Journal of Chromatography B, 1035 (2016) 104–110

Contents lists available at ScienceDirect

Journal of Chromatography B

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

Development and application of a multi-component LC–MS/MS method for determination of designer benzodiazepines in urine

Madeleine Pettersson Bergstrand[a,b,*], Anders Helander[a,b,c], Olof Beck[a,c]

[a] Department of Laboratory Medicine, Division of Clinical Pharmacology, Karolinska Institutet, Stockholm, Sweden
[b] Department of Laboratory Medicine, Division of Clinical Chemistry, Karolinska Institutet, Stockholm, Sweden
[c] Department of Clinical Pharmacology, Karolinska University Laboratory, Stockholm, Sweden

ARTICLE INFO

Article history:
Received 17 July 2016
Received in revised form 22 August 2016
Accepted 27 August 2016
Available online 19 September 2016

Keywords:
Designer benzodiazepines
LC–MS/MS
NPS
Urine
Drug testing

ABSTRACT

New psychoactive substances (NPS) have become an increasing drug problem in the past decade. For detection of NPS, new analytical methods have to be developed, and the methods also have to be updated regularly. This study aimed at developing a multi-component LC–MS/MS method for detection and quantification of 11 NPS of the benzodiazepine sub-class ("designer benzodiazepines") in urine specimens. The method involves dilution of urine with internal standard and hydrolysis of any glucuronide conjugated forms. Separation of the compounds was achieved on a BEH Phenyl column, followed by MS/MS detection in positive electrospray mode. Method validation was performed following the EMA guideline. The method was applied to study the occurrence of designer benzodiazepines in Sweden in 2014–2015, by analysis of 390 samples retrieved from a routine drug testing laboratory. In 40% of these samples, selected based on a positive immunoassay benzodiazepine screening but a negative MS confirmation for the standard set of prescription benzodiazepines, intake of designer benzodiazepines was revealed. These results stress the importance of using and updating confirmation methods to include the increasing number of designer benzodiazepines appearing on the NPS market.

© 2016 Elsevier B.V. All rights reserved.

1. Introduction

In a joint report from the European Monitoring Center for Drugs and Drug Addiction (EMCDDA) and Europol published in 2016, it was noted that a sub-class of the new psychoactive substances (NPS), benzodiazepines, has become an increasing drug problem [1]. EMCDDA first reported these substances as a separate class in a report from 2014 [2] and they are now generally referred to as "designer benzodiazepines". The first benzodiazepine reported as an NPS to the EMCDDA was phenazepam in 2007 [3] and the first substance reported that was not already a pharmaceutical product was pyrazolam [4], which was reported in 2012 [5].

Therapeutically, benzodiazepines are prescribed to treat several disorders, such as anxiety, insomnia and epilepsy [6]. However, it is well-known that caution must be taken since they can induce drug dependence [6]. Benzodiazepines are commonly misused together with other psychoactive drugs, e.g. opioids [7]. Patients undergoing substitution therapy for opioid dependence have been reported to co-administer benzodiazepines with methadone or buprenorphine, to achieve a heroin-like effect [7].

Abuse of the designer benzodiazepine etizolam has been reported as an increasing problem in Japan [8], Europe, and the USA [9]. Additionally, designer benzodiazepines have been reported in cases concerning driving under the influence of drugs [10] and in drug-related deaths [11–13], and classical benzodiazepines are also known to be used in drug related crimes [14]. Use of several designer benzodiazepines has been reported from the Swedish STRIDA project, which is aimed at investigating the occurrence of NPS use in the country and to assess the associated clinical symptoms and health risks [15].

For analysis of classical benzodiazepines, a common approach is to use initial screening by immunochemical assays followed by confirmation using methods based on mass spectrometry (MS). It was recently shown that also several designer benzodiazepines can be detected using commonly used immunoassays [16]. Furthermore, MS, and other techniques such as electron-capture detection, nitrogen-phosphorus detection, and UV detection, coupled to separation techniques such as gas and liquid chromatography (LC), have been used for analysis of designer benzodiazepines [4,17–33]. However, most methods were designed to measure only one or a few

* Corresponding author at: Department of Laboratory Medicine, Division of Clinical Pharmacology, Karolinska Institutet, Stockholm, Sweden.
E-mail address: madeleine.pettersson.bergstrand@ki.se (M. Pettersson Bergstrand).

http://dx.doi.org/10.1016/j.jchromb.2016.08.047
1570-0232/© 2016 Elsevier B.V. All rights reserved.

substances. In routine clinical toxicology, multi-component methods are necessary since a large number of analytes must be screened for in a short time.

The aim of this study was to develop and validate a multi-component LC–MS/MS confirmation method for detection and quantification of 11 designer benzodiazepines in urine; clonazolam, deschloroetizolam, diclazepam, etizolam, flubromazepam, flubromazolam, flutazolam, meclonazepam, nifoxipam, phenazepam, and pyrazolam. In addition, another aim was to apply the method for analysis of urine samples from a routine drug testing laboratory to obtain an indication of their use in Sweden.

2. Experimental

2.1. Chemicals

Methanol of LC–MS grade and ammonium acetate of ≥98% purity were obtained from Thermo Fisher Scientific (Waltham, MA, USA), formic acid for MS and *E. coli* ß-glucuronidase from Sigma-Aldrich (St. Louis, MO, USA), and acetonitrile of HPLC gradient grade, acetic acid and sodium hydroxide of pro analysis grade, and 25% ammonia solution from VWR (Radnor, PA, USA). Ultra-pure water (>18 MΩ/cm) was prepared in-house using a Milli-Q water purification system (Millipore, Bedford, MA, USA).

Clonazolam, deschloroetizolam, flubromazolam, meclonazepam, nifoxipam, and pyrazolam were obtained from Chiron AS (Trondheim, Norway) and diclazepam, etizolam, flubromazepam and phenazepam from LGC standards (Teddington, UK), all as 1 mg/mL solutions in methanol. Flutazolam was obtained as tablets (Swedish customs service; the identity was confirmed by NMR, and the assumed amount was 4 mg/tablet). Temazepam-d5 and estazolam-d5 (internal standards) were obtained as 100 μg/mL solutions in methanol from Sigma-Aldrich.

2.2. Preparation of solutions

A stock solution of flutazolam was prepared by dissolving a piece of the tablet in methanol to achieve a concentration of 100 μg/mL. The mixture was sonicated for 10 min, allowed to stand in room temperature overnight, and filtered through a 0.45 μm acrodisc filter (Gelman Sciences, Ann Arbor, MI, USA). The stock solution was stored at −20 °C.

Standard and quality control (QC) samples were prepared by first mixing all analytes in methanol at a concentration 50 times the final target concentration. The mixtures were then diluted with urine to achieve 9 concentration levels for the standard curve at 1, 2, 5, 10, 20, 40, 60, 80, and 100 times the lower limit of quantification (LLOQ), and 4 levels for the QC samples (1, 2, 50, and 75 times the LLOQ). The blank urine used to prepare standard and QC samples was first set to pH 6.0 using sodium hydroxide and acetic acid, filtered through a Whatman Quality 1 filter paper (VWR), and finally ultra-filtered through a 0.45-μm MF HA membrane filter (Merck, Darmstadt, Germany). The methanol mixtures containing all analytes and the urine standard and QC solutions were stored at −20 °C.

The internal standard (IS) solution was prepared by diluting methanol solutions of temazepam-d5 and estazolam-d5 with 10 mmol/L ammonium acetate buffer (pH 5.8) to achieve final concentrations of 20 ng/mL temazepam-d5 and 6.4 ng/mL estazolam-d5. The final IS solution in 10 mmol/L ammonium acetate buffer pH 5.8 was stored at 4 °C.

2.3. Instrumentation

Analyses were performed using an ACQUITY UPLC system with a Xevo TQ tandem mass spectrometer from Waters (Milford, MA, USA) operating in positive electrospray mode using an auto sampler set at 10 °C, a column oven set at 60 °C and needle washes consisting of 800 μL formic acid:acetonitrile/methanol:Milli-Q water (5:900:95, v/v/v, = strong needle wash) and 1200 μL mobile phase A (=weak needle wash).

Separations were performed using an Acquity UPLC BEH Phenyl column (1.0 × 50 mm; d_p 1.7 μm) with a 0.2 μm in-line filter from Waters. Mobile phase A consisted of 0.1% formic acid in Milli-Q water, mobile phase B of acetonitrile and the flow rate was 0.3 mL/min. The 3.10 min gradient was run in the following way: 3% B for 0.2 min; linear increase to 20% B over 0.5 min; linear increase to 25% B over 0.7 min; held at 25% B for 0.25 min; linear increase to 30% B over 0.29 min; held at 30% B for 0.25 min; linear increase to 40% B over 0.41 min; linear increase to 99% B over 0.04 min; held at 99% B for 0.05 min; back to 3% B over 0.01 min; and finally equilibrated at 3% B for 0.4 min.

The MS settings included a capillary voltage of 0.60 kV, extractor voltage of 3.00 V, source temperature of 120 °C, desolvation temperature of 500 °C, nitrogen desolvation gas flow of 1100 L/h, argon collision gas flow of 0.15 mL/min, and a dwell time of 0.01 s. The analyte specific MS settings are shown in Table 1. All data were recorded and processed using MassLynx V 4.1 SCN 901 software from Waters.

2.4. Urine samples

The urine samples used for this study were de-identified left-over aliquots among samples sent to the Department of Clinical Pharmacology, at Karolinska University Laboratory for routine drug testing. The samples were initially screened with the CEDIA benzodiazepine screening assay (Thermo Fisher Scientific), and all samples testing positive were confirmed using a routine LC–MS/MS method that covered the following benzodiazepines and metabolites: α-hydroxymidazolam, α-hydroxyalprazolam, α-hydroxytriazolam, oxazepam, lorazepam, desmethyldiazepam, temazepam, 7-aminonitrazepam, 7-aminoflunitrazepam, and 7-aminoclonazepam. The majority of urine samples (88%) had been stored for a maximum of 1 month at 4 °C and thereafter frozen at −20 °C, while the others were stored for a maximum of 4 months at 4 °C before being frozen.

A few samples were also obtained from the STRIDA project [15] and were frozen at −20 °C directly upon arrival to the laboratory.

2.5. Sample preparation

Samples were prepared for analysis by adding 170 μL IS solution followed by 30 μL ß-glucuronidase and 50 μL urine into a 1.2 mL auto-sampler vial. The vials were mixed for 10 s, allowed to stand in room temperature (≈25 °C) for 10 min, centrifuged at 4350 rpm for 5 min using a Heraeus Multifuge 3S centrifuge (Thermo Fisher Scientific), and finally transferred to the auto sampler for injection of 5 μL into the LC–MS/MS system. The following identification criteria were applied: a relative retention time within 1% of samples spiked with reference substance, and an ion ratio between two selected reaction monitoring (SRM) transitions according to published recommendations [34].

2.6. Method validation

Validation of the method was performed following the European Medicines Agency (EMA) guideline [35]. The limit of detection (LOD) was set as the concentration that had a signal-to-noise ratio (S/N) > 3 for both SRM transitions and fulfilled the identification criteria of the method for at least 67% of the samples in a dilution series obtained from the lower limit of quantification (LLOQ, CV ≤20% and

Download English Version:

https://daneshyari.com/en/article/5136687

Download Persian Version:

https://daneshyari.com/article/5136687

Daneshyari.com