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A quick method for determination of psychoactive agents in serum and hair by using capillary electrophoresis and mass spectrometry

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ABSTRACT

The aim of the research was to develop a new sensitive method for simultaneously the determination of psychoactive drugs: 1-benzylpiperazine, 7-aminoclonazepam, alprazolam, clonazepam, diazepam, estazolam, lorazepam and tetrazepam in human serum and hair samples. In the preparation step, microwave-assisted extraction (MAE) was used. Extracts were analyzed by means of capillary electrophoresis with mass spectrometry time-of-flight detection (CE-TOF-MS). In the validation study of the MAE/CE-TOF-MS analytical method, three concentration levels of analytes (10, 100 and 250 ng/mL for serum and 0.2, 2.2 and 5.6 ng/mL for hair) were taken into account. Such parameters as limit of detection (0.4–1.2 ng/mL for serum, 6.0–23.0 pg/mg for hair), limit of quantification (1.3–4.1 ng/mL for serum, 20.0–77.0 pg/mg for hair), precision (3.0–11.3% for serum, 2.4–14.2% for hair), accuracy of the assay (RE) (−8.0 to 12.0% for serum, −8.0 to 11.0% for hair), recovery (88.6–113.4% for serum, 86.1–107.4% for hair) and matrix effects (87.9–110.7% for serum, 85.1–108.4% for hair) were calculated for the studied compounds. Then, the MAE/CE-TOF-MS method was successfully applied to the analysis of hair samples taken from patients treated with benzodiazepines.

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1. Introduction

In recent years, liquid chromatography combined with mass spectrometry (LC–MS) has been the most popular technique to screen, identify and quantify psychoactive compounds or their metabolites in various bio-materials [1]. However, the capillary electrophoresis technique coupled with mass spectrometry detection (CE-MS) is increasingly becoming an alternative technique to LC–MS separation [2,3]. The first description of a coupling of CE to MS was reported by Smith et al. in 1987 [4]. Four years later, in 1991, Johansson et al. published a report on the application of the CE-UV-MS method to the separation and identification of flurazepam metabolites in human urine [5]. This was one of the first researches describing the application of the CE-MS technique to the determination of psychoactive substances in a biological matrix [6]. Nowadays, the CE-MS technique can be a useful tool for monitoring various psychoactive compounds (e.g. illegal drugs, psychotropic drugs, and biomarkers) in biological materials (e.g. urine, plasma, hair, and saliva), which is a crucial area in clinical toxicology, forensic science and doping control [6–12]. Despite this, there are only a few published papers on the application of the CE-MS technique to the separation, identification and determination of benzodiazepines (BZDs) and/or 1-benzylpiperazine (BZP) in biological material [13–18] (see Table S-1 presented in 'Electronic supplementary materials' for further details). However, to the best of the authors' knowledge, there has been no publication describing the application of the CE-MS technique to the separation and determination of BZP or BZDs in human serum and hair.

The main aim of this research was the development and validation of a sensitive MAE/CE-TOF-MS method for the determination of BZP and seven BZDs in human serum and hair. BZP belongs to a group of designer drugs of abuse commonly called "party pills". It can cause amphetamine-like effects and possesses abuse potential [19], while BZDs are a large group of psychoactive drugs whose long-term use can induce physical and mental addiction. Moreover, a combination of this class of drugs with illicit drugs or ethyl alcohol can lead to toxic or even fatal effects, when overdosed [20]. Finally, the MAE/CE-TOF-MS method was applied to the analysis of natural hair samples obtained from patients treated with BZDs.

2. Materials and methods

2.1. Reagents, standards and materials

LC–MS grade reagents: acetonitrile, methanol, isopropyl alcohol, dichloromethane, n-hexane, ammonium acetate, and sodium

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tetraborate decahydrate were purchased from Sigma–Aldrich (St. Louis, MO, USA). Analytical grade formic acid and ammonia solution were supplied by Merck (Darmstadt, Germany). Ethyl acetate (HPLC-grade), chloroform, toluene, isoamyl alcohol and 30% NaOH water solution, all analytical grade, were purchased from Avantor Performance Materials (Gliwice, Poland). Water (18.2 MΩ cm, TOC < 5 ppm) was ultrapurified in a Milli-Q Plus system (Millipore, Bedford, MA, USA).

Drug standards: 1-benzylpiperazine (BZP), 7-aminoclonazepam (7-aClo), alprazolam (Alp), clonazepam (Clo), diazepam (Dia), estazolam (Est), lorazepam (Lor) and tetrazepam (Tetr) were purchased from LGC Standards (Teddington, UK). The deuterated analogs of benzylpiprazine-d8 (BZP-d8) and BZDs: 7-aminoclonazepam-d4 (7-aClo-d4), clonazepam-d4 (Clo-d4), diazepam-d5 (Dia-d5), estazolam-d5 (Est-d5) and lorazepam-d4 (Lor-d4) were obtained from Lipomed AG (Arlesheim, Switzerland). Drug stock solutions of BZP and BZDs (1 mg/mL) and internal standards (IS, 0.1 mg/mL) were stored in methanol in −20 °C. The stability of the drug stock solution was evaluated by comparative analysis of 2-weeks- and 4-weeks-old solutions with freshly prepared ones, which revealed no significant differences in the analytical signal (*t*-Student test, confidence level of 95%). Spiking solutions were prepared daily by appropriately diluting stock solutions with water. Standard drug solutions, used in the experiments on extraction efficiency, matrix effects, and recovery, were prepared by diluting stock solutions with the background electrolyte (BGE) to concentrations of 75, 150, 750 and 1880 ng/mL (corresponding to 10, 20, 100 and 250 ng/mL, respectively, in 1 mL of serum sample, or 0.2, 0.8, 2.2 and 5.6 ng/mg, respectively, in 45 mg of hair sample).

Human serum was provided by the local blood bank (Kraków, Poland) and it was stored frozen at −20 °C. The reference lyophilized serum samples (Mid Lot: 5780 and High Lot: 5779 Range Benzodiazepines 2, Quality Control Materials: Clinical & Forensic) containing five of the studied BZDs-7-aClo, Alp, Clo, Dia and Lor – were purchased in UTAK Laboratories, INC (Hague, the Netherlands). Drug-free, control hair samples (dark blond and brown) were received from healthy volunteers with no history of psychoactive drugs intake. The samples were stored in sealed polyethylene bags, at room temperature in a dry and dark place until the analysis. Natural hair samples positive for BZDs were collected from patients (male, age 34–53 years) who had been treated with Myolastan (tetrazepam, Sanofi-Aventis, France) or Clonazepamum (clonazepam, Polfa, Poland).

2.2. Sample preparation and extraction conditions

2.2.1. Hair pre-treatment

Before analysis, all hair samples were rinsed in the following sequence: methanol, water, and then methanol again in order to eliminate any external contamination. After drying at room temperature, each hair sample was cut into c.a. 2 mm long pieces, ground with a ball-grinder (Retsch, Haan, Germany), and 45 mg of powdered hair sample was weighed in an extraction vessel. Due to no available hair reference material for determination of BZP and BZDs, spiked hair samples were used throughout in the optimization and validation steps.

2.2.2. Microwave-assisted extraction

A MARS 5 microwave-assisted sample preparation system (CEM, Matthews, NC, USA) equipped with 24 Xpress® PFA vessels (75 mL) was used for isolation of the drugs from serum and hair. 1 mL of borate buffer (pH = 9.5) and 3 mL of extraction solvent (ethyl acetate) were added to 1 mL serum sample or 45 mg hair sample. Then, the MAE process was carried out for 5 min at 55 °C (serum) or for 15 min at 65 °C (hair). The temperature was ramped from room temperature in 5 min, using microwave power ranging from 480 to 800 W. After the MAE process, the content of each vessel was transferred to a plastic conical tube and centrifuged (10 min, 4000 rpm, 4 °C). In the next step, 3 mL of the organic layer was separated and evaporated to dryness under a stream of nitrogen at 40 °C. The residue was then dissolved in 100 μL of the ten times diluted BGE by a mixture of water: organic component. The centrifuged solution (10 min, 10,000 rpm, 4 °C) was transferred to other vials and centrifuged again (13,000 rpm, 5 min) for degassing and then taken for capillary electrophoretic analysis.

2.2.3. Liquid–liquid extraction

1 mL of borate buffer (pH = 9.5) and 3 mL of extraction solvent (ethyl acetate) were added to 1 mL of serum sample. Then, the content of the plastic vessels was gently agitated for 5 min using the Promax 1020 reciprocating platform shaker (Heidolph, Schwabach, Germany). The subsequent steps were the same as in case of the MAE extraction.

2.3. CE-TOF-MS conditions

The separation of studied compounds was performed using the PA 800 plus capillary electrophoresis system (Beckman Coulter, Brea, CA, USA). The MicrOTOF II (Bruker, Bremen, Germany) mass spectrometer (MS) with electrospray ionization source (ESI) and time of flight analyzer (TOF) was applied as the detector. The CE instrument was coupled with MS using an external detector adapter cartridge for the CE capillary (Beckman Coulter) and CE ESI-MS Sprayer Kit (Agilent Technologies, Santa Clara, CA, USA), which was used for precise positioning of the capillary outlet in the ESI source. The syringe pump (kdScientific, Holliston, MA, USA) delivered the sheath liquid into the ESI source (a mixture of isopropanol/H_2O, 1:1, v/v with 0.2% HCOOH, flow rate 180 μL/h). The separation of BZP and BZDs was performed in a polyamide-coated fused silica capillary (75 μm i.d., 100 cm length, Beckman Coulter) using a mixture of 100 mM formic acid and acetonitrile (80:20, v/v) as BGE. The injection of samples was hydrodynamic, applying a pressure of 4.83 kPa (0.7 psi) for 6 s. The separation process was carried out for 20 min using a voltage of +30 kV. The capillary temperature was set to 25 °C (ca. 75 cm in the cartridge [the remaining part of the capillary, ca. 25 cm, was out the liquid cooling system, at room temperature, ca. 22 °C]). The temperature of the sample garage was set to 15 °C. Before daily operations, the capillary (with the capillary outlet outside the ESI source) was conditioned in a sequence of 5 min long steps: 0.1 M NaOH, water, MeOH, water and BGE, using a pressure of 137.90 kPa (20 psi). Between runs, the capillary (with the capillary outlet placed in the ESI source) was rinsed with water (2 min, 172.34 kPa, 25 psi), 1 M NaOH (0.5 min, 10.34 kPa, 1.5 psi), water (2 min, 172.34 kPa, 25 psi), MeOH (2.5 min, 206.84 kPa, 30 psi) and then water (5 min, 137.90 kPa, 20 psi). Vials with BGE solution and all solvents used for the CE-MS system were replaced after every four sample injections. Before usage, BGE and NaOH solutions were filtered through 0.45 μm Minisart RC15 cellulose syringe filters (Sartorius, Goettingen, Germany) and then all solvents were degased by centrifugation (5 min, 10,000 rpm).

MS detection was performed in the positive ion mode and profile spectra were acquired in the mass range 50–1450 m/z. The ESI conditions were as follows: nebulizer pressure: 0.4 bar, dry gas: 4.0 L/min heated to 180 °C, and capillary voltage −4500 V. The mass resolving power of the instrument was over 16,000. Mass calibration was carried out using sodium formate clusters after each run according to the procedure given by Bruker. Data were collected by Compass DataAnalysis 3.2 software (Bruker). The results were recalculated on the basis of an extracted ion chromatogram for all

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