Journal of Pharmaceutical and Biomedical Analysis 127 (2016) 49–59

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

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

Comparison of a triple-quadrupole and a quadrupole time-of-flight mass analyzer to quantify 16 opioids in human plasma

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ARTICLE INFO

Article history:
Received 28 October 2015
Received in revised form
27 December 2015
Accepted 29 December 2015
Available online 3 January 2016

Keywords:
Opioids
UHPLC–MS
Triple-quadrupole mass analyzer (QqQ)
Quadrupole time-of-flight mass analyzer (QToF)
Accuracy profile
Matrix effects

ABSTRACT

The aim of this work is to study whether a quadrupole time-of-flight (QToF) mass analyzer, coupled to an ultra high performance liquid chromatography (UHPLC) system, can be a valuable alternative for a triple-quadrupole (QqQ) mass analyzer, for quantitative toxicological purposes. The case study considered was the quantification of 16 opioids (6-monoacetylmorphine, buprenorphine, codeine, dihydrocodeine, ethylmorphine, fentanyl, hydrocodone, hydromorphone, morphine, norbuprenorphine, norcodeine, norfentanyl, oxycodone, oxymorphone, pholcodine and tilidine) in human plasma. Both methods were validated in parallel in terms of selectivity, matrix effects, extraction recovery, carry-over, bias, precision and sensitivity. Accuracy-profile methodology was used to determine the optimal calibration model, and to estimate bias, repeatability, intermediate precision and total error. Selectivity was demonstrated for all opioids and deuterated analogues, except for codeine-d3 on the UHPLC-QTOF. For most compounds, extraction recoveries were in the range 60 to 80% on both systems, except for the synthetic analogues, buprenorphine, fentanyl and tilidine, where large variability is observed. Carry-over was negligible on both systems. For different opioids, the optimal calibration model was different between the systems. The accuracy profiles of the majority of the opioids indicated that, over the entire tested concentration range, for more than 5% of the future measurements, total errors are expected to exceed the a priori defined 15% acceptance limit. For some exceptions, however, the measurements even suffer from total errors above 30%, which can be attributed to the solid phase extraction procedure that was applied as sample pretreatment technique. Sensitivity was generally tenfold better on the LC-QToF system, probably due to the difference in ion choice for quantification between both systems. In conclusion, the best performing system seemed to depend on the compound, on the parameter and even on the concentration. Accuracy profiles clearly provided valuable information complementary to that obtained in classical validation tests, and therefore preferably are taken into account when deciding on a method's performance.

1. Introduction

In the last decade, ultra high performance liquid chromatography–tandem mass spectrometry (UHPLC–MS/MS) became a popular analysis technique for targeted screening and quantitation of drugs and drugs of abuse in a clinical toxicological laboratory. Often, a triple-quadrupole (QqQ) MS, applied in multiple-reaction-monitoring (MRM) mode [1–9], is considered as perfect work horse for quantitative analysis, because of its wide dynamic range and good sensitivity. Methods involving very simple [1–3] to rather extensive (automated) [4–9] sample preparation steps have been reported for several types of biological matrices [1–9].

Modern evolutions, both in terms of UHPLC instrumentation and hybrid MS, have led to the introduction of new and improved equipment on the market, e.g. UHPLC quadrupole time-of-flight (QToF) MS. Along with the instrumental evolutions, new acquisition modes, such as MS^E, were introduced. In MS^E mode, both low and high fragmentation data are obtained, providing full scan and

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http://dx.doi.org/10.1016/j.jpba.2015.12.055
0731-7085/

fragmentation information from the analytes of interest [10]. This mode is usually applied for both targeted and untargeted screening.

In this paper, the performance of a UHPLC–QToF, used in MS^E mode is evaluated for the quantification of 16 opioids and metabolites in human plasma. The opioids of interest are 6-monoacetylmorphine (6-MAM), buprenorphine, codeine, dihydrocodeine, ethymorphine, fentanyl, hydrocodone, hydromorphone, morphine, norbuprenorphine, norcodeine, norfentanyl, oxycodone, oxymorphone, pholcodine and tilidine. A UHPLC–MS/MS method, which is currently used in the toxicology laboratory of the UZ Brussel for the targeted screening of 16 opioids and metabolites in human plasma, is taken as case study. This method includes a solid phase extraction (SPE) step and deuterated analogues are used as internal standards (IS). Method validation experiments were performed in parallel on a UPLC–QqQ MS/MS (system I) and on a UPLC–QToF MS/MS (system II). Selectivity, matrix effects, extraction recovery, accuracy, precision, carry-over and sensitivity were compared [11–16]. The accuracy-profile methodology proposed by Hubert et al. [17–20] was used to determine the optimal calibration model and to estimate the total error. Accuracy profiles were developed for both systems covering therapeutic and toxic plasma concentrations [21–25]. These profiles enable an easy graphical interpretation, indicating in which concentration range the 95% confidence interval of the total error of the measurements is acceptable [17–20].

In the literature, several research groups have focused on the comparison between QqQ and QToF quantifications in different matrices [10,26–30]. Concerning quantitative analysis of pharmaceuticals in plasma, Peng et al. [26] have compared determinations of the antiviral drug 2′-deoxy-2′-β-fluoro-4′-azidocytidine using QToF (in rat plasma) and QqQ (in dog plasma), and validated both methods in terms of specificity, recovery, matrix effects, linearity, limit of quantification, precision, accuracy and stability. Garcia-Villalba et al. [27] compared QToF and QqQ analyses of a selection of urolithins in plasma (and other biological matrices) in terms of selectivity, recovery, matrix effect, linearity, sensitivity, precision, and stability. Rosano et al. [10] have compared quantifications using a QqQ in MRM mode and a QToF in MS^E mode for a selection of 17 drugs and metabolites, including six opioids. Selectivity, extraction recovery, matrix effects, precision and bias were addressed for codeine, dihydrocodeine, hydrocodone, hydromorphone, morphine, oxycodone and oxymorphone. Being the most relevant paper in terms of comparison of both systems for opioids, the paper of Rosano et al. [10] was taken as a reference. No problems in terms of selectivity/specificity were reported. Extraction recoveries for the opioids determined using the QToF ranged between 60 and 95%. The matrix effect was expressed as (area spiked post extraction/area neat standards $-1) \times 100\%$, and ranged for the QToF within -20 and +10%. Interday precisions, expressed as RSD%, ranged from 2.5 to 10%, and from 2.5 to 11% for the QqQ and QToF, respectively. The accuracies ranged for the opioids from -15 to 20% for the QqQ and from -10 to 15% for the QToF. In terms of sensitivity, the authors reported LODs of 10 μg/L and LOQs of 25 μg/L. The general conclusion was that quantitative assays on both systems were acceptable and comparable.

In the above mentioned published comparison studies, the analytical methods have been properly validated and compared. However, method validation applying accuracy profiles [17–20] is an upcoming trend nowadays. It is able to detect problems in analytical methods that are not revealed with classic validation protocols [11–14]. To the best of our knowledge, total-error approaches and accuracy profiles have not been used in previous comparison studies. In this current study, this methodology was additionally addressed and used to compare the QqQ and QToF determinations.

2. Materials and methods

2.1. Standard solutions

2.1.1. Stock solutions

Certified 1 g/L standard solutions in methanol or acetonitrile of codeine, dihydrocodeine hydrochloride, hydrocodone, hydromorphone, morphine, norcodeine, norfentanyl and oxycodone were bought from LGC (Teddington, United Kingdom), of buprenorphine hydrochloride, fentanyl and pholcodine monohydrate from Lipomed (Arlesheim, Switzerland), and of 6-MAM, ethylmorphine and oxymorphone from Cerilliant (Round Rock, Texas, USA). Standard solutions in methanol or acetonitrile containing 0.1 g/L free base of codeine-d3, dihydrocodeine-d6, morphine-d6 and tilidine were purchased from LGC; of norbuprenorphine from Lipomed; and of 6-MAM-d6, buprenorphine-d4, fentanyl-d5, hydrocodone-d3, hydromorphone-d6, norbuprenorphine-d3, oxycodone-d6 and oxymorphone-d3 from LGC. All above stock solutions were stored at −20 °C.

2.1.2. Working solutions

2.1.2.1. Solutions used to develop accuracy profiles.

2.1.2.1.1. Opioid mixture solutions. On each of three days, five identical opioid mixture solutions, containing the opioids of interest, were prepared by diluting the stock solutions, in 0.1% aqueous formic acid, FA (UPLC/MS grade, Biosolve, Dieuze, France). The mixture solution contained fentanyl, norbuprenorphine, norfentanyl and tilidine in concentrations of 1 mg/L, while those of the other opioids were 10 mg/L. This tenfold concentration difference was applied for all mixture solutions throughout the study.

2.1.2.1.2. Calibration standards working solutions. On each of three days, two series (prepared from two opioid mixture solutions) of calibration standards working solutions were prepared with concentrations of 0.05, 0.1, 1, 5 and 10 mg/L in 0.1% aqueous FA.

2.1.2.1.3. Validation standards working solutions. On each of three days, three series (each from one of the opioid mixture solutions) of validation standards working solutions were prepared with concentrations of 0.5, 2.5 and 7.5 mg/L in 0.1% aqueous FA.

2.1.2.2. Internal standards mixture. Deuterated analogues were used as internal standards (IS). The IS stock solution contained 1 mg/L 6-MAM-d6, buprenorphine-d4, codeine-d3, dihydrocodeine-d6, hydrocodone-d3, hydromorphone-d6, morphine-d3, norbuprenorphine-d3, oxycodone-d6, oxymorphone-d3, and 0.5 mg/L fentanyl-d5 in methanol. The deuterated IS used for each opioid are listed in Table 1.

2.1.2.3. Spiked plasma standards. Plasma calibration standards (CS) and validation standards (VS) were prepared by spiking 900 μL blank plasma, obtained from healthy volunteers with 100 μL working solution. CS were prepared at five concentration levels: 5, 10, 100, 500 and 1000 μg/L, and a blank. VS were prepared at three concentration levels: 50, 250 and 750 μg/L.

2.2. Sample pretreatment

2.2.1. Reagents

For the solid phase extraction process, phosphate (0.1 M; pH 6.0) and acetate (0.1 M; pH 4.0) buffers were prepared. They were prepared using MilliQ water (Millipore, Overijse, Belgium), sodium hydroxide (Anala R Normapur, Leuven, Belgium), sodium azide (Sigma), potassium dihydrogenium phosphate p.a. (Merck, Darmstadt, Germany) and glacial acetic acid (Anala R Normapur). The SPE eluent, prepared ex tempore, was a mixture of 2-propanol, 28% ammonia solution (both Anala R Normapur) and dichloromethane