non-target screening workflow was tested also in combination with LDTD-APCI instrument. Full scan acqnuisition was performed within scan range 300–500 Da to enhance method sensitivity and resolving power 70000 FWHM (LDTD-APCI-HRMS). Processing of full scan spectra with Compound Discoverer identified same most abundant transformation products but total number of resolved compounds were much lower according to lack of compound separation. Moreover, figure 3a shows analyte response of dihydroxy irbesartan, which in case of LDTD-APCI-HRMS screening method structural isomers cannot be resolved. Despite to absence of compound separation providing additional structural confirmation and lower sensitivity, LDTD-APCI-HRMS show promising preliminary results for non-target screening application. Acquisition in full scan mode with adequate scan range is capable to provide data for quantitation analysis and qualitative information about dominant transformation products. Up to our knowledge, there is no method for non-target screening using LDTD-APCI published in the literature.

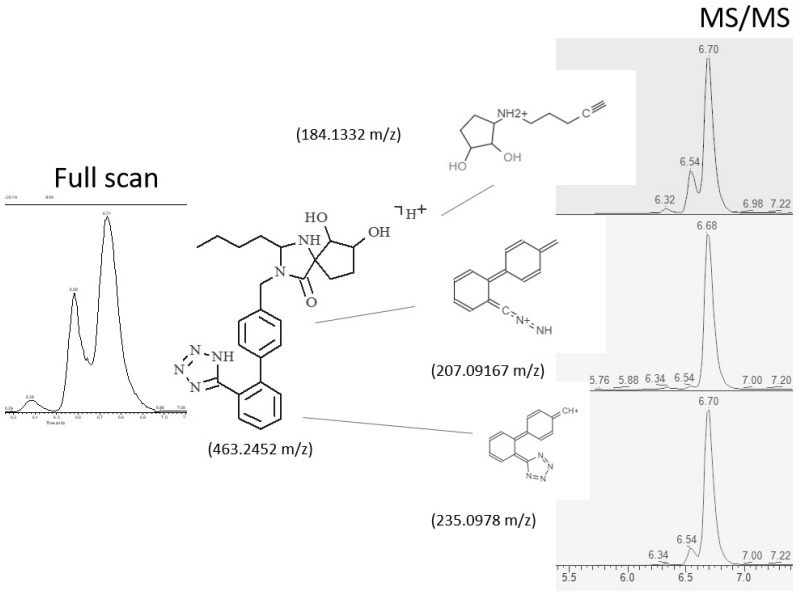


Figure 2a*. Chromatograms and predicted MS/MS products of dihydroxy irbesartan. (LC-HRMS).*

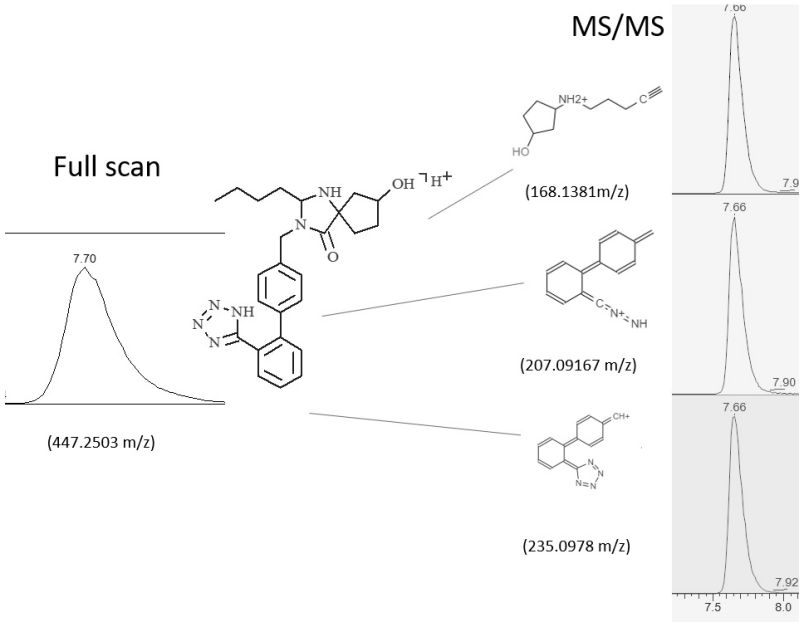


Figure 2b. *Chromatograms and predicted MS/MS products of hydroxy irbesartan (LC-HRMS).*

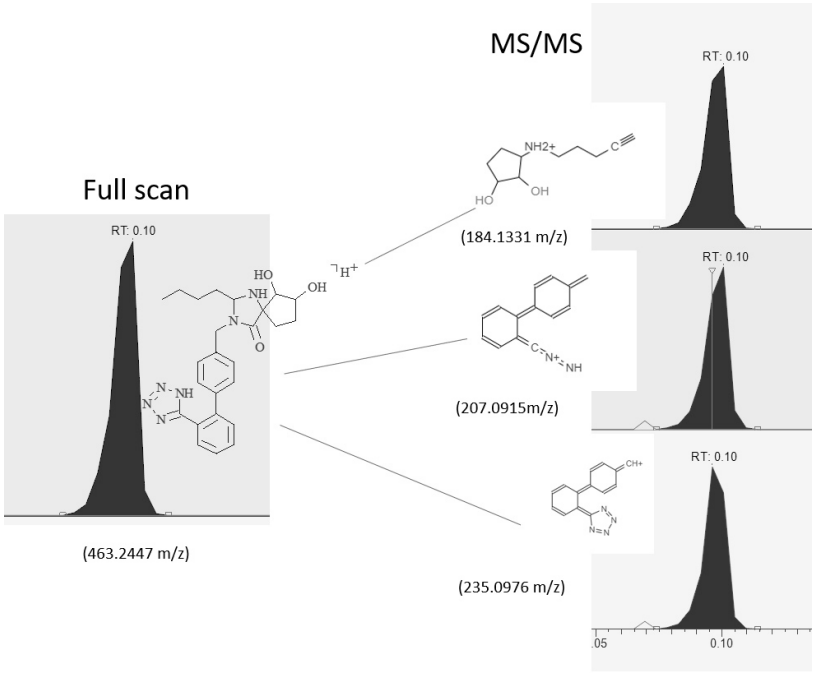


Figure 3a. *Extracted ion chromatograms and predicted MS/MS products of dihydroxy irbesartan. (LDTD-APCI-HRMS).*

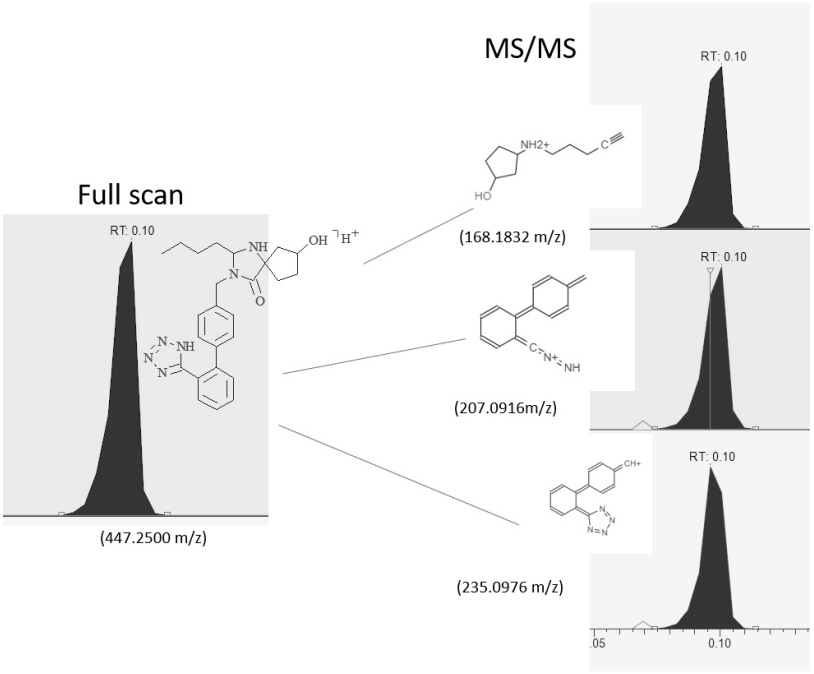


Figure 3b. *Extracted ion chromatograms and predicted MS/MS products of hydroxy irbesartan. (LDTD-APCI-HRMS.*

Conclusions

The thesis aims we defined as development of novelty analytical approaches for environmental research, enhancing time efficiency and reducing requirement of material and production of waste. These methods are presented through three scientific articles. Although beneficial properties of LDTD-APCI instrumentation are obvious, omitting compound separation process involve into analysis some negative aspects. Specific compound feature like presence of structural isomers can be identified via different affinity on stationery phase during separation. Consequently, identidacation of isomers can by challenging or even impossible for LDTD-APCI. Also ultrafast sample analysis concurentlly reduce time for data aqusition and number of recorded MS-MS experiments is typically reduced to units, when LC-MS methods allow up to thousands individual compound detection.

Direct sample introduction technique LDTD-APCI have been confirmed as powerful tool for specific investigation purposes when target analysis of large sample set is part of experimental design. Also, connection of LDTD-APCI with high-resolution mass spectrometry and straight forward extraction method for biological samples has been confirmed as additionally beneficial. LDTD-APCI with high-resolution mass spectrometry showed promising potential for ultra-fast non-target screening of PPCPs transformation products, which have to be carefully evaluated.

References

Albaigés, Joan, Josep M. Bayona, and Jagoš R. Radović. 2016. "Photochemical Effects on Oil Spill Fingerprinting." In *Standard Handbook Oil Spill Environmental Forensics*, Academic Press, 917–59. https://www.sciencedirect.com/science/article/pii/B9780128038321000209#bib0555 (May 22, 2020).

Alharbi, Sultan K. et al., 2017. "Photolysis and UV/H2O2 of Diclofenac, Sulfamethoxazole, Carbamazepine, and Trimethoprim: Identification of Their Major Degradation Products by ESI–LC–MS and Assessment of the Toxicity of Reaction Mixtures." *Process Safety and Environmental Protection* 112: 222–34. https://www.sciencedirect.com/science/article/pii/S095758201730229X?casa_token=EQKUXVEYX7IAAAAA:rL47zdnPXvFnnWcTdV2ufQSOQyJA3dYzl1nHus3D0FKUBl7DNbrcfrj9Q8d9zM2Il6YhA2eYkBQ (May 17, 2020).

Ashfaq, Muhammad et al., 2019. "Occurrence, Spatial Variation and Risk Assessment of Pharmaceuticals and Personal Care Products in Urban Wastewater, Canal Surface Water, and Their Sediments: A Case Study of Lahore, Pakistan." *Science of the Total Environment* 688: 653–63.

Badami, Bharati V. 2008. "Concept of Green Chemistry." *Resonance* 13(11): 1041–48.

Bai, Ying, Zhengguo Cui, Rongguo Su, and Keming Qu. 2018. "Influence of DOM Components, Salinity, PH, Nitrate, and Bicarbonate on the Indirect Photodegradation of Acetaminophen in Simulated Coastal Waters." *Chemosphere* 205: 108–17. https://www.sciencedirect.com/science/article/pii/S0045653518307367 (May 22, 2020).

Beattie, Iain et al., 2012. "Evaluation of Laser Diode Thermal Desorption (LDTD) Coupled with Tandem Mass Spectrometry (MS/MS) for Support of *in Vitro* Drug Discovery Assays: Increasing Scope, Robustness and Throughput of the LDTD Technique for Use with Chemically Diverse Compound Libraries." *Journal of Pharmaceutical and Biomedical Analysis* 59: 18–28. https://www.sciencedirect.com/science/article/pii/S0731708511005905 (March 27, 2019).

Behera, Shishir Kumar, Hyeong Woo Kim, Jeong Eun Oh, and Hung Suck Park. 2011. "Occurrence and Removal of Antibiotics, Hormones and Several Other Pharmaceuticals in Wastewater Treatment Plants of the Largest Industrial City of Korea." *Science of the Total Environment* 409(20): 4351–60. https://www.sciencedirect.com/science/article/pii/S0048969711007327 (May 16, 2020).

Beretta, Magda et al., 2014. "Occurrence of Pharmaceutical and Personal Care Products (PPCPs) in Marine Sediments in the Todos Os Santos Bay and the North Coast of Salvador, Bahia, Brazil." *Journal of Soils and Sediments* 14(7): 1278–86.

Białk-Bielińska, Anna et al., 2012. "Hydrolysis of Sulphonamides in Aqueous Solutions." *Journal of Hazardous Materials* 221–222: 264–74.

Blair, Benjamin et al., 2015. "Evaluating the Degradation, Sorption, and Negative Mass Balances of Pharmaceuticals and Personal Care Products during Wastewater Treatment." *Chemosphere* 134: 395–401. https://www.sciencedirect.com/science/article/pii/S0045653515004245?via%3Dihub (May 19, 2020).

Bletsou, Anna A. et al., 2015. "Targeted and Non-Targeted Liquid Chromatography-Mass Spectrometric Workflows for Identification of Transformation Products of Emerging Pollutants in the Aquatic Environment." *TrAC - Trends in Analytical Chemistry* 66: 32–44.

Boreen, Anne L, William A Arnold, and Kristopher McNeill. 2003. "Photodegradation of Pharmaceuticals in the Aquatic Environment: A Review." *Aquatic Sciences* 65(4): 320–41. https://link.springer.com/content/pdf/10.1007/s00027-003-0672-7.pdf (May 21, 2020).

Borik, Adam et al., 2020. "Determination of Citalopram in Fish Brain Tissue: Benefits of Coupling Laser Diode Thermal Desorption with Low- and High-Resolution Mass Spectrometry." *Analytical and Bioanalytical Chemistry* 412: 4353–4361.

Brew, David W. et al., 2020. "Metabolomic Investigations of the Temporal Effects of Exposure to Pharmaceuticals and Personal Care Products and Their Mixture in the Eastern Oyster (Crassostrea Virginica)." *Environmental Toxicology and Chemistry* 39(2): 419–36.

Brodin, Tomas et al., 2017. "Environmental Relevant Levels of a Benzodiazepine (Oxazepam) Alters Important Behavioral Traits in a Common Planktivorous Fish, (Rutilus Rutilus)." *Journal of Toxicology and Environmental Health - Part A: Current Issues* 80(16–18): 963–70.

Brunner, Andrea Mizzi et al., 2019. "Monitoring Transformation Product Formation in the Drinking Water Treatments Rapid Sand Filtration and Ozonation." *Chemosphere* 214: 801–11.

Buser, Hans Rudolf, Thomas Poiger, and Markus D. Müller. 1998. "Occurrence and Fate of the Pharmaceutical Drug Diclofenac in Surface Waters: Rapid Photodegradation in a Lake." *Environmental Science and Technology* 32(22): 3449–56. https://pubs.acs.org/doi/abs/10.1021/es980301x (May 22, 2020).

Bynum, Nichole D., Katherine N. Moore, and Megan Grabenauer. 2014. "Evaluation of Laser Diode Thermal Desorption-Tandem Mass Spectrometry (LDTD-MS-MS) in Forensic Toxicology." *Journal of Analytical Toxicology* 38(8): 528–35. https://academic.oup.com/jat/article-lookup/doi/10.1093/jat/bku084 (May 30, 2020).

Caldwell, Daniel J., Frank Mastrocco, Luigi Margiotta-Casaluci, and Bryan W. Brooks. 2014. "An Integrated Approach for Prioritizing Pharmaceuticals Found in the Environment for Risk Assessment, Monitoring and Advanced Research." *Chemosphere* 115(1): 4–12.

Calisto, Vânia, M. Rosário M. Domingues, and Valdemar I. Esteves. 2011. "Photodegradation of Psychiatric Pharmaceuticals in Aquatic Environments - Kinetics and Photodegradation Products." *Water Research* 45(18): 6097–6106. https://www.sciencedirect.com/science/article/pii/S0043135411005276 (May 22, 2020).

Carballa, Marta et al., 2004. "Behavior of Pharmaceuticals, Cosmetics and Hormones in a Sewage Treatment Plant." *Water Research* 38(12): 2918–26.

Chen, Xijuan et al., 2012. "Ozonation Products of Triclosan in Advanced Wastewater Treatment." *Water Research* 46(7): 2247–56.

Chen, Yi Jie et al., 2020. "GC-MS/MS Analysis for Source Identification of Emerging POPs in PM2.5." *Ecotoxicology and Environmental Safety* 193: 110368.

Dabić, Dario, Sandra Babić, and Irena Škorić. 2019. "The Role of Photodegradation in the Environmental Fate of Hydroxychloroquine." *Chemosphere* 230: 268–77. https://www.sciencedirect.com/science/article/pii/S0045653519309294 (May 22, 2020).

Daughton, Christian G., and Thomas A. Ternes. 1999. "Pharmaceuticals and Personal Care Products in the Environment: Agents of Subtle Change?" *Environmental Health Perspectives* 107(SUPPL. 6): 907–38.

Ding, Jiannan et al., 2015. "Biological Fate and Effects of Propranolol in an Experimental Aquatic Food Chain." *Science of the Total Environment* 532: 31–39.

Dion-Fortier, Annick et al., 2019. "Signal Enhancement in Laser Diode Thermal Desorption-Triple Quadrupole Mass Spectrometry Analysis Using Microwell Surface Coatings." *Journal of Mass Spectrometry* 54(2): 167–77.

Ejhed, H. et al., 2018. "The Effect of Hydraulic Retention Time in Onsite Wastewater Treatment and Removal of Pharmaceuticals, Hormones and Phenolic Utility Substances." *Science of the Total Environment* 618: 250–61. https://www.sciencedirect.com/science/article/pii/S004896971733070X?casa_token=jHKWjtfVwXwAAAAA:N9nXq18wNMIITZ1XI-t1j-8onxeq72QYiLC1Y5Bu9Q85kHiDcvGm8stmVJoRIm9iyc0c-5dWNFA (May 18, 2020).

Ellepola, Nishanthi et al., 2020. "A Toxicological Study on Photo-Degradation Products of Environmental Ibuprofen: Ecological and Human Health Implications." *Ecotoxicology and Environmental Safety* 188: 109892. https://www.sciencedirect.com/science/article/pii/S0147651319312230 (May 22, 2020).

Facey, Sandra J. et al., 2018. "Rapid and Complete Degradation of Diclofenac by Native Soil Microorganisms." *Environmental Technology and Innovation* 10: 55–61.

Fayad, Paul B., Michèle Prévost, and Sébastien Sauvé. 2010. "Laser Diode Thermal Desorption/Atmospheric Pressure Chemical Ionization Tandem Mass Spectrometry Analysis of Selected Steroid Hormones in Wastewater: Method Optimization and Application." *Analytical Chemistry* 82(2): 639–45. http://www.ncbi.nlm.nih.gov/pubmed/20000768 (February 12, 2019).

Fedorova, Ganna, Oksana Golovko, Tomas Randak, and Roman Grabic. 2014. "Storage Effect on the Analysis of Pharmaceuticals and Personal Care Products in Wastewater." *Chemosphere* 111: 55–60. https://ac.els-cdn.com/S0045653514003361/1-s2.0-S0045653514003361-main.pdf?_tid=96085d26-b337-11e7-a055-00000aab0f02&acdnat=1508243878_f9c26bc7c6e78030c8cacb4563a64e1a (October 17, 2017).

Fent, Karl. 2015. "Progestins as Endocrine Disrupters in Aquatic Ecosystems: Concentrations, Effects and Risk Assessment." *Environment International* 84: 115–30.

Gan, Zhiwei et al., 2014. "Transformation of Acesulfame in Water under Natural Sunlight: Joint Effect of Photolysis and Biodegradation." *Water Research* 64: 113–22. https://www.sciencedirect.com/science/article/pii/S0043135414004862?via%3Dihub#bib40 (May 23, 2020).

Gerba, Charles P., and Ian L. Pepper. 2015. "Municipal Wastewater Treatment." In *Environmental Microbiology: Third Edition*, Academic Press, 583–606. https://www.sciencedirect.com/science/article/pii/B9780128147191000227 (May 17, 2020).

Golovko, Oksana et al., 2014a. "Seasonal Changes in Antibiotics, Antidepressants/Psychiatric Drugs, Antihistamines and Lipid Regulators in a Wastewater Treatment Plant." *Chemosphere* 111: 418–26. https://www.sciencedirect.com/science/article/pii/S0045653514005104#t0005 (November 21, 2018).

Golovko, Oksana et al., 2014b. "Seasonal Changes in Antibiotics, Antidepressants/Psychiatric Drugs, Antihistamines and Lipid Regulators in a Wastewater Treatment Plant." *Chemosphere* 111: 418–26. https://www.sciencedirect.com/science/article/pii/S0045653514005104 (May 19, 2020).

Gros, Meritxell et al., 2019. "Veterinary Pharmaceuticals and Antibiotics in Manure and Slurry and Their Fate in Amended Agricultural Soils: Findings from an Experimental Field Site (Baix Empordà, NE Catalonia)." *Science of the Total Environment* 654: 1337–49.

Guo, Wei et al., 2020. "Psychoactive Compounds at Environmental Concentration Alter Burrowing Behavior in the Freshwater Crayfish." *Science of the Total Environment* 711: 135138.

Hamdi El Najjar, Nasma et al., 2013. "Levofloxacin Oxidation by Ozone and Hydroxyl Radicals: Kinetic Study, Transformation Products and Toxicity." *Chemosphere* 93(4): 604–11.

Harahap, Yahdiana, Innet Maysyarah, and Herman Suryadi. 2017. "Analytical Validation of Clopidogrel in Human Plasma through Ultrahigh-Performance Liquid Chromatography-Tandem Mass Spectrometry." *International Journal of Applied Pharmaceutics* 9: 163–67.

Hashemi, Beshare, Parvin Zohrabi, and Mojtaba Shamsipur. 2018. "Recent Developments and Applications of Different Sorbents for SPE and SPME from Biological Samples." *Talanta* 187: 337–47.

Haunschmidt, Manuela, Christian W. Klampfl, Wolfgang Buchberger, and Robert Hertsens. 2010. "Determination of Organic UV Filters in Water by Stir Bar Sorptive Extraction and Direct Analysis in Real-Time Mass Spectrometry." In *Analytical and Bioanalytical Chemistry*, Springer, 269–75.

Heudi, Olivier et al., 2011. "Laser Diode Thermal Desorption-Positive Mode Atmospheric Pressure Chemical Ionization Tandem Mass Spectrometry for the Ultra-Fast Quantification of a Pharmaceutical Compound in Human Plasma." *Journal of Pharmaceutical and Biomedical Analysis* 54(5): 1088–95. https://www.sciencedirect.com/science/article/pii/S0731708510006680 (May 30, 2020).

Holm, John V., Kirsten Rugge, Poul L. Bjerg, and Thomas H. Christensen. 1995. "Occurrence and Distribution of Pharmaceutical Organic Compounds in the Groundwater Downgradient of a Landfill (Grindsted, Denmark)." *Environmental Science and Technology* 29(5): 1415–20. https://pubs.acs.org/doi/abs/10.1021/es00005a039 (May 10, 2020).

Huber, Sandra et al., 2016. "A First Screening and Risk Assessment of Pharmaceuticals and Additives in Personal Care Products in Waste Water, Sludge, Recipient Water and Sediment from Faroe Islands, Iceland and Greenland." *Science of the Total Environment* 562: 13–25.

Ivanová, Lucia et al., 2018. "Pharmaceuticals and Illicit Drugs – A New Threat to the Application of Sewage Sludge in Agriculture." *Science of The Total Environment* 634: 606–15. https://www.sciencedirect.com/science/article/pii/S0048969718311720 (November 21, 2018).

Jackson, Latonya M., and Paul L. Klerks. 2019. "Impact of Long-Term Exposure to 17A-Ethinylestradiol in the Live-Bearing Fish Heterandria Formosa." *Archives of Environmental Contamination and Toxicology* 77(1): 51–61.

Joss, Adriano et al., 2006. "Biological Degradation of Pharmaceuticals in Municipal Wastewater Treatment: Proposing a Classification Scheme." *Water Research* 40(8): 1686–96. https://www.sciencedirect.com/science/article/pii/S0043135406001126 (January 22, 2019).

Kagle, Jeanne et al., 2009. "Chapter 3 Biodegradation of Pharmaceutical and Personal Care Products." *Advances in Applied Microbiology* 67(C): 65–108. https://pubmed.ncbi.nlm.nih.gov/19245937/ (May 20, 2020).

Kibuye, Faith A. et al., 2019. "Fate of Pharmaceuticals in a Spray-Irrigation System: From Wastewater to Groundwater." *Science of the Total Environment* 654: 197–208.

Klement, Aleš et al., 2018. "Sorption of Citalopram, Irbesartan and Fexofenadine in Soils: Estimation of Sorption Coefficients from Soil Properties." *Chemosphere* 195: 615–23. https://www.sciencedirect.com/science/article/pii/S0045653517320684 (June 7, 2019).

Kodešová, Radka et al., 2015. "Pharmaceuticals' Sorptions Relative to Properties of Thirteen Different Soils." *Science of The Total Environment* 511: 435–43. https://www.sciencedirect.com/science/article/pii/S0048969714017999 (April 30, 2019).

Košinová, Anna. 2019. 56 "Odpověď Půdního Mikrobního Společenstva Na Aplikaci Farmak Přítomných v Zavlažovacích Vodách Se Zaměřením Na Irbesartan." University of South Bohemia, České Budějovice, Czech Republic.

Kunkel, Uwe, and Michael Radke. 2008. "Biodegradation of Acidic Pharmaceuticals in Bed Sediments: Insight from a Laboratory Experiment." *Environmental Science and Technology* 42(19): 7273–79.

Lange, Anke et al., 2009. "Sexual Reprogramming and Estrogenic Sensitization in Wild Fish Exposed to Ethinylestradiol." *Environmental Science and Technology* 43(4): 1219–25.

Lange, Vinzenz, Paola Picotti, Bruno Domon, and Ruedi Aebersold. 2008. "Selected Reaction Monitoring for Quantitative Proteomics: A Tutorial." *Molecular Systems Biology* 4(1): 222. https://onlinelibrary.wiley.com/doi/abs/10.1038/msb.2008.61 (May 25, 2020).

Lanshoeft, Christian et al., 2014. "Laser Diode Thermal Desorption Atmospheric Pressure Chemical Ionization Tandem Mass Spectrometry Applied for the Ultra-Fast Quantitative Analysis of BKM120 in Human Plasma." *Analytical and Bioanalytical Chemistry* 406(22): 5413–23. http://link.springer.com/10.1007/s00216-014-7966-6 (May 30, 2020).

Li, Mingyue et al., 2016. "Simultaneous Analysis of 45 Pharmaceuticals and Personal Care Products in Sludge by Matrix Solid-Phase Dispersion and Liquid Chromatography Tandem Mass Spectrometry." *Analytical and Bioanalytical Chemistry* 408(18): 4953–64.

Lin, Angela Yu Chen, and Martin Reinhard. 2005. "Photodegradation of Common Environmental Pharmaceuticals and Estrogens in River Water." *Environmental Toxicology and Chemistry* 24(6): 1303–9. http://doi.wiley.com/10.1897/04-236R.1 (May 22, 2020).

Loftin, Keith A., Craig D. Adams, Michael T. Meyer, and Rao Surampalli. 2008. "Effects of Ionic Strength, Temperature, and PH on Degradation of Selected Antibiotics." *Journal of Environmental Quality* 37(2): 378–86.

Lohne, Jack J. et al., 2012. "Laser Diode Thermal Desorption Mass Spectrometry for the Analysis of Quinolone Antibiotic Residues in Aquacultured Seafood." *Rapid Communications in Mass Spectrometry* 26(24): 2854–64. http://www.ncbi.nlm.nih.gov/pubmed/23136016 (March 27, 2019).

Lonappan, Linson et al., 2016. "Diclofenac in Municipal Wastewater Treatment Plant: Quantification Using Laser Diode Thermal Desorption—Atmospheric Pressure Chemical Ionization—Tandem Mass Spectrometry Approach in Comparison with an Established Liquid Chromatography-Electrospray Ionization–Tandem Mass Spectrometry Method." *Journal of Chromatography A* 1433: 106–13. https://www.sciencedirect.com/science/article/pii/S0021967316000625 (April 9, 2018).

Mandalakis, Manolis, Manolis Tsapakis, and Euripides G. Stephanou. 2001. "Optimization and Application of High-Resolution Gas Chromatography with Ion Trap Tandem Mass Spectrometry to the Determination of Polychlorinated Biphenyls in Atmospheric Aerosols." *Journal of Chromatography A* 925(1–2): 183–96.

Mohapatra, D.P. et al., 2012. "Carbamazepine in Municipal Wastewater and Wastewater Sludge: Ultrafast Quantification by Laser Diode Thermal Desorption-Atmospheric Pressure Chemical Ionization Coupled with Tandem Mass Spectrometry." *Talanta* 99: 247–55. https://www.sciencedirect.com/science/article/pii/S0039914012004274 (March 27, 2019).

van Nuijs, Alexander L.N., Isabela Tarcomnicu, and Adrian Covaci. 2011. "Application of Hydrophilic Interaction Chromatography for the Analysis of Polar Contaminants in Food and Environmental Samples." *Journal of Chromatography A* 1218(35): 5964–74.

Ohoro, C. R., A. O. Adeniji, A. I. Okoh, and O. O. Okoh. 2019. "Distribution and Chemical Analysis of Pharmaceuticals and Personal Care Products (PPCPs) in the Environmental Systems: A Review." *International Journal of Environmental Research and Public Health* 16(17). / pmc/articles/PMC6747491/?report=abstract (July 22, 2020).

Van der Oost, Ron, Jonny Beyer, and Nico P.E. Vermeulen. 2003. "Fish Bioaccumulation and Biomarkers in Environmental Risk Assessment: A Review." *Environmental Toxicology and Pharmacology* 13(2): 57–149.

Oulton, Rebekah L., Tamar Kohn, and David M. Cwiertny. 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): 1956–78.

Paul, Samir C. et al., 2014. "Sorption Behavior of Ibuprofen and Naproxen in Simulated Domestic Wastewater." *Water, Air, and Soil Pollution* 225(1): 1821. http://link.springer.com/10.1007/s11270-013-1821-9 (May 18, 2020).

Petersen, Karina, Harald Hasle Heiaas, and Knut Erik Tollefsen. 2014. "Combined Effects of Pharmaceuticals, Personal Care Products, Biocides and Organic Contaminants on the Growth of Skeletonema Pseudocostatum." *Aquatic Toxicology* 150: 450–54.

Prosser, R. S., and P. K. Sibley. 2015. "Human Health Risk Assessment of Pharmaceuticals and Personal Care Products in Plant Tissue Due to Biosolids and Manure Amendments, and Wastewater Irrigation." *Environment International* 75: 223–33.

Ramirez, Alejandro J., Mohammad A. Mottaleb, Bryan W. Brooks, and C. Kevin Chambliss. 2007a. "Analysis of Pharmaceuticals in Fish Using Liquid Chromatography-Tandem Mass Spectrometry." *Analytical Chemistry* 79(8): 3155–63.

Ramirez, Alejandro J., Mohammad A. Mottaleb, Bryan W. Brooks, and C. Kevin Chambliss. 2007b. "Analysis of Pharmaceuticals in Fish Using Liquid Chromatography-Tandem Mass Spectrometry." *Analytical Chemistry* 79(8): 3155–63.

Sabourin, Lyne et al., 2009. "Runoff of Pharmaceuticals and Personal Care Products Following Application of Dewatered Municipal Biosolids to an Agricultural Field." *Science of the Total Environment* 407(16): 4596–4604.

Santoke, Hanoz, and William J. Cooper. 2017. "Environmental Photochemical Fate of Selected Pharmaceutical Compounds in Natural and Reconstituted Suwannee River Water: Role of Reactive Species in Indirect Photolysis." *Science of the Total Environment* 580: 626–31. https://www.sciencedirect.com/science/article/pii/S0048969716326948 (May 22, 2020).

Šauer, Pavel et al., 2018. "Two Synthetic Progestins and Natural Progesterone Are Responsible for Most of the Progestagenic Activities in Municipal Wastewater Treatment Plant Effluents in the Czech and Slovak Republics." *Water Research* 137: 64–71. https://www.sciencedirect.com/science/article/pii/S0043135418301787 (September 18, 2018).

Schlüter-Vorberg, Lisa et al., 2015. "Toxification by Transformation in Conventional and Advanced Wastewater Treatment: The Antiviral Drug Acyclovir." *Environmental Science and Technology Letters* 2(12): 342–46.

Solliec, Morgan, Daniel Massé, and Sébastien Sauvé. 2014. "Analysis of Trimethoprim, Lincomycin, Sulfadoxin and Tylosin in Swine Manure Using Laser Diode Thermal Desorption-Atmospheric Pressure Chemical Ionization-Tandem Mass Spectrometry." *Talanta* 128: 23–30.

Sparkman, O. David. 1996. "Evaluating Electron Ionization Mass Spectral Library Search Results." *Journal of the American Society for Mass Spectrometry* 7(4): 313–18.

Strittmatter, Nicole, Rolf Alexander Düring, and Zoltán Takáts. 2012. "Analysis of Wastewater Samples by Direct Combination of Thin-Film Microextraction and Desorption Electrospray Ionization Mass Spectrometry." *Analyst* 137(17): 4037–44.

Subedi, Bikram, Mohammad A. Mottaleb, C. Kevin Chambliss, and Sascha Usenko. 2011. "Simultaneous Analysis of Select Pharmaceuticals and Personal Care Products in Fish Tissue Using Pressurized Liquid Extraction Combined with Silica Gel Cleanup." *Journal of Chromatography A* 1218(37): 6278–84.

Sun, Qian et al., 2016. "PPCPs in Jiulong River Estuary (China): Spatiotemporal Distributions, Fate, and Their Use as Chemical Markers of Wastewater." *Chemosphere* 150: 596–604.

Tarpani, Raphael Ricardo Zepon, and Adisa Azapagic. 2018. "A Methodology for Estimating Concentrations of Pharmaceuticals and Personal Care Products (PPCPs) in Wastewater Treatment Plants and in Freshwaters." *Science of the Total Environment* 622–623: 1417–30.

Tarr, Matthew A., Junhua Zhu, and Richard B. Cole. 2000. "Atmospheric Pressure Ionization Mass Spectrometry." In *Encyclopedia of Analytical Chemistry*, Chichester, UK: John Wiley & Sons, Ltd. http://doi.wiley.com/10.1002/9780470027318.a6003 (May 25, 2020).

Ternes, Thomas A. et al., 2004. "A Rapid Method to Measure the Solid-Water Distribution Coefficient (K d) for Pharmaceuticals and Musk Fragrances in Sewage Sludge." *Water Research* 38(19): 4075–84.

Ternes, Thomas A et al., 2003. "Ozonation: A Tool for Removal of Pharmaceuticals, Contrast Media and Musk Fragrances from Wastewater?" *Water Research* 37(8): 1976–82. https://www.sciencedirect.com/science/article/pii/S0043135402005705 (May 17, 2020).

Tran, Ngoc Han, Martin Reinhard, and Karina Yew Hoong Gin. 2018. "Occurrence and Fate of Emerging Contaminants in Municipal Wastewater Treatment Plants from Different Geographical Regions-a Review." *Water Research* 133: 182–207.

Valenti, Theodore W. et al., 2012. "Human Therapeutic Plasma Levels of the Selective Serotonin Reuptake Inhibitor (SSRI) Sertraline Decrease Serotonin Reuptake Transporter Binding and Shelter-Seeking Behavior in Adult Male Fathead Minnows." *Environmental Science and Technology* 46(4): 2427–35.

Vasquez, M. I. et al., 2014. "Environmental Side Effects of Pharmaceutical Cocktails: What We Know and What We Should Know." *Journal of Hazardous Materials* 279: 169–89.

Viglino, Liza, Michèle Prévost, and Sébastien Sauvé. 2011. "High Throughput Analysis of Solid-Bound Endocrine Disruptors by LDTD-APCI-MS/MS." *Journal of Environmental Monitoring* 13(3): 583–90.

Wang, Jianlong, and Shizong Wang. 2016. "Removal of Pharmaceuticals and Personal Care Products (PPCPs) from Wastewater: A Review." *Journal of Environmental Management* 182: 620–40.

Wenning, Richard J., and Linda Martello. 2013. "POPs in Marine and Freshwater Environments." In *Environmental Forensics for Persistent Organic Pollutants*, Elsevier B.V., 357–90.

de Wilt, Arnoud et al., 2018. "Enhanced Pharmaceutical Removal from Water in a Three Step Bio-Ozone-Bio Process." *Water Research* 138: 97–105. https://www.sciencedirect.com/science/article/pii/S0043135418302240 (May 17, 2020).

Xuan, Richeng et al., 2010. "Hydrolysis and Photolysis of Oxytetracycline in Aqueous Solution." *Journal of Environmental Science and Health - Part B Pesticides, Food Contaminants, and Agricultural Wastes* 45(1): 73–81.

Zhang, Yi et al., 2017. "Rapid Determination of 54 Pharmaceutical and Personal Care Products in Fish Samples Using Microwave-Assisted Extraction—Hollow Fiber—Liquid/Solid Phase Microextraction." *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences* 1051: 41–53.

Zhou, D., W. R. Porter, and G. G.Z. Zhang. 2017. "Drug Stability and Degradation Studies." In *Developing Solid Oral Dosage Forms: Pharmaceutical Theory and Practice: Second Edition*, Academic Press, 113–49. https://www.sciencedirect.com/science/article/pii/B9780128024478000054 (May 22, 2020).

Zwiener, Christian, Thomas Glauner, and Fritz H. Frimmel. 2000. "Biodegradation of Pharmaceutical Residues Investigated by SPE-GC/ITD-MS and on-Line Derivatization." *HRC Journal of High Resolution Chromatography* 23(7–8): 474–78.

English summary

Tracing pharmaceuticals and personal care products (PPCPs) from sources to recipients

Adam Bořík

Increasing consumption and production of new PPCPs step up pressure on environmental scientists and need to identify the fate and impact of these pollutants. LC-MS methods applied in this research have been staying as gold standard since nineties when problematics of PPCPs in context of environmental pollution raised and they are still irreplaceable for large number of applications even during recent time. Despite good performance, some disadvantage properties should be considered as reasons for replacement with possible alternatives. The negative aspects like organic solvent consumption, long analysis time and waste production, which is directly dependent on additional sample preparation for LC system can be avoided by replacement liquid chromatography technique with direct sample introduction approach, where separation is completely omitted.

Laser diode thermal desorption with atmospheric pressure chemical ionization (LDTD-APCI) can be suitable alternative. This technique was applied for quantification of sulfamethoxazole, irbesartan, fexofenadine, and carbamazepine in soil leachate samples as part of experimental methodology describing pharmaceutical fate in soil environment that can indirectly contaminated surface water. The evaluation process involved statistical comparison with LC method with comparable results and performance parameters when for example both methods quantification limits were units of ng g^{-1}.

Different types of toxicology tests are frequently employed for investigation of PPCP's impact on non-target aquatic organisms. Such type of experiment usually exposes experimental organisms to one or a few compounds of interest and then potential negatives effects are evaluated. As part of evaluation is frequently applied quantification of target analytes in organisms to estimate bioaccumulation/bioconcentration effects of pollutants. LDTD-APCI combined with high-resolution mass spectrometry shown to be effective approach, especially in combination with straightforward extraction technique previously developed for 74 pharmaceuticals isolation from fish tissue. This methodology simplifies sample preparation with avoiding additional solid-phase extraction or purification, rapidly accelerates analysis time because of fast sample desorption (usually low tens of seconds) and high-resolution mass spectrometer provides method selectivity important for resolving analyte of interest from complex biological matrix.

Results reached with LDTD-APCI presented in this thesis are encouraging for further development of advanced analytical applications for environmental research.

Czech summary

Sledování farmaceutik a produktů osobní péče od zdrojů až po recipienty

Adam Bořík

Narůstající spotřeba a produkce nových sloučenin spadajících do kategorie PPCPs neustále zvyšuje tlak na výzkumné kapacity a stoupá nutnost identifikace dopadu těchto látek na životní prostředí. LC-MS metody dlouhodobě představují klíčový nástroj analýzy PPCPs a pro mnohé aplikace jsou stále nenahraditelné. Navzdory přesnosti a robustnosti existují určité limitace, které mohou být podnětem k hledání efektivnějších alternativ. Hlavní negativní aspekty jsou spjaty především se separační metodou. Kapalinová chromatografie vyžaduje pro svůj provoz mobilní fáze zahrnující organická rozpouštědla a dále také relativně náročnou úpravu vzorků spojenou s využitím nutného spotřebního materiálu.

Nahrazením kapalinové chromatografie metodou přímé introdukce vzorku je možné tyto negativa vyloučit. Takovou metodou je například termální desorpce diodovým laserem kombinovaná s chemickou ionizací za atmosférického tlaku (LDTD-APCI). Tato metoda byla aplikována pro potřeby kvantifikace sulfamethoxazolu, irbesartanu, fexofenadinu a karbamazepinu v půdních výluzích jako součást experimentu zaměřeného na popis osudu farmak v tomto prostředí, které může mít nepřímou souvislost s kontaminací recipientu. Nově vyvinutá LDTD-APCI metoda byla porovnána s konvenční LC s obdobnými výsledky validačních parametrů, a to včetně kvantifikačních limitů v řádu jednotek ng g^{-1}.

Sledování konkrétních dopadů PPCPs na vodní organizmy je často spojeno s toxikologickými testy. Jedním z možných modelů je chronický *in vivo* test. Tento typ experimentu zahrnuje sledování jedné nebo pouze několika málo vybraných sloučenin, jejichž efekt je následně vyhodnocován. Experimentální výstupy potom mohou zahrnovat determinaci bioakumulačního/biokoncentračního potenciálu polutantu, které vyžaduje kvantitativní stanovení analytu ve vybraných tkáních. LDTD-APCI v kombinaci s vysoko rozlišující hmotnostní spektrometrií se ukázalo být efektivní metodou, a to zvláště v kombinaci se zjednodušenou a účinnou extrakční metodou, původně vyvinutou pro extrakci 74 farmak z tkáně ryb. Tato metoda urychluje přípravu vzorků vynecháním purifikačních kroků, dále výrazně zkracuje celkový čas analýzy použitím přímé introdukce vzorku termální desorpcí (obvykle méně než 30 sekund na vzorek) a vysoko rozlišující hmotnostní spektrometr zajišťuje selektivitou potřebnou pro rozlišení cílových analytů v komplexní matrici.

Výsledky dosažené s LDTD-APCI prezentované v této dizertační práci jsou povzbudivé a podmětné k vývoji dalších pokročilých analytických přístupů pro environmentální výzkum.

Acknowledgements

I would like to express my deepest gratitude to my supervisor Assoc. Prof. Roman Grabic for his advices, always constructive criticism, patience, endless support and for taught me to be self-sufficient. Thank you Roman for show me the amazing world of analytical chemistry and turned my life upside down but in right direction.

I am grateful to head of our lab Prof. Tomáš Randák. He is always supportive and taking care about all laboratory members.

I owe a warm thanks to all my laboratory colleagues for made all aspects of my professional life easier and also for their friendship, namely Assoc. Prof. Vladimír Žlábek, Jitka Kolářová, DVM, Helena Švecová, Ph.D., Jan Turek, Ph.D., Kateřina Grabicová, Ph.D., Ganna Fedorova, Ph.D., Oksana Golovko, Ph.D.,. Viktoriia Burkina, Ph.D., Andrea Vojs Staňová, Ph.D., Daniel Červený, Ph.D., Petra Nováková, Dipl.-Ing. Marie Šandová, M.Sc. Mladen Avramovič, M.Sc. Tuyen Van Nguyen, Anna Koubová, Ph.D. and also to all members of Laboratory Aquatic Toxicology and Ichtyopathology.

I am indebted to Prof. Bryan W. Brooks and his team for gave me study opportunity during my internship in Baylor University, Waco Texas.

One more special thanks to Prof. Brooks, Andrea Vojs Staňová, Ph.D., and Kateřina Grabicová, Ph.D., for their advices and overall help with my scientific papers.

Warmest thanks go to my family and friends providing me mental support during my study.

I will never be able to handle that without support from all of you.

The thesis was financial supported from following sources:

- Czech Science Foundation (projects No. 17-08937S, 18-15802S, and 16-06498S),
- Ministry of Education, Youth and Sports of the Czech Republic – projects „CENAKVA" (No. LM2018099); "CENAKVA Center Development (No. CZ.1.05/2.1.00/19.0380)"; "CENAKVA" (No. CZ.1.05/2.1.00/01.0024), and "CENAKVA II" (No. LO1205 under the NPU I program),
- Grant Agency of the University of South Bohemia (No. 061/2019/Z, No.097/2017/Z, and 012/2016/Z).

List of publications

Peer-reviewed journal with IF

Borik, A., Staňová, A.V., Brooks, B.W., Grabicová, K., Randák, T., Grabic, R., 2020. Determination of citalopram in fish brain tissue: benefits of coupling laser diode thermal desorption with low- and high-resolution mass spectrometry. Anal. Bioanal. Chem. 412, 4353–4361. (IF 2019 = 3.637)

Borik, A., Vojs Stanova, A., Kodesova, R., Brooks, B.W., Grabicova, K., Novakova, P., Grabic, R., 2020. Ultrafast laser diode thermal desorption method for analysis of representative pharmaceuticals in soil leachate samples. Talanta 208, 120382. (IF 2019 = 5.339)

Mordačíková, E., Vojs, M., Grabicová, K., Marton, M., Michniak, P., Řeháček, V., **Bořík, A**., Grabic, R., Bruncko, J., Mackuľak, T., Vojs Staňová, A., 2020. Influence of boron doped diamond electrodes properties on the elimination of selected pharmaceuticals from wastewater. J. Electroanal. Chem. 862, 114007. (IF 2019 = 3.807)

Grabicova, K., Vojs Staňová, A., Koba Ucun, O., **Borik, A**., Randak, T., Grabic, R., 2018. Development of a robust extraction procedure for the HPLC-ESI-HRPS determination of multi-residual pharmaceuticals in biota samples. Anal. Chim. Acta 1022, 53–60. (IF 2017 = 5.123)

Šauer, P., **Bořík, A**., Golovko, O., Grabic, R., Staňová, A.V., Valentová, O., Stará, A., Šandová, M., Kocour Kroupová, H., 2018. Do progestins contribute to (anti-)androgenic activities in aquatic environments? Environ. Pollut. 242, 417–425. (IF 2017 = 4.358)

Šauer, P., Stará, A., Golovko, O., Valentová, O., **Bořík, A**., Grabic, R., Kroupová, H.K., 2018. Two synthetic progestins and natural progesterone are responsible for most of the progestagenic activities in municipal wastewater treatment plant effluents in the Czech and Slovak republics. Water Res. 137, 64–71. (IF 2017 = 7.051)

Abstracts and coference proceedings

Borik, A., Chronakova, A., Grabic, R., Kodesova, R., 2019. Combination of high throughput target analysis by laser diode thermal desorption with screening analysis of irbesartan and its degradation products. ICCE 2019, Greece Thessaloniki.

Borik, **A.,** Vojs Stanova, A., Grabicova, K., Randak, T., Grabic, R., 2018. Application of LDTD/TQMS-HRMS methods for quantification of psychoactive compounds in tissue microsamples. 22nd International Mass Spectrometry Conference. 26–31 August 2018, Florence, Italy.

Vojs Staňová, A., Medvecká, E., **Bořík, A.,** Grabic, R., Škvára, P., Mackuľak, T., Marton, M., Vojs, M., 2018. Identification of electrochemical degradation products of selected pharmaceuticals by high resolution mass spectrometry. 22nd International Mass Spectrometry Conference. 26–31 August 2018, Florence, Italy.

Borik, A., Grabicova, K., Randak, T., Grabic, R., 2017. Application of LDTD/TQMS-HRMS methods for quantification of psychoactive compounds in tissue microsamples. ICCE 2017, Norway, Oslo.

Training and supervision plan during study

Name	Adam Bořík
Research department	Laboratory of Environmental Chemistry and Biochemistry FFPW
Supervisor	Assoc. Prof. Roman Grabic
Period	October 2015 – September 2020

Ph.D. courses	**Year**
Basic of scientific communication	2016
Aquatic toxicology	2016
Applied hydrobiology	2016
Ichtiology and systematic of fish	2016
English language	2019

Scientific seminars	**Year**
Seminar days of RIFCH and FFPW	2015 2016 2017 2018

International conferences	**Year**
Borik, A., Chronakova, A., Grabic, R., Kodesova, R., 2019. Combination of high throughput target analysis by laser diode thermal desorption with screening analysis of irbesartan and its degradation products. ICCE 2019, Greece Thessaloniki. (Poster presentation)	2019
Borik, A., Vojs Stanova, A., Grabicova, K., Randak, T., Grabic, R., 2018. Application of LDTD/TQMS-HRMS methods for quantification of psychoactive compounds in tissue microsamples. 22nd International Mass Spectrometry Conference. 26–31 August 2018, Florence, Italy. (Poster presentation)	2018
Borik, A., Grabicova, K., Randak, T., Grabic, R., 2017. Application of LDTD/TQMS-HRMS methods for quantification of psychoactive compounds in tissue microsamples. ICCE 2017, Norway, Oslo. (Poster presentation)	2017

Internship	**Year**
Baylor University, Waco Texas USA, Department of environmental science January – April 2018	2018

Curriculum vitae

PERSONAL INFORMAITON

Surname: Bořík
First name: Adam
Title: Dipl.-Ing.
Born: 26. 1. 1990, Mělník, Czech Republic
Nationality: Czech
Contact: aborik@frov.jcu.cz

PRESENT POSITION

Researcher and Ph.D. student in the Laboratory of Environmental Chemistry and Biochemistry University of South Bohemia in Ceske Budejovice Faculty of Fisheries and Protection of Waters, Research Institute of fish Culture and Hydrobiology

EDUCATION

2010–2013 Bachelor's degree – University of South Bohemia in Ceske Budejovice, Faculty of Fisheries and Protection of Waters

2013–2015 Master's degree – University of South Bohemia in Ceske Budejovice, Faculty of Fisheries and Protection of Waters

2015–2020 Ph.D. study – Laboratory of Environmental Chemistry and Biochemistry, University of South Bohemia in Ceske Budejovice, Faculty of Fisheries and Protection of Water

Ph.D. COURSES

Basic of scientific communication, Aquatic toxicology, Applied hydrobiology, Ichtiology and systematic of fish, English language

KNOWLEDGE OF LANGUAGES

Czech- native speaker, English- B2

FOREIGN STAY DURING Ph.D.

Baylor University, Waco Texas USA, Department of environmental science Team of Prof. Bryan W. Brooks. (Passive sampling in hurricane impacted area) January–April 2018