Journal of Chromatography A, xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Journal of Chromatography A

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

Post-column infusion of internal standard quantification for liquid chromatography-electrospray ionization-tandem mass spectrometry analysis – Pharmaceuticals in urine as example approach

Julia Rossmann[a,*], Lars David Renner[b], Reinhard Oertel[a], Ali El-Armouche[a]

[a] Institute of Clinical Pharmacology, Medical Faculty Carl Gustav Carus, Dresden University of Technology, 01307 Dresden, Germany
[b] Leibniz Institute of Polymer Research Dresden and the Max Bergmann Center of Biomaterials, 01069 Dresden, Germany

ARTICLE INFO

Article history:
Received 21 October 2017
Received in revised form
21 December 2017
Accepted 1 January 2018
Available online xxx

Keywords:
Post-column-infusion quantification
Electrospray ionization mass spectrometry
Internal standard
Urine
Matrix effect

ABSTRACT

Liquid chromatography with electrospray ionization tandem mass spectrometry (LC-ESI–MS/MS) technique is gaining more and more attraction as the method of choice for multi-sample analysis. However, it is strongly susceptible to the influence of matrix components. Matrix effects are the main source of substantial losses in detection sensitivity and have to be compensated via complex quantification methods In this work, we introduce a sophisticated quantification method for the LC-ESI–MS/MS analysis of 16 substances in urine samples using a single continuously post-column infused internal standard (PCI-IS) for matrix effect correction. The performance of the introduced technique was proven by the simultaneous quantification using internal standards. Our results demonstrate that a single post-column infused internal standard suffices to analyze multiple target analytes. The introduced method is a new approach to analyze complex matrices and represents a powerful alternative to the classic internal standard methodology. The proposed technique significantly reduces the required steps for sample preparation, costs of additional stable isotopically-labeled internal standards, and self-induced matrix effects.

1. Introduction

Liquid chromatography coupled with tandem mass spectrometric detection and electrospray ionization (LC-ESI–MS/MS) is the method of choice for rapid drug analysis in biological and environmental samples [1,2]. However, the sensitivity of the method is strongly affected by variations in sample composition [3], sample preparation [4–6] and the type of ionization [7,8]. Common indicators for occurring matrix effects (ME) during MS measurement are reductions in signal intensity, accuracy and reproducibility. The presence of matrix compounds can result in either lower or higher ionization rates of the target analytes, which implies that the actual analyte concentration is inaccurately determined. The influence of matrix compounds on the molecular detection in MS is not completely understood; however, it is clear that those effects are prone to electrospray ionization [2,9,10]. In the last decades, other contributing factors during ESI have been examined, such as enhancing and suppressing effects by the sample matrix [11–15], solvent additives [16–18] electrospray source design [19], ionization mode [20], mobile phase composition [21,22] and chromatographic techniques [23–25]. However, also internal standards (IS) can be common sources of interferences, for example isotopically labeled analog internal standards (SIL-IS) of the target analytes [26–28].

In order to compensate for MEs, alternative methods for quantification have been developed, e.g. the external matrix matched calibration (external calibration), standard addition method, or the use of IS or SIL-IS. While external matrix matched calibration does not compensate for the individual sample matrix and standard addition method is induced with higher sample numbers and volume, the use of analog SIL-IS is currently the method of choice to compensate for ME and quantitative analysis [29]. The method is based on the assumption that analyte and analog SIL-IS have the highest probability of similar physical and chemical properties, and thus share comparable ME.

However, limited availability and high costs of analog SIL-IS led to quantify several analytes by one SIL-IS or IS substances with comparable behavior in response and retention time that are not present in the actual sample [30,31]. However, even minimal differences in retention time, even of the SIL-IS analog of the analyte, can in some cases result in different ME and cause substantial problems during quantification [32–37]. It has been conclusively shown

* Corresponding author at: Fiedlerstraße 46, 01307 Dresden, Germany.
E-mail address: julia.rossmann@tu-dresden.de (J. Rossmann).

https://doi.org/10.1016/j.chroma.2018.01.001
0021-9673/

that increased concentrations and variety of target molecules and IS during calibration and in sample solutions can strongly affect the ionization efficiency due to self-induced ME which can ultimately result in the loss of the linear relationship [26–28,37].

To avoid the above-mentioned disadvantages, a new technique was introduced: post-column infusion of IS (PCI-IS). The PCI-IS method was first described by Choi et al. for the quantification of the insecticide tebufenozide and the corresponding metabolite hydroxyl-tebufenozide by the post column infused isotopically labeled analog tebufenozide [^{13}C] in extracted wheat hay samples. They succinctly showed that PCI-IS provides the opportunity to quantify analytes independent of elution times [38]. Another study–exemplifying the high similarity of the ME for more than 100 pesticides–showed that ME were predominantly depending on retention time [39]. Therefore, the applicability of the PCI-IS correction by a single monitor substance was shown, however it did not result in the development of a defined methodology. Gonzales et al. developed a matrix compensation method by using eight isotopically labeled PCI-ISs targeting 18 different drugs (i.e. β-blockers, hypertension drugs, analgesics, lipid-lowering drugs, opioid, antidiabetic and antidepressant) at a concentration range 3–1000 mg/L in urine sample matrices [28]. The effectivity of this approach was rather low as eight PCI-ISs were required to quantify 18 drugs and they did not clearly demonstrate the suitability of the selected PCI-IS. Liao et al. adapted the PCI-IS technique to successfully analyze six benzodiazepines (10–500 μg/L) in urine samples and to analyze eleven amino acids (3–200 mg/L) in plasma matrices [40,41], showing an improvement using a single PCI-IS at low infusion concentrations (10–100 ng/min) for quantification. In our method development, we aimed to further reduce the selected SIL-IS in a multi-target analytical method in the lower ng/L range [31]. Previously, we found high comparability of signal suppression for more than 20 pharmaceuticals in different wastewater, urine and plasma matrices [42].

In this work, we decided to use a urine matrix to establish an PCI-IS approach using two isotopically labeled analogs for 16 analyte substances (antidepressants, anticonvulsants, neuroleptics, two metabolites and one antibiotic) with comparable ME profiles that can be successfully separated and detected at low concentration (ng/L) within three minutes. Our PCI-IS method showed vast improvements in accuracy compared to the quantification by IS and SIL-IS. The successful development strongly suggests that the PCI-IS method is useful to investigate large numbers of analytes using few PCI-IS in the presence of complex ME and that it is suitable for detecting trace levels during high-throughput analyses.

2. Experimental section

2.1. Targets, standards and chemicals

To develop the PCI-IS method, we have chosen 14 pharmaceuticals that are active compounds of anticonvulsants, antidepressants, neuroleptics (antipsychotic drugs), antibiotic and two related metabolites of Fluoxetine and Venlafaxine.

The selected substances and 11 SIL-ISs, the corresponding chemical information and their manufacturer are listed in Table S1. Stock solutions (1 mg/mL) of each target substance were prepared in methanol or purchased directly from the producer and stored at −20 °C for up to twelve months, until a decrease in the response was detected. The SIL-ISs were purchased at 1 mg/mL or 0.1 mg/mL stock solution and stored at −20 °C for up to twelve months. Acetonitrile (Merck, Darmstadt, Germany), 2 mM ammonium acetate solution (Merck, Darmstadt, Germany) and formic acid (Sigma, St. Louis, MO, USA) (conc.) 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 working solution and as mobile phase for LC-ESI–MS/MS. For ME profiles, sample spiking and calibration solutions, a standard mixture containing all target analytes was prepared (1 μg/mL) in solvent A and B (A/B; 50:50; v/v) and stored for up to four weeks at 4–8 °C. We used the same procedure for the SIL-ISs. For the PCI-IS method, Venlafaxine d6 and Trimipramine d3 (Cerilliant, Round Rock, TX, USA) were combined in one infusion solution while the concentrations were adjusted at 1.25 ng/mL for Venlafaxine d6 and at 2.5 ng/mL for Trimipramine d3 in solvent A/B (50:50). Information on used additives and chemicals are given in the supporting information.

2.2. Sample preparation and solid phase extraction

Urine samples were received from healthy, non-medicated volunteers. The urine samples were diluted 5-, 10- and 20-fold and were adjusted to pH 3.5 with formic acid prior to solid phase extraction (SPE) procedure or standard spiking. For the study of recovery (RE) and process efficiency (PE) the water samples were also adjusted to pH 3.5 with formic acid prior to standard spiking and SPE procedure.

The applied SPE procedure was modified from a prior sample preparation method for antibiotic substances detection in sewage water [43]. 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). More detailed information on the SPE procedure is described in the supporting information. The SPE extracts were dried in an air stream at 60 °C and dissolved in a final volume of 250 μL solvent A/B (50:50; v/v) for LC–MS/MS analysis.

2.3. Continuous post-column infusion methodology and data processing

The post-column infusion was operated with a P680 HPLC pump (Thermo Scientific Dionex, Idstein, Germany). An ABSciex quadrupole mass spectrometer (Sciex API4000, ABSciex, Framingham MA, USA) was used as detector. The system was interfaced by an orthogonal ESI source that operates in the positive ionization mode. The system was controlled with Analyst 1.6 software. Quantification by SIL-IS, external calibration methodology, and calculated target analyte area for PCI-IS method were prepared with the MultiQuant software version 3.0. Detailed information on the chromatographic system, the reversed phase gradient, the MS system and detection parameters (Table S2 and Table S3) can be found in the supporting information.

For PCI-IS correction and ME visualization, the listed signal intensity data information of the infused PCI-ISs was exported of the Analyst software. The data points of the signal intensity of the infused substances were smoothed by building the mean value of five consecutive data points. For the so-called ME profile the signal intensity of the injected sample matrix was normalized to the signal intensity of the injection of pure solvent and plotted versus the corresponding chromatographic run time.

For the PCI-IS method the intensities of the PCI-IS were summarized for a time window that was adjusted to the peak width of the corresponding target analyte. The area of the analyte was divided by the sum of the PCI-IS intensity.

A standard calibration in 10-fold diluted urine was used for the quantification by linear regression of target analyte concentration and the individual ratio of the target analyte area to PCI-IS intensity sum. Finally, the derived function of the linear regression was applied to quantify unknown samples by their ratio of target analyte area to PCI-IS intensity sums that were similarly affected by ME.

Please cite this article in press as: J. Rossmann, et al., Post-column infusion of internal standard quantification for liquid chromatography-electrospray ionization-tandem mass spectrometry analysis – Pharmaceuticals in urine as example approach, J. Chromatogr. A (2017), https://doi.org/10.1016/j.chroma.2018.01.001