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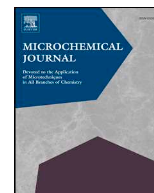
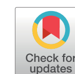


Screening and quantitation of forty-six drugs of abuse and toxic compounds in human whole blood by capillary electrophoresis: Application to forensic cases



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ABSTRACT

A novel capillary electrophoresis method, coupled with photo-diode array detection, was established for the analysis of forty-six drugs of abuse, including opiates, cocaine, amphetamines, benzodiazepines, and alkaloids, in human whole blood. Doxapram was used as the internal standard, and an Oasis HLB column was used for solid-phase extraction of drugs from human whole blood. This method was applied to analyze real blood samples in forensic cases. The separation was performed on a fused-silica capillary (75 μm I.D. × 50.2 cm, with 40 cm of effect length). In this study, the running buffer was 150 mM phosphate buffer (pH 2.4) containing 20% methanol. Samples were injected at a pressure of 0.5 psi for 10 s and separated at a running voltage of 16 kV. The precision, accuracy, sensitivity, linear range, limit of detection, and recovery of the method were evaluated. The time taken for the entire separation process was 18.5 min. All compounds were identified by relative retention times, and the peak height ratios (the ratio between the target analyte peak height and the internal standard peak height) were monitored at 200 nm and 210 nm in corresponding ultraviolet spectra. 210 nm was used as quantitative detection wavelength. Calibration curves were established for all compounds of interest. For human blood samples, the detection limits were 8–80 ng/mL, and the linear range was 0.03–10 μg/mL. The intraday and interday precisions were both less than 13%. This method offers simple and effective screening and quantitation of drugs of abuse and toxic compounds in human blood at toxicologically relevant concentrations, and was applied to analyze blood samples from forensic cases.

1. Introduction

Drugs of abuse are an increasing health and societal problem in China. Identification and quantitation of drugs of abuse and other toxic compounds in human whole blood are important facets of forensic toxicology [1–3].

Numerous analytical methods have been published for the analysis of only a few species of compounds [4]. Immunoassays are utilized as quick screening methods, but they lack specificity and only a limited number of drugs can be detected simultaneously [5–8]. Gas chromatography–mass spectrometry (GC/MS) is the most commonly used quantitative technique [9–12]. High-performance liquid chromatography–mass spectrometry (HPLC/MS) is also used for screening and quantitation [5,13–15]. Compared with these methods, capillary electrophoresis (CE) [16–21] and capillary electrophoresis–mass spectrometry (CE/MS) [22–25] have many advantages, such as high efficiency, fast analysis, low cost, ease of operation, and small sample volume. Many CE and CE/MS methods have been established to analyze drugs. Imran Ali et al. have reviewed the use of capillary electrophoresis, including analysis of anticancer drugs [19], chiral resolution of racemic environmental pollutants [26], pharmaceutical and biomedical analysis [20], and chiral separations of small peptides [27]. A simple and rapid

Abbreviations: CE, capillary electrophoresis; Rs, resolution; rtm, relative migration time; rh, the peak height ratio; PDA, photo-diode array; LOD, limit of detection; IS, internal standard; MDA, 3,4-Methylenedioxyamphetamine; MDMA, 3,4-Methylenedioxymethamphetamine

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capillary zone electrophoresis method was reported by Arin Gül Dal et al. to detect piroxicam in tablets. In-line solid-phase extraction-capillary electrophoresis coupled with mass spectrometric detection has been used for the determination of 2-ethylidene-1, 5-dimethyl-3, 3-diphenylpyrrolidine, codeine, hydrocodeine, and 6-acetylmorphine in urine. A method was developed for the screening of nineteen drugs of abuse in human urine by CE, with a sequential injection-solid-phase extraction system. Gianpiero Boatto et al. used CE with diode array detection to detect ten amphetamine designer drugs in human whole blood, and Wu and Tsai et al. analyzed morphine and morphine-3β-D glucuronide in human urine [17,22,28–30]. Hanwen Sun et al. used the solid-phase extraction–capillary electrophoresis to detect melamine residues in milk [31]. Qian Chen et al. detected clenbuterol and salbutamol from complex extracts of swine feed by capillary electrophoresis [32]. CE methods have also been developed for the determination of six quinolone residues in chicken, hen, and swine tissue samples, as well as lomefloxacin, gatifloxacin, enoxacin, ciprofloxacin, ofloxacin, enrofloxacin, and pefloxacin residues in porcine tissue [33,34]. CE methods have been applied in food samples successfully, and these methods have made great contributions to the study of drugs of abuse in blood.

Though there are many CE methods for the analysis of drugs of abuse in urine and food samples, few methods exist for the analysis of blood. Meanwhile, few methods have been developed to screen drugs, and most CE methods mainly focus on the same class of drug or a few compounds. Therefore, we proposed a CE method using photo-diode array (PDA) detection that can screen a wide range of drugs (46 compounds) in human whole blood. The analytes included opioids, cocaine, amphatamines, phenothiazines, tricyclic antidepressants, benzodiazepines, and alkaloids, which can be usually encountered in forensic practice.

The method presented in this study is capable of screening and quantifying 46 drugs of abuse and toxic compounds in human whole blood via CE coupled with PDA detection. We believe this method can improve the analysis of unknown compounds and be implemented as a novel screening and quantitation tool.

2. Experimental

2.1. Reagents and materials

The 46 target compounds (as listed in Table 1) and the internal standard, doxapram, were of pharmaceutical purity and purchased from China Pharmaceutical and Biological Products Institute (No.2 Tiantan Xili, Chongwen District, Beijing, China). All drugs applied in this study are listed in Tables 1 and 2. Ethyl acetate (Shanghai chemical reagent co. Ltd., China), methanol (Shanghai Fudan Chemical Factory Yonghua Reagent Factory, China), ammonium hydroxide (Taicang Yonghua Special Reagent Factory, China), hydrochloric acid (Jinshan Chemical Factory, China), phosphoric acid (Zhenya Chemical Factory, China), sodium dihydrogen phosphate (Shantou Jinshan Chemical Factory, China), sodium borate (Shanghai Lingfeng Chemical Reagent Co., Ltd., China), and boric acid (Shanghai Dongpu Chemical Reagent Company, China) were used. Distilled water was purified by a Milli-Q system (Millipore, Bedford, MA, USA). Oasis HLB columns (1 mL/30 mg) were purchased from Waters (Milford, MA, USA).

2.2. Instrumentation

CE experiments were performed with a P/ACE system MDQ (Beckman Instruments, Fullerton, CA, USA), equipped with a PDA detector The CE instrument was operated by a PC, with 32 karat System Software from Beckman Instruments. Fused-silica capillaries (75 μm I.D. × 375 μm O.D. × 50.2 cm length, effective separation length of 40 cm), obtained from Beckman Coulter, were employed in all experiments.

2.3. Standard solution preparation

Drugs of abuse and toxic compounds were prepared in methanol at a concentration of 1.0 mg/mL. Doxapram (IS) was prepared in methanol at a concentration of 1.0 mg/mL. A testing solution (0.05 mg/mL) containing a mixture of drugs and toxic compounds, including pholcodine, amphetamine, methamphetamine, 3,4-Methylenedioxyamphetamine (MDA), 3,4-Methylenedioxymethamphetamine (MDMA), pseudoephedrine, ephedrine, ketamine, pethidine, promethazine, imipramine, chlorprothixene, thebaine, codeine, morphine, O6-monoacetylmorphine, heroin, noscapinum, haloperidol, and diphenoxylate, was used to optimize the CE conditions.

2.4. Sample pretreatment

The solid-phase extraction (SPE) column was equilibrated with 1 mL of methanol, followed by 1 mL of water. Next, 0.5 mL of human whole blood was diluted with 1.5 mL of water (including 20 μL of phosphoric acid and 10 μL of doxapram) and was loaded onto the cartridge. Then, the cartridge was washed with 1 mL of water containing 2% NH_4OH and 5% methanol, followed by 1 mL of *n*-hexane and 1 mL of water with 5% methanol, successively. Next, 1 mL of ethyl acetate:MeOH (12:1) was used to elute target analytes. Twenty microliters of 1% HCl were added to the eluate, and the eluate was evaporated to dryness at 50 °C under air stream. Finally, the evaporated eluate was redissolved with methol:water (1:4).

2.5. CE conditions

The capillary thermostat was set at 25 °C. Samples were injected at a pressure of 0.5 psi for 10 s. The running buffer was 150 mM phosphate (pH 2.4) with 20% methanol.

Prior to use, the capillary was rinsed with 1 M NaOH, water, and running buffer for 10 min each. Prior to each sample injection, the capillary was flushed with the running buffer for 3 min. At the end of each experiment, the capillary was rinsed with 1 M NaOH and water for 10 min each.

All compounds could be identified by relative retention times, and the normalized peak height (the ratio between the target analyte peak height and the internal standard peak height) was monitored at 200 nm and 210 nm in corresponding ultraviolet spectra.

2.6. Application to real blood samples

The developed analytical method was applied to two human blood samples from forensic cases, which were obtained from Shanghai Key Laboratory of Crime Science Evidence.

3. Result and discussion

3.1. Optimization of CE operating conditions

3.1.1. Optimization of the running buffer

In CE separations, the separation buffer pH influences the net charge of target analytes [24]. Phosphate was used as the buffer system for alkaline drugs in order to ionize the drugs under acidic conditions. In this study, separation efficiency with buffer pH ranging from 4.0–2.2 was investigated. As the pH decreased, the separation efficiency between the compounds changed. Fig. 1 shows the changes in resolution (Rs) of drugs in the testing solution with different pH values. At pH 2.4, the separation efficiency of each compound was optimal.

In addition, buffer concentration is an important factor in improving resolution and analytical sensitivity [35]. In this study, the concentration of phosphate was optimized using concentrations ranging from 50 mM to 200 mM. As phosphate concentration increased, the

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