Journal of Chromatography B, 959 (2014) 36–41

Contents lists available at ScienceDirect

Journal of Chromatography B

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

Small molecule adduct formation with the components of the mobile phase as a way to analyse valproic acid in human serum with liquid chromatography-tandem mass spectrometry

Marek Dziadosz*, Michael Klintschar, Jörg Teske

Institute of Legal Medicine, Hannover Medical School (MHH), Carl-Neuberg-Str. 1, D-30625 Hannover, Germany

ARTICLE INFO

Article history:
Received 17 January 2014
Received in revised form 25 March 2014
Accepted 27 March 2014
Available online 4 April 2014

Keywords:
Valproic acid
Adduct formation
Liquid chromatography
Mass spectrometry

ABSTRACT

A valproic acid (VPA) LC-MS/MS analytical method using analyte adduct formation was developed and validated in human serum. The fragmentation of the sodium acetate adduct (mass transition: 225/143) and acetic acid adduct (mass transition: 203/143) were used as the target and qualifier mass transition, respectively. A Luna 5 μm C18 (2) 100 A, 150 mm × 2 mm analytical column and a mobile phase consisting of A (H_2O/methanol = 95/5, v/v) and B (H_2O/methanol = 3/97, v/v), both with 10 mM ammonium acetate and 0.1% acetic acid (pH = 3.2) were used. A binary flow pumping mode with a total flow rate of 0.4 mL/min was applied. Protein precipitation with 1 mL of the mobile phase B was used as sample preparation. The calculated limit of detection/quantification was 0.45/1.0 μg/mL and the inter-/intra-day precision was <6%. The application of a deuterated internal standard resulted in a good adduct formation reproducibility. The strategy applied made the VPA LC-MS/MS analysis in human serum on the basis of two mass transitions possible. Therefore, it is an interesting alternative for the VPA pseudo multiple reaction monitoring methods (mass transition 143/143) and a proof that the developed strategy is also useful for the analysis of compounds which do not produce any stable ion fragments detectable by tandem mass spectrometry.

1. Introduction

Valproic acid (VPA) is a small molecule used as an antiepileptic agent in the treatment of primary generalised, partial and myoclonic seizures [1]. The simple chemical structure of this aliphatic compound (Fig. 1) is a challenge for the detection with tandem mass spectrometry. Its acidic nature is the reason why the ionisation in the negative multiple reaction monitoring (MRM) mode in analyses performed in different matrices is favoured. However, since no stable ion fragments are created during VPA ionisation the developed LC-MS/MS analytical methods are run as pseudo MRM methods with a pseudo mass transition (143/143) [2–7]. A different strategy was presented by Cheng et al. [8]. This method based on a pre-column derivatisation with 4-dimethylaminobenzylamine dihydrochloride. A positive electrospray (ESI) ionisation and multiple reaction monitoring were used. Since the derivatisation step was performed in 1 h, the sample preparation in the procedure developed by Cheng et al. was more time consuming, in comparison to the validated pseudo MRM methods [2–7]. However, the possibility to use a mass transition on the basis of VPA derivative fragmentation (277/120) was an advantage for the valproic acid detection by tandem mass spectrometry. Similar to other methods only one VPA mass transition could be used in the method validation [8].

In this work we investigated the valproic acid adduct formation with the components of the mobile phase. Further, our aim was also to examine the applicability of the VPA adduct ion fragmentation for its quantification in human serum by a developed and validated LC-MS/MS method. A valproic acid quantification method based on two real mass transitions would make a better drug identification possible. Therefore, it would be a benefit for its analysis in biological material (clinical applications) with this technique. It would be also an advantage for the field of the forensic toxicology, because fatal VPA intoxication cases occur [9]. Adduct formation is fundamental in the electrospray ionisation and leads to different ion peaks observed in the positive and negative ESI mode [10]. However, in MRM methods the $[M\text{-}H]^-$ ion (-ESI mode) and the $[M+H]^+$ adduct (+ESI mode) are most common used and other adduct ions are often ignored.

Adduct formation is based on complicated equilibria reactions between the analyte and its adducts. A main problem is the pro-

* Corresponding author. Tel.: +49 511 532 4558; fax: +49 511 532 5635.
E-mail addresses: Dziadosz.Marek@mh-hannover.de, analytiker@chemist.com (M. Dziadosz).

http://dx.doi.org/10.1016/j.jchromb.2014.03.033
1570-0232/

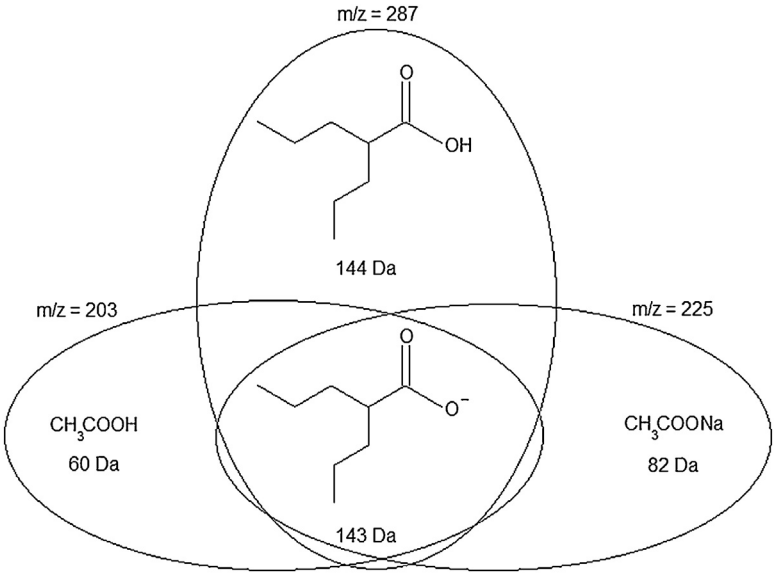

Fig. 1. VPA adduct formation with the components of the mobile phase.

cess reproducibility under certain analytical conditions, especially when one single ion adduct is used. Li et al. showed that the relative standard deviation could be improved from <17% to <6% when a summation of $[M+H]^+$, $[M+NH_4]^+$ and $[M+Na]^+$ adducts was performed [11]. However, adduct signal summation is inconvenient and additionally assumes that the response factor for all adduct ions is equal, which has not been demonstrated [12]. Application of a deuterated analyte as internal standard (ISTD) is a good alternative to minimise the process reproducibility problem, because similar adduct distribution can be expected for such ISTD. Generally, multiple adduct formation is categorised as a pitfall in liquid chromatography-tandem mass spectrometry. Different strategies used to minimise the problems concerned with this process were described elsewhere [13,14].

We previously developed a γ-hydroxybutyrate (GHB) quantification method in human serum which is based on adduct formation in the negative MRM mode [15]. Further, we described the analyte adduct formation process closer and announced the preparation of two publications which will describe the mentioned problems in detail [16]. One of the mentioned problems is the possibility to analyse drugs with LC-MS/MS which do not produce any stable ion fragments during electrospray ionisation-like VPA. In this work we wanted not only to improve the VPA analysis in human serum with liquid chromatography-tandem mass spectrometry by the use of two real mass transitions but also to prove if the developed adduct identification strategy is applicable for the analysis of other small drugs which do not produce stable ion fragments detectable by tandem mass spectrometry.

2. Materials and methods

2.1. Chemicals

All chemicals/solvents used were of analytical/LC-MS grade and purchased from: Biozol (Eching, Germany)—VPA, VPA-D6; Merck (Darmstadt, Germany)—ammonium acetate; Fluka (Steinheim, Germany)–H_2O, methanol; J.T. Baker (Deventer, Netherlands)—acetic acid. Blank human serum was purchased from the blood bank of the Hannover Medical School.

2.2. Equipment

An Applied Biosystems API 4000 QTrap tandem mass spectrometer with electrospray ionisation (ESI) and a Shimadzu UFLC Prominence System equipped with two solvent delivery units (LC-20AD), Communication Bus Module (CBM-20A), Autosampler (SIL-20AC HT), degasser (DGU-20 A3) and column oven (CTO-10 AS VP) were used for all purposes. Data acquisition and integration were performed by the Analyst 1.5 software.

2.3. Conditions

Chromatographic separation was performed with a Luna 5 μm C18 (2) 100 A, 150 mm × 2 mm column (Phenomenex, Aschaffenburg, Germany). A binary flow pumping mode with a total flow rate of 0.4 mL/min was used. The elution was performed with a mobile phase consisting of A (H_2O/methanol = 95/5, v/v) and B (H_2O/methanol = 3/97, v/v), both with 10 mM ammonium acetate and 0.1% acetic acid (pH = 3.2). The elution program was as follows: starting with 20% of eluent B, ramping to 80% B from 1.0 to 5.0 min, holding 80% B from 5.0 to 9.0 min, reducing to 20% B from 9.0 to 12.0 min, holding 20% B from 12.0 to 15.0 min. ESI-MS/MS was performed in the negative multiple reaction monitoring mode with the following source/gas parameters: curtain gas (N_2)—25 psi, collision gas (N_2)—medium, ion source gas 1 and 2 (N_2)—80 psi, temperature—200 °C, ion spray voltage—−4500 V. The pauses between mass ranges were set at 5 ms. A continuous infusion at a flow rate of 10 μL/min of each 0.1 and 10 μg/mL VPA/VPA-D6 in 80% B made the adduct identification and optimisation of the compound dependant ESI-MS/MS parameters for each target (T) and qualifier (Q) mass transition possible (Table 1).

2.4. Sample preparation and extraction procedure

A 10 mg/mL methanol VPA stock solution was used for the sample preparation. The appropriate dilutions in the range of 40–200 μg/mL were prepared in human serum. A 500 μg/mL VPA-D6 methanol solution was used as internal standard (ISTD). A volume of 10 μL of ISTD was added to a microcentrifuge tube with 100 μL of human serum spiked with VPA. After that, 1 mL of mobile phase B was added. The protein precipitation was performed by vigorous sample shaking for 15 min. After 5 min of centrifugation the