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Evaluation of the matrix effect of different sample matrices for 33 pharmaceuticals by post-column infusion

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ABSTRACT

Matrix effects that occur during quantitative measurement by liquid chromatography mass spectrometry specifically when using electrospray ionization are a widely recognized phenomenon. Sample matrix compounds affect the ionization process of the target analytes, lead to a low signal response, and flawed analytical results. How these matrix compounds directly influence the ionization process has not yet been completely understood. In the present study, we determined the matrix effect for 33 pharmaceutical substances in sample extracts of urine, plasma and wastewater. Most of the investigated substances were subject to a signal suppression effect. Only for a small subset of the compounds we detected a signal enhancement effect. We investigated the matrix effect profiles in detail to disentangle the influence of different matrices and to correlate the impact of specific components and groups of the analyzed extract in suppressing or enhancing effects in the profile.

Most signal suppression effects were detected in the first half of the chromatographic run-time for the matrix extracts of urine and wastewater. The observed effects are caused by high mass flow of salts and other diverse matrix components that were contained in high concentrations in those biological matrices. We also found signal suppression in the matrix effect profile of plasma samples over a wide time range during the chromatographic separation that were associated with a high content of triglycerides of diverse carbohydrate chain lengths. Here, we provide a broader picture of how 33 substances were influenced during analysis. Our results imply that a high number of the investigated substances had comparable effects of matrix compounds, despite differences in their chemical structure.

1. Introduction

The analysis of pharmaceutical residues in biological and environmental samples has become a major objective in many biological and water chemical research areas [1]. Mainly, these analyses are performed using the LC–MS/MS technique because of its high accuracy and reproducibility [2,3]. However, during the measurement, the ionization of the target analytes is affected by matrix compounds which are dissolved within the sample extracts. Different ionization techniques (e.g., electro spray ionization (ESI) or atmospheric pressure chemical ionization (APCI)) respond differently in dependence to the matrix components [4–7]. The signal intensity can be suppressed by high mass flows and co-elution of specific compounds, e.g., high concentrations of sugars, proteins, lipids, salts, amines, glycopeptides, phosphocholines or metabolites of the targets. Alternatively, the signal can be enhanced by the accumulation of positively charged ions or by neutralizing charge of the target molecules [8–13]. Moreover, system variables like mobile phase additives, solid phases for extraction or analyte derivatives influence the mechanism of ionization [14–18]. The mechanisms, by which these matrix compounds and variables influence the analytes, are not properly understood, but we understand that they are prone to ionization by ESI [4,7]. First reports based on LC-MS/MS procedures described the quantification of the so-called matrix effect (ME), using techniques such as post-extraction addition or the post-column infusion [19–22]. Those methods enable us to systematically evaluate the matrix effect and to study the influence

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on the instrumental measurement system can. Likewise, the efficiency of the sample preparation procedure can be determined. Many sample species of biological origin such as urine, plasma, oral fluids or food have been probed for their matrix impact on the measurement. Others have evaluated the matrix effect focusing on different plant matrices [23,24]. Stahnke et al. showed that the ME differed less than 10% in suppression effects for 80% of the 129 studied pesticide substances [23].

However, the above mentioned studies provided no insights into the correlation between the dependence between matrix influence and compounds. In those studies [23,24], the ME was investigated with different substances but the authors failed to connect the ME to the chemical structure of the individual target analyte.

To reduce the matrix influence, protein participation, solid phase extraction (SPE), liquid–liquid extraction, sample dilution or flow reduction are used for sample purification [24–29]. The matrix evaluation by Kittlaus et al. [24] demonstrated the beneficial effect of multiple sequential sample preparation steps. However, sample clarification could not completely remove the matrix influence on the measurement [17,21,24,27,30,31]. Due to incomplete removal of matrix compounds, methods for quantification have been implemented to compensate the occurring matrix impact, for example the use of isotopically labeled internal standards.

The aim of this study was to determine the ME that occurred during the measurement of biological and environmental samples by LC–MS/MS using positive ESI. The study was carried out through post-column infusion of 33 pharmaceuticals of different action groups featuring diverse chemical structures. The selected matrices were SPE-extracts of urine, plasma and wastewater samples. The wastewater samples were composite samples of the communal influent and effluent of the sewage treatment plant (STP) Dresden Kaditz, Germany and industrial sewage water of a pharmaceutical producer.

Furthermore, in this study we identified mechanisms of the ME. Thus, we focused our evaluation of the matrix effect on compounds with signal enhancement. The subsets of investigated drugs consisted of β-blocker nadolol, the structurally related atenolol, the metabolite of the anticonvulsant oxcarbazepine, the mono-hydroxy-derivate (MHD) and the structurally related anticonvulsant carbamazepine, the anticonvulsants gabapentin and pregabalin, and the antibiotics clindamycin and sulfamethoxazole.

To our knowledge, the present study is one of the first investigations on the origins of matrix effects that relates the influence of the functional groups of the target analytes using ESI-LC–MS/MS. We show substantial differences in the matrix profiles for the tested compounds in urine, plasma and urban wastewater matrix. For these matrices, the matrix effects were predominantly induced by high mass flows of matrix components. Matrix effects induced by chemical interactions were detected only for several target analytes. The understanding of the underlying causes of the matrix effect will enable novel developments in purification steps and higher sensitivity for the detection in derivatisation applications of the analyzed targets.

2. Materials and methods

2.1. Chemicals, pharmaceutical standards and standard mixtures

Acetonitrile (ACN, HPLC-grade), methanol (MeOH, HPLC-grade) and ammonium acetate (p.A.) were purchased from Merck (Darmstadt, Germany), formic acid (conc., LC–MS grade) and disodium ethylenediamine tetraacetate (Na_2EDTA, ACS reagents) from Sigma (St. Louis, MO, USA) and water (HPLC-grade) from VWR (Darmstadt, Germany). ACN, 2 mM ammonium acetate solution and formic acid were used to prepare solvent A (3/97/0.05, v/v/v) and B (95/5/0.05, v/v/v), respectively, which were used for the preparation of the pharmaceutical working solution and as mobile phase for LC–MS/MS.

The 33 selected pharmaceuticals, the corresponding chemical information and the producer are listed in Table 1. The selection of the 33 pharmaceutical substances was based on a previous method development for the residue analysis of central nervous active substances out of wastewater (WW) samples. This selection was enlarged by antibiotic, antifugal and beta blocker substances that circumstantially occur in WW.

The stock solution (1 mg/mL) of every substance was prepared in methanol and stored at −20 °C for up to three months. For the post-column infusion and sample spiking, a standard mixture of all pharmaceuticals was prepared (1 μg/mL) in solvent A/B (50:50; v/v) and stored for four weeks at 4–8 °C.

2.2. Samples

Sample matrices of plasma, urine and 24 h composite WW were selected for the evaluation. Every matrix type was prepared at least twice (No 1. and No 2.) to ensure a confirmed effect for the selected matrix. Sample extracts were prepared at different days and for different sampling dates.

The WW samples were collected from January to May 2013 from communal and industrial sewage influent at the STP Dresden-Kaditz and its effluent to the river Elbe. The wastewater samples were supplemented with 10 mM Na_2EDTA-solution to a final 0.8 μg/mL concentrated sample solution, centrifuged at 6000 × *g* for 5 min, filtered with a glass fiber filter (<0.7 μm, WICOM, Heppenheim, Germany) and adjusted with formic acid to pH 3.5 prior to analysis. Plasma and urine samples were diluted 10 and 40 times and adjusted with formic acid to pH 3.5. An aliquot of 2.5 mL of the adjusted samples was taken for preparation with SPE. Each sample was run in triplicates.

2.3. Sample matrix preparation

Samples were prepared with a SPE procedure, which was developed for antibiotic substances in sewage water [32]. The sample cleanup was performed with 30 mg Oasis HLB Vac cartridges (Waters, Milford, MA, USA), using the automatic sample processor Abimed ASPEC XL (Gilson, Middleton, WI, USA). The cartridges were conditioned with 1 mL methanol/10% formic acid (9:1), 1 mL water and 1 mL of 10 mmol/L Na_2EDTA-solution. Afterwards, the sample aliquot was loaded and washed with 1 mL water. The substances were eluted with 1 mL of methanol/10% formic acid (9:1) and evaporated until completely dry in a gentle air stream at 60 °C. The dry extracts were redissolved in a total volume of 250 μL solvent A/B (50:50; v/v).

2.4. Instrumentation

The chromatographic system UltiMate® 3000 Intelligent LC series, containing binary pump, vacuum solvent degasser unit and sample injector (Thermo Scientific Dionex, Idstein, Germany) was selected for chromatographic separation and controlled with a Chromeleon Chromatography Data System (Dionex Softron, Idstein, Germany). The column oven (Shimadzu, Kyoto, Japan) was maintained at 40 °C. The post-column infusion was operated with a P680HPLC Pump of Dionex (Thermo Scientific Dionex, Idstein, Germany). An ABSciex quadrupole mass spectrometer (Sciex API4000, ABSciex, Framingham MA, USA) was used as analytical detector. The system was interfaced by a Z-Spray ESI source that operated in the positive ionization mode. The system was controlled with Analyst 1.6 software.