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Determination of some psychotropic drugs in serum and saliva samples by HPLC-DAD and HPLC MS

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ABSTRACT

A simple, rapid and sensitive HPLC-DAD method has been developed and validated for the simultaneous determination of seven psychotropic drugs (risperidone, citalopram, clozapine,quetiapine, levomepromazine, perazine and aripiprazole) in human serum or saliva samples. The chromatographic analyses were performed on a XSELECT CSH Phenyl-Hexyl column with a mobile phase containing methanol, acetate buffer at pH 3.5 and 0.025 mL^{-1} diethylamine. The influence of concentration of methanol in injection samples and injection volume on peak symmetry and system efficiency was examined.The full separation of all investigated drugs, good peaks' symmetry and simultaneously high systems efficiency were obtained in applied chromatographic system. The method is suitable for the analysis of investigated drugs in human plasma or saliva for psychiatric patients for control of pharmacotherapy, particularly in combination therapy. HPLC–MS was applied for verification of the presence of drugs and their metabolites in serum and saliva samples from patients.

1. Introduction

Therapeutic drug monitoring is a useful tool for the clinical management of patients receiving a pharmacotherapy, particularly in psychiatry. Therapeutic drug monitoring of antidepressants is necessary for an optimal supervision of patient drug regimen to avoid medical complications, intoxication, non responsiveness or noncompliance. A significant percentage of psychiatric patients who are treated with psychotropic drugs are treated with more than one drug. Thus, it is advantageous to use a rapid and reliable assay that is suitable for determination of multiple antipsychotic drugs in biological samples in a single run. The concentrations of the drugs in biological samples are often very low. For these reasons, to monitor psychoactive drugs, the analytical methods have to be highly sensitive and selective for accurate and precise quantification.

The general methods for analyzing psychotropic drugs in different biological fluids are based on a combination of efficient separation method with a sensitive detection technique. At present, numerous separation techniques, including high-performance liquid chromatography (HPLC) [1], gas chromatography (GC) [2,3] and capillary electrophoresis (CE) [4,5], have been employed for the analysis of psychotropic drugs. Among those methods, HPLC has been considered as the most efficient and robust specific technique to some advantages including convenience, high separation ability and simple operation.

Various detection methods have also been applied to accurate determination of psychotropic drugs in different samples such as: voltammetry, chemiluminescence [6], fluorescence [7], UV adsorption [8–11], and mass spectrometry [12–20]. Nowadays most HPLC methods are developed with mass spectrometric detection techniques.

Psychotropic drugs were quantified in different biological samples e.g., in plasma [7,11,21,22], serum [10,13,23,24], whole blood [16], urine [1,7], saliva [3,25].

Most of the published methods allow quantification of a single drug, sometimes with its related metabolites [6,11,18]. Simulta-

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neous quantification of various psychotropic drugs have also been published [1,9,10], mostly in the same therapeutic class. In recent years there is a trend in clinical and forensic toxicology toward simultaneous quantification of a various compounds in one analytical run [16].

In most cases psychotropic drugs separation and determination were performed on alkyl bonded stationary phase (C18, rarely C8) [1,8,10,12,13,15,16,21]. Protonated basic psychotropic drugs can interact with residual silanol groups of the stationary phases. Thus, besides the reversed phase retention mechanism also an ion-exchange retention mechanism occurs, which often results in asymmetry of peaks, irreproducible retention, poor efficiency and worse separation. The silanol ion-exchange interaction can be reduced by using mobile phase with buffer at low pH, when the silanol ionization is suppressed, mobile phase with buffers at high pH to suppress solutes ionization, addition of an anionic ion-pairing reagents, making neutral associates or addition of organic amines as silanol blockers. Good results were also obtained by selecting a stationary phase to minimize the interaction between analyte and residual silanols. The introduction of hydrophobic π–π active aromatic moieties to the common *n*-alkyl chain RP-sites generates a concerted π–π reversed-phase retention mechanism, which diversivies the common RP-interaction properties. Analysis of some psychotropic drugs was also successfully performed on CN [26], Phenyl [27], CycloHexyl [28], Polar RP [16] columns.

Described in literature mobile phases applied to analysis of basic psychotropic drugs often contained organic modifier and addition of salts e.g., ammonium acetate [12], ammonium formate [17,18], acids [9,15,16,21], buffer at acidic pH [9,15,16,21,24], buffer at basic pH [29]. Mobile phases with addition of ion-pairing reagents [7] or amines e.g., triethylamine, tetramethylethylenediamine as silanol blockers were also used [8,11].

In most cases, owing to complex matrix such as biological fluids interference and insufficient instrumental detection limit for trace psychotropic drugs in real biological samples, direct HPLC determination of those compounds is difficult.Therefore, a separation from matrices and preconcentration is often required prior to chromatographic analysis of the analytes. Different sample preparation methods for analysis of psychotropic drugs in various biological samples e.g., protein precipitation [14,16,20,21], liquid–liquid extraction (LLE) [8,9,17], dispersive liquid–liquid microextraction (DLLME) [1], Ultrasound-assisted emulsification microextraction [30], solid-phase extraction (SPE) [11,12,18,24], stir bar sorptive extraction (SBSE) [7], some methods using on-line SPE with a column-switching system or methods with a simple protein precipitation have also been developed.

The aim of this study was to develop and validate a method for the simultaneous quantification of some psychotropic drugs in serum and saliva. The HPLC-DAD method was fully validated including function response, linearity, limit of detection and quantification, recovery, matrix effects, process efficiency, repeatability and intermediate precision. The presence of some investigated drugs in samples from patients receiving medication were confirmed by HPLC–MS.

2. Experimental

2.1. Chemicals

Drug standards: Risperidone, Quetiapine, Clozapine, Levomepromazine, Perazine, Citalopram were obtained respectively from Janssen-Cilag International NV (Beerse, Belgium), Adamed Sp. z o.o. (Pieńków, Poland), EGIS Pharmaceuticals PLC (Budapest, Hungary), PROTERAPIA Sp z o.o. (Warszawa, Poland), HASCO-LEK S.A. (Wrocław, Poland), Ranbaxy Sp. z o. o. (Warszawa, Poland), Otsuka Pharmaceutical Europe Ltd. (Wexham, Great Britain). Methanol (MeOH) of chromatographic quality and diethylamine (DEA), acetic acid, sodium acetate, phosphoric acid, ammonium (25%), ammonium chloride were purchased from Merck (Darmstadt, Germany). Water was double distilled.

2.2. Apparatus and HPLC-DAD conditions

There were used BAKERBOND^{TM} speOctadecyl (C18) J.T. Baker (Phillipsburg, USA) cartridges (100 mg/1 mL) and SPE chamber – Baker SPE – 12 G J.T. Baker (Philipsburg, USA) for sample preparation.

Chromatographic analysis was performed using liquid chromatograph LaChrom Elite (Merck) equipped with an autosampler, column oven L-7350, solvent degasser L-7612 and DAD detector. The chromatographic measurements were carried out at 22 °C with an eluent flow rate of 1.0 mL/min. The chromatographic separation was performed on Phenyl-Hexyl column from Phenomenex (5 μm,150 mm × 4.6 mm). The following eluents were used: eluent (A) H_2O and 0.025 mL^{-1} DEA, eluent (B) MeOH and 0.025 mL^{-1} DEA, eluent (C) acetate buffer at pH 3.5 and 0.025 mL^{-1} DEA. Samples were injected onto HPLC column and eluted with following gradient elution program: 40% B from 0 to 15 min, from 40 to 45% B from 15 to 35 min, from 45 to 65% B from 35 to 45 min. The concentration of the eluent C was constant and was 20% during the whole analysis.

The DAD detector was set in the 200–400 nm range. The chromatographic data was acquired and processed with EZchrom Elite software.

2.3. HPLC–MS conditions

Determination of target compounds was carried out using an HPLC system equipped with the Phenyl-Hexyl analytical column. The column was maintained at 20 °C. The injected sample volume was 20 μL, while the mobile phase was composed of MeOH, acetate buffer at pH 3.5 and 0.025 mL^{-1} mixed and dosed at a flow rate of 0.6 mL/min.The mass spectral analysis was performed on a 6410 triple quadrupole mass spectrometer from Agilent (Santa Clara, USA) equipped with an ESI interface operating in positive ion mode, with the following set of operation parameters: capillary voltage, 4000 V; nebulizer pressure, 40 psi; drying gas flow, 9 L/min; drying gas temperature, 310 °C; LC–MS mass spectra were recorded across the range 50–1000 *m*/*z*. Quadrupole 1 was fixed at a set parent ion, quadrupole 2 was used as a collision chamber to induce fragmentation, and quadrupole 3 was fixed at a set daughter ion. The HPLC–MS data were collected and processed by MassHunter software (Agilent). The data were further processed using Microsoft Excel.

The instrument was operated in selected ions monitoring mode (SIM) and multiple reaction monitoring (MRM) as well. The monitored pseudomolecular ions $[M+H]^+$ are presented in Table 1 .

2.4. Serum and saliva sample collection

Serum and saliva samples for SPE procedure optimization and validation were taken from healthy volunteers. After blood coagulation the samples was centrifuged for 10 min at 1500 × *g*. The serum was separated and stored at −20 °C until analysis. The saliva was also stored at the same temperature.

Human body fluid samples were collected from psychiatric patients at Autonomous Public Clinical Hospital No. 1 in Lublin (Poland). The study was approved by Bioethical Commission. The