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Determination of co-administrated opioids and benzodiazepines in urine using column-switching solid-phase extraction and liquid chromatography–tandem mass spectrometry

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ABSTRACT

Co-administration of opioids with benzodiazepines is very common around the world. A semi-automated method was developed for the determination of four opioids and two benzodiazepines as well as their metabolites (including glucuronide metabolites) in human urine, based on on-line column-switching-solid-phase extraction (CS-SPE) and liquid chromatography–tandem mass spectrometry (LC–MS/MS). The CS-SPE was performed by loading 200 μL of urine sample to an Oasis HLB cartridge. Detection was achieved using a LC–MS/MS system equipped with an electrospray ionization source (ESI). For unequivocal identification and confirmation, two selected reaction monitoring transitions were registered for each compound, and no co-elution of interferences was observed at the expected retention time. Significant ion suppressions were observed for most analytes during chromatographic runs, but isotope-labeled internal standards (ISs) were used and found to be useful to compensate for the determination error caused by the matrix effect. The assay's linearity ranged from 1–20 ng/mL to 800–1000 ng/mL for 23 compounds, except for lorazepam (LOR), whose linearity was in the range of 1–100 ng/mL. This method showed to be precise and accurate. The relative standard deviation (RSD) % values of within-run precision, between-run precision and total precision were not greater than 10.4% ($n=3$), 12.9% ($n=5$) and 15.1% ($n=15$), respectively. Accuracy values were in the range of 87.5–110%. Limits of detection (LODs) ranged from 0.2 ng/mL to 5 ng/mL, and limits of quantification (LOQs) ranged from 1 ng/mL to 20 ng/mL. The method was applied to the assay of 12 samples from forensic cases, which exemplified the co-administration of benzodiazepines (BZDs) by some heroin abusers. This method was of high sensitivity, selectivity and reliability, minimum sample manipulation, semi-automation, and fairly high throughput (analysis time per sample was 20 min). The method developed will be useful for the detection of co-administrated drugs and the study of the interactions of BDZs with opioids.

1. Introduction

Benzodiazepines (BZDs), such as diazepam, are prescription drugs that are used for the treatment of anxiety, sleep disorders, and drug and alcohol withdrawal symptoms [1]. Co-administration of BZDs with narcotic pain relievers is very common around the world, which can cause the most notable drug interaction and may result in overdose lethality due to severe respiratory depression [2–5]. For this population, drug screening should be conducted to assess the recent BZD and opioids use, which can help doctors to take better treatment approaches [6]. Besides clinical purpose, the results of identification and quantification of these commonly co-administered drugs and their metabolites could be served as

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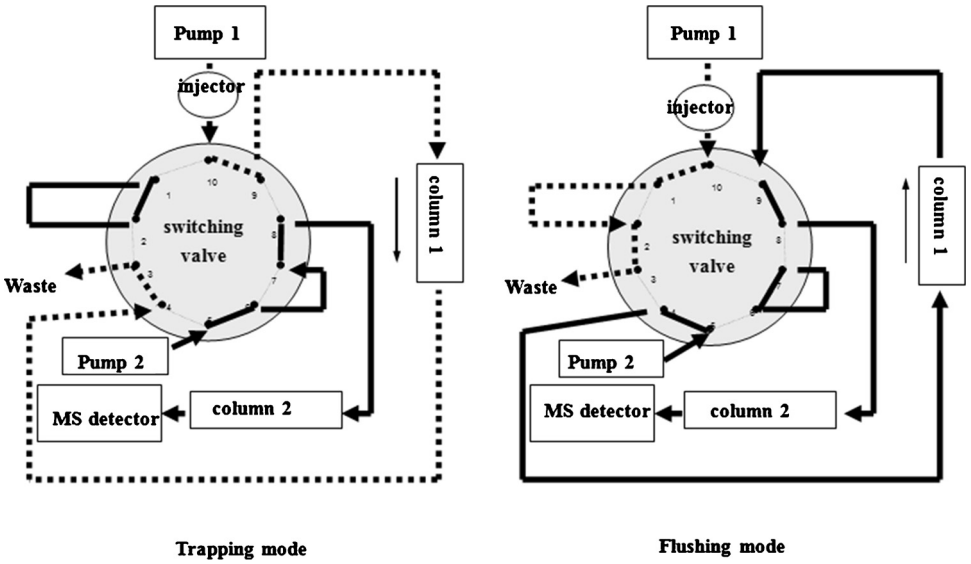

Fig. 1. Trap/flush configuration of the on-line column switching solid-phase extraction system.

a key factor in interpreting the causes of death for forensic purpose. For instance, co-administration of BZDs may play some role in morphine-associated death, and simultaneous determination of BZDs and morphine can provide useful information for case investigation [3,4,7].

Urine is always the first choice for the screening of abused drugs, providing higher concentrations and relatively longer detection window [8,9]. Immunoassay is an initial urine screening method adopted by many laboratories. For the confirmation another independent analytical method must be performed, such as gas chromatography–mass spectrometry (GC–MS) or liquid chromatography–mass spectrometry (LC–MS). Sample preparations are then subsequently required. Solid-phase extraction (SPE) exhibits superiority than liquid–liquid extraction, in terms of reduced matrix effects, high sensitivity and robustness. Specifically, basic, neutral, and weakly acidic drugs can be eluted using one SPE cartridge [10], making SPE very suitable for drug screening. But the reported off-line SPE methods can be time-consuming, labor-intensive, and thus resulting in long reporting time.

On-line column-switching SPE (CS-SPE) method can overcome the shortcomings of off-line SPE methods while highlighting its advantages [11]. The CS-SPE consists of two high-performance-liquid chromatography (HPLC) systems connected by a six- or ten-port switching valve (Fig. 1). In the first step, analytes of the interest are retained on column 1 (trapping column), whereas the matrix components can be washed off. In the second step, column 1 is switched on-line to column 2 (analytical column), where the analytes are separated in the isocratic or gradient mode [12].

Several methods using the CS-SPE method for the determination of BZDs and/or opioids in urine have already been published [13,14], as well as methods developed for the direct detection of the glucuronide metabolites of opioids [15,16]. However, hydrolysis procedure for the glucuronide metabolites of BZDs was still employed in the reported methods, which could lead to the change of the metabolite profiling, due to possibly incomplete hydrolysis of the glucuronide metabolites, and molecular structure conversion between drugs and/or metabolites [11,17].

Here a CS-SPE combined with liquid chromatography–tandem mass spectrometry (LC–MS/MS) based method was described for the simultaneous determination of four frequently prescribed opioids and two most commonly abused BZDs, including heroin, codeine, buprenorphine, tapentadol, diazepam, and lorazepam, as well as their glucuronide metabolites in human urine. The glucuronide conjugates were determined directly without chemical or enzymatic hydrolysis, which could provide accurate and reliable information of the metabolite profiling for clinical or forensic purpose.

To our knowledge, no CS-SPE combined with LC–MS/MS method for the direct identification and quantitation of the glucuronide metabolites of BZDs and opioids without hydrolysis has been presented in the literature. Hence, this method was novel, and the simple and semi-automated procedure allowed a quick start-up of assays for the analytes of interest. It was reliable for clinical and forensic investigations of drug abuse and the interaction of BZDs and opioids.

2. Materials and methods

2.1. Chemicals, reagents, and solutions

Oxazepam (OXA), morphine (MOR), O^6-acetylmorphine (6-MOR), O^3-acetylmorphine (3-MOR), codeine (COD), acetylcodeine (ACOD) were obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Lorazepam (LOR, 1.0 mg/mL), lorazepam-3-O-glucuronide (LORG, 100 μg/mL), nordiazepam (NDIA, 1.0 mg/mL), temazepam (TEM, 1.0 mg/mL), oxazepam glucuronide (OXAG, 100 μg/mL), temazepam glucuronide lithium salt (TEMG, 100 μg/mL), morphine-3-glucuronide (M3G, 1.0 mg/mL), morphine-6-glucuronide (M6G, 100 μg/mL), codeine-6-glucuronide (CODG, 100 μg/mL), buprenorphine (BUP, 100 μg/mL), norbuprenorphine (NBUP, 1.0 mg/mL), buprenorphine glucuronide (BUPG, 100 μg/mL), norbuprenorphine glucuronide (NBUPG, 100 μg/mL), tapentadol (TAP, 1.0 mg/mL), tapentadol-β-D-glucuronide (TAPG, 100 μg/mL), DIA-d_5 (1.0 mg/mL), OXA-d_5 (1.0 mg/mL), TEM-d_5 (1.0 mg/mL), OXAG-d_5 (100 μg/mL), NDIA-d_3 (1.0 mg/mL), MOR-d_3 (1.0 mg/mL), M3G-d_3 (100 μg/mL), M6G-d_3 (100 μg/mL), TAPG-d_3 (100 μg/mL), 6-MOR-d_3 (1.0 mg/mL), NBUP-d_3 (1.0 mg/mL), BUP-d_4 (100 μg/mL), COD-d_3 (1.0 mg/mL), NCOD-d_3 (1.0 mg/mL), LOR-d_4 (1.0 mg/mL), and TAP-d_3 (100 μg/mL) were all purchased from Cerilliant (Round Rock, TX, USA).

Ultra-pure water was prepared using an ELGA Labwater PURELAB Ultra system (Bucks, UK). HPLC-grade acetonitrile and methanol were purchased from Merck Co., Inc. (Darmstadt, Germany). HPLC-grade ammonium formate, formic acid and acetic acid were purchased from CNW Technologies GmbH (Düsseldorf, Germany). Analytical grade ammonia was purchased