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Comparison of conventional liquid chromatography–tandem mass spectrometry versus microflow liquid chromatography–tandem mass spectrometry within the framework of full method validation for simultaneous quantification of 40 antidepressants and neuroleptics in whole blood

Andrea E. Steuer*,[1], Michael Poetzsch[1], Magdalena Koenig, Eva Tingelhoff, Sandra N. Staeheli, Andreas T. Roemmelt, Thomas Kraemer

Department of Forensic Pharmacology & Toxicology, Institute of Forensic Medicine, University of Zurich, Switzerland

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ABSTRACT

Microflow liquid chromatography (MFLC) coupled to mass spectrometry (MS) is claimed to improve analysis throughput, reduce matrix effects and lower mobile phase consumption. This statement was checked within the framework of method validation of a multi-analyte procedure in clinical and forensic toxicology employing MFLC–MS/MS and conventional LC–MS/MS. 200 μL whole blood were spiked with 50 μL internal standard mixture and extracted by protein precipitation. The concentrated extract was separated into two vials. One was analyzed using a Thermo Fisher Ultimate liquid chromatography system coupled to an ABSciex 5500 QTrap mass spectrometer (LC–MS/MS) and one by an ABSciex Eksigent Microflow LC system coupled to an ABSciex 4500 linear ion trap quadrupole MS (MFLC–MS/MS). Both methods were fully validated and compared in terms of selectivity, stability, limits, calibration model, recovery (RE), matrix effects (ME), bias, imprecision and beta tolerance interval for 40 antidepressants and neuroleptics including 9 metabolites. Both methods had comparable LODs, LOQs and calibration models with some exceptions. The MFLC system showed slightly higher coefficients of variation (CVs) in the RE experiments. ME were reproducible in both systems but with lower CVs in the conventional LC system. Acceptance criteria for imprecision and bias were fulfilled for 32 analytes on the LC and for 28 analytes on the MFLC system. Beta tolerance intervals indicated better reproducibility in terms of narrower intervals for the conventional LC system. The advantages of the MFLC system were low mobile phase consumption, short run time, and better peak separation. The systems were comparable in terms of peak interference, LOD, ME, bias and imprecision. The advantages of the conventional LC system were more data points per peak, linear calibration models, stable retention times and better beta tolerance intervals. Due to higher robustness, the conventional LC system was finally chosen for routine application in forensic toxicology.

1. Introduction

Conventional liquid chromatography (LC) with flow rates of 200–1000 μL/min coupled to different kinds of mass spectrometric (MS) apparatus are nowadays the most commonly used analytical techniques in clinical and forensic toxicology. Scaling down flow rates to nano- or microflow LC (MFLC) systems lead to certain benefits like reduced solvent consumption, higher throughput through decreased run times, a higher ionization yield, and reduced ion suppression/enhancement effects [1,2]. However, in the field of clinical and forensic toxicology such methods are currently scarce, possibly because these techniques are suspected to lack ruggedness [2]. Only tetrahydrocannabinol (THC), vitamin D, and methotrexate methods have been described [1–5]. Only two studies aimed to actually compare standard validation parameters such as limits of detection (LODs) and quantification (LOQs), linearity, matrix effects, bias and imprecision for both systems. For analysis of methotrexate

* Corresponding author at: Department of Forensic Pharmacology & Toxicology, Institute of Forensic Medicine, University of Zurich, Winterthurerstrasse 190/52, CH-8057 Zurich, Switzerland. Tel.: +41 446355679; fax: +41 446356852.
E-mail address: andrea.steuer@irm.uzh.ch (A.E. Steuer).
[1] These authors contributed equally to this work.

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as a single analyte method, MFLC was shown to be accurate and precise comparable to conventional LC and about 14 times more sensitive [2]. Reduction of matrix effects and improved precision compared to conventional LC were observed studying four analytes in one method [1]. However, in these two methods only one (methotrexate) or four analytes (alprazolam, clopidogrel, buspirone, and terfenadine) were evaluated.

In recent years there is a trend in clinical and forensic toxicology towards simultaneous quantification of a variety of compounds in one analytical run (multi-analyte procedures). These procedures allow faster and more effective analyses e.g. if only a limited amount of sample is available and the number of different analytes is unknown in the beginning of a typical analysis in clinical and forensic toxicology [6–14]. Antidepressant and neuroleptic drugs are widely used in the treatment of psychiatric diseases such as depression, mood and anxiety disorders, and schizophrenia. Due to steadily increasing prescription of these drugs, their analysis within the framework of different questions in clinical and forensic toxicology such as therapeutic drug monitoring, driving under the influence of drugs (DUID), influence at the time of an offence, poisoning and lethal overdoses is becoming more and more important. Several methods using conventional LC–MS/MS in different matrices such as serum, plasma or whole blood have been published in the literature [15–23].

Therefore the aims of the present study were firstly to develop and validate accurate, precise and robust methods for the quantification of 40 antidepressant and neuroleptic drugs in whole blood based on fast and simple protein precipitation, and secondly to evaluate the usefulness of MFLC–MS/MS compared to conventional LC–MS/MS in terms of standard method validation parameters, especially matrix effects, LODs, bias and imprecision.

2. Material and methods

2.1. Chemicals and reagents

Methanolic or acetonitrilic solutions (1 mg/mL) of agomelatine, amisulpride, amitriptyline, nortriptyline, amitriptyline oxide, aripiprazole, bupropion, hydroxybupropion, chlorprothixene, citalopram, clomipramine, norclomipramine, clozapine, norclozapine, doxepine, nordoxepine, duloxetine, fluoxetine, norfluoxetine, flupentixol, haloperidol, imipramine, levomepromazine, mirtazapine, olanzapine, opipramol, paroxetine, perazine, pipamperone, promazine, quetiapine, risperidone, 9-hydroxyrisperidone, sertraline, trazodone, trimipramine, venlafaxine, O-desmethylvenlafaxine, and zuclopenthixol and methanolic solutions of the deuterated internal standards (IS, 0.1 mg/mL) amisulpride-d5, aripiprazole-d8, citalopram-d6, clomipramine-d3, duloxetine-d3, fluoxetine-d6, paroxetine-d6, quetiapin-d8, risperidone-d4, trazodone-d6, trimipramine-d3, and venlafaxine-d6 were obtained from AdipoGen (Liestal, Switzerland), Lipomed (Arlesheim, Switzerland), LGC (Wesel, Germany), and Cerilliant (delivered by Sigma-Aldrich, Buchs, Switzerland). Water was purified with a Purelab Ultra millipore filtration unit (Labtech, Villmergen, Switzerland) and acetonitrile of HPLC grade was obtained from Fluka (Buchs, Switzerland). All other chemicals used were from Merck (Zug, Switzerland) and of the highest grade available.

2.2. Biosamples

Human blank whole blood samples were used for method development and validation and were obtained from healthy volunteers. Authentic blood samples from DUID, other criminal offence and postmortem cases were submitted to the authors' laboratory by the local police and by the state attorneys. Three proficiency tests (TCA, TDMA, TDMD) were obtained from the society of toxicological and forensic chemistry.

2.3. Sample preparation

Whole blood samples were extracted by protein precipitation (PP). Briefly, to 200 μL whole blood, 50 μL of the IS-mixture (amisulpride-d5 200 ng/mL, aripiprazole-d8 250 ng/mL, citalopram-d6 80 ng/mL, clomipramine-d3 200 ng/mL, duloxetine-d3 75 ng/mL, fluoxetine-d6 300 ng/mL, norfluoxetine-d6 300 ng/mL, haloperidol-d4 10 ng/mL, paroxetine-d6 80 ng/mL, quetiapine-d8 450 ng/mL, risperidone-d4 30 ng/mL, trazodone-d6 1200 ng/mL, trimipramine-d3 150 ng/mL, venlafaxine-d6 300 ng/mL), 50 μL spiking solution A (MeOH) and 50 μL spiking solution B (ACN) were added. PP was performed by slow addition of 600 μL acetonitrile during vortexing. The mixture was vortexed, shaken for 10 min, centrifuged (5 min, 10,000 rpm) and 550 μL of the supernatant were transferred into an autosampler vial. After addition of 50 μL formic acid (20%, v/v) the supernatant was evaporated to dryness under a gentle stream of nitrogen at 40 °C, reconstituted in 200 μL of a mixture of eluent A and B (85:15, v/v), separated into two vials and analyzed by LC–MS/MS and MFLC–MS/MS as described in Sections 2.4.1 and 2.4.2, respectively.

2.4. Apparatus and procedure for LC–MS/MS

2.4.1. Apparatus and procedure for LC–MS/MS

The analysis was performed using a Thermo Fischer Ultimate 3000 UHPLC system (Thermo Fisher, San Jose, California, USA) coupled to an ABSciex 5500 QTrap linear ion trap quadrupole mass spectrometer (ABSciex, Darmstadt/Germany).

The LC settings were as follows: Phenomenex (Aschaffenburg, Germany) Synergy Polar RP column (100 mm × 2.0 mm, 2.5 μm), gradient elution with 50 mM ammonium formate buffer in water containing 0.3% (v/v) formic acid (pH 3.5, A) and acetonitrile containing 0.1% (v/v) acetic acid (B). The flow rate was 0.7 mL/min with the following gradient: start conditions 15% B for 1 min, 1–11 min to 70% B, 11–14 min to 90% B, hold at 90% B for 1 min, at 16 min reequilibrating to 15% B for 2 min. Injection volume was 10 μL.

The Turbo V ion source equipped with a stainless steel electrode (100 μm internal diameter) was operated in positive ESI mode with the following MS conditions: gas 1, nitrogen (50 psi); gas 2, nitrogen (60 psi); ion spray voltage, 5500; ion-source temperature, 450 °C; curtain gas, nitrogen (30 psi), collision gas, medium. The MS was operated in the scheduled multiple reaction monitoring (MRM) mode with an MRM detection window of 60 s and a target scan time of 1.2 s using 2–3 transitions for each analyte except for the ISs where 1 MRM transition was used. The MS settings for each analyte are given in Supplementary Data. The MS was controlled by analyst 1.5.2 software.

2.4.2. Apparatus and procedure for microflow-LC–MS/MS

The analysis was performed using an ABSciex Eksigent Microflow LC system (Redwood City, California, USA) coupled to an ABSciex 4500 QTtrap linear ion trap quadrupole mass spectrometer (ABSciex, Darmstadt/Germany).

The MFLC settings were as follows: Halo® Phenyl Hexyl column (50 mm × 0.5 mm, 2.7 μm), gradient elution with 10 mM ammonium formate buffer in water pH 3.5 (C) and acetonitrile containing 0.1% (v/v) acetic acid (D). The flow rate was 30 μL/min with the following gradient: start conditions 13% D for 0.4 min, 0.4–1.5 min to 30% D, 1.5–4 min to 37% D, 4–4.4 min to 80% D, hold at 80% B till 5 min. Reequilibrating is performed for 1 min before the next injection. Injection volume 5 μL.

The Turbo V ion source equipped with a hybrid electrode (50 μm internal diameter) was operated in positive ESI mode with the