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Ionisation efficiencies can be predicted in complicated biological matrices: A proof of concept

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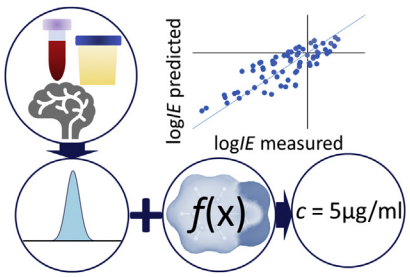
HIGHLIGHTS

- 10 compounds were analysed in neat solvent, blood, plasma, urine, cerebrospinal fluid, brain and liver tissue homogenates.
- Assuming equal ionisation efficiencies lead to mismatch of 660 times between actual and predicted ESI/MS responses.
- Ionisation efficiencies were predicted via charged delocalisation and degree of ionisation.
- Ionisation efficiencies allowed reducing prediction mismatch to 8 times.

GRAPHICAL ABSTRACT



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ABSTRACT

The importance of metabolites is assessed based on their abundance. Most of the metabolites are at present identified based on ESI/MS measurements and the relative abundance is assessed from the relative peak areas of these metabolites. Unfortunately, relative intensities can be highly misleading as different compounds ionise with vastly different efficiency in the ESI source and matrix components may cause severe ionisation suppression. In order to reduce this inaccuracy, we propose predicting the ionisation efficiencies of the analytes in seven biological matrices (neat solvent, blood, plasma, urine, cerebrospinal fluid, brain and liver tissue homogenates). We demonstrate, that this approach may lead to an order of magnitude increase in accuracy even in complicated matrices. For the analyses of 10 compounds, mostly drugs, in negative electrospray ionisation mode we reduce the predicted abundance mismatch compared to the actual abundance on average from 660 to 8 times. The ionisation efficiencies were predicted based on i) the charge delocalisation parameter *WAPS* and ii) the degree of ionisation α, and the prediction model was subsequently validated based on the cross-validation method 'leave-one-out'.

1. Introduction

Mass spectrometry (MS) coupled to electrospray ionisation (ESI) is intensively used for the analysis of pharmaceutical drugs in biological matrices [1]. The ability to analyse samples almost

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directly with direct infusion [2] or flow injection experiments [3] coupled with high-resolution MS has provided a tremendous increase in sample throughput. This technique has proven useful for the analyses of a wide range of samples from human blood plasma [4] to historic wines [5] to ecological samples [6]. For quantitative analysis, nevertheless, standard substances are required due to the large differences in ionisation efficiencies observed in ESI/MS [7–10]. Unfortunately, standard substances are often not available for metabolites and degradation products; therefore, knowing or predicting the ionisation efficiency of these compounds would be extremely useful for estimating their relative importance.

Several research groups have demonstrated that ionisation efficiencies can be correlated with various molecular properties of the compound (pK_a [11,12], log*P* [12,13], surface area [12], charge delocalisation [14,15], gas-phase proton affinity [16,17], etc.). Additionally, our group has shown that measured ionisation efficiencies are transferable from one setup to another [18] and from one solvent system to another [19,20]. Based on these outcomes different models predicting ionisation efficiencies have been developed [10,14,19,21–23]. These models use analytes physicochemical parameters and solvent properties as input parameters. Most commonly used physicochemical parameters are related to the hydrophobicity (log*P*, *WAPS*, *WANS*, C/H ratio) and ionizability of the analyte (pK_a, the degree of ionisation α, etc). We have lately shown [14] that ionisation efficiency can be predicted with high accuracy in ESI negative mode via the degree of charge delocalisation (*WAPS*) and degree of ionisation in solution (α). This approach has been applied for 62 compounds in 10 different solvent systems and serves, therefore, as a good starting point for analysis of complex samples.

In spite of significant research carried out in the field these approaches have remained inapplicable for biological sample analysis. So far, all research groups have predicted ionisation in solvent mixtures without the presence of matrix compounds. However, most analyses are performed in complex matrices. Matrix compounds may significantly decrease or increase the ESI/MS signal of the compound of interest [24,25], this effect is known as matrix effect. The decrease of the signal is much more common and even though the mechanism of ionisation suppression is not completely clear, several trends have been identified. Firstly, matrix effect is expected to arise from the competition of compounds for the surface charge in the ESI droplets [26]. The more hydrophobic the matrix compounds are, the more efficient they are in occupying the droplets' surface and, therefore, these compounds are expected to cause more ionisation suppression [20]. Secondly, the presence of non-volatile solutes causes a severe decrease in ESI/MS response via precipitation of the analyte on the ESI interface [27]. Lastly, gas phase charge transfer from the analyte to matrix components may alter analyte signal [28]. These effects are expected to be even more pronounced for measurements carried out without any or with minimal chromatographic separation [29].

In order to be of practical value for real sample analyses, the ionisation efficiency models should be able to account for the matrix effect. Therefore, it needs to be evaluated whether ionisation efficiency models can also be constructed in matrices relevant for real sample analyses. Based on the previously obtained promising results for ESI negative mode in various solvents we aim to go one step further by predicting the ionisation efficiencies for analysis in biological matrices. Therefore, the aim of this paper is to study whether ionisation efficiencies in ESI negative mode can be predicted in biological matrices (plasma, urine, whole blood, cerebrospinal fluid (CSF), liver and brain tissue homogenates). For this purpose, ionisation efficiency values of 10 compounds, predominantly pharmaceuticals, were measured in different biological matrices with flow injection analyses. The ionisation efficiency model was fitted in each matrix. We use the worst-case scenario, a simple protein precipitation sample preparation without any chromatographic separation of the analyte and matrix compounds, as a proof of concept that ionisation efficiencies can be predicted under severe matrix effect conditions. The method is cross-validated by the 'leave-one-out' validation method.

2. Experimental section

2.1. Compounds and sample pretreatment

Lincomycin hydrochloride (purity ≥95%), dodecanoic acid and fumaric acid (both ≥99%) were obtained from Sigma (Steinheim, Germany) and warfarin (≥99%) from DuPont Pharma (Wilmington, DE, USA). Naproxen (≥98%) was obtained from Synthex Research Center (Edinburgh, UK) and taurocholic acid sodium salt hydrate (≥95%) from Acros Organics (Geel, Belgium). Salicylic acid, benzoic acid and sorbic acid (all ≥99%) were obtained from Reakhim (Moscow, Russia), and 3-[(trifluoromethyl)sulphanyl]benzoic acid (3-CF_3SO_2-benzoic acid, purified by recrystallisation) is a kind gift from prof. L. M. Yagupolskii. Dilution of the samples was performed on pipetting instrument Freedom EVO (TECAN, Switzerland). The structures are shown in Supporting Information.

Liver and brain tissue, urine, and blood from a healthy dog (beagle) were obtained from in-house sources at Janssen Pharmaceutica (Beerse, Belgium), plasma and CSF of a healthy dog (beagle) were obtained from Bioreclamation IVT, USA. For brain and liver tissue, 1 part of tissue was homogenised with 9 parts of MilliQ water to form tissue homogenates. Biological matrices were stored frozen at −20 °C, except for blood which was used fresh (within 2 h). For plasma and blood K_2EDTA was used as anticoagulant. A neat solvent which was a solution of 20/80 0.1% ammonia solution/acetonitrile was used as an example of a simple matrix. Ammonia solution (25% puriss) was obtained from Lach:Ner, Czech Republic and acetonitrile (LC grade) from Merck, Darmstadt, Germany. The mobile phase directed to ESI/MS consisted also of 20/80 0.1% ammonia solution/acetonitrile.

A simple standard protein precipitation sample preparation was carried out: 50 μL of the stock solution of the compound was added to a mixture of 400 μL of acetonitrile and 50 μL of biological matrix (plasma, urine, whole blood, cerebrospinal fluid (CSF), liver or brain tissue (1 part of tissue homogenised with 9 parts of water)). This mixture was thoroughly mixed and centrifuged for 10 min at 13 000 g. The supernatant (injection volume 5 μL) was used for MS analysis. Linear range was 1–200 μM depending on the compound and matrix; the exact concentrations are described in Supporting Information.

2.2. Ionisation efficiency measurements

Ionisation efficiencies were measured in flow injection mode with an Accela liquid chromatograph (Thermo Fisher Scientific, San Jose, USA) coupled with an LTQ ion trap (Thermo-Fisher Scientific, San Jose, USA) mass spectrometer. All measurements were carried out in ESI negative MS scan mode. Sheath gas flow rate 35 psi, auxiliary gas flow 10 a. u., sweep gas flow rate 5 a. u. spray voltage −3.5 kV, and capillary temperature 275 °C were used. The flow rate was 0.2 mL/min.

The measurement of absolute ionisation efficiencies is complicated; therefore, we measured relative ionisation efficiencies (RIE). In order to provide ionisation efficiency values comparable to previous and upcoming studies, all values are provided relative to benzoic acid. The logarithmic ionisation efficiency (log*IE*) of benzoic acid in 20/80 0.1% ammonia solution/acetonitrile has been previously taken as 0 [14].

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