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Sequential cloud-point extraction for toxicological screening analysis of medicaments in human plasma by high pressure liquid chromatography with diode array detector

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ABSTRACT

A complex extraction system with the use of cloud-point extraction technique (CPE) was developed for sequential isolation of basic and acidic/neutral medicaments from human plasma/serum, screened by HPLC/DAD method. Eight model drugs (paracetamol, promazine, chlorpromazine, amitriptyline, salicyclic acid, opipramol, alprazolam and carbamazepine) were chosen for the study of optimal CPE conditions. The CPE technique consists in partition of an aqueous sample with addition of a surfactant into two phases: micelle-rich phase with the isolated compounds and water phase containing a surfactant below the critical micellar concentration, mainly under influence of temperature change. The proposed extraction system consists of two chief steps: isolation of basic compounds (from pH 12) and then isolation of acidic/neutral compounds (from pH 6) using surfactant Triton X-114 as the extraction medium. Extraction recovery varied from 25.2 to 107.9% with intra-day and inter-day precision (RSD %) ranged 0.88–1087 and 5.32–17.96, respectively. The limits of detection for the studied medicaments at λ 254 nm corresponded to therapeutic or low toxic plasma concentration levels. Usefulness of the proposed CPE-HPLC/DAD method for toxicological drug screening was tested via its application to analysis of two serum samples taken from patients suspected of drug overdosing.

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1. Introduction

Screening drug procedures play first-rate role in systematic toxicological analysis (STA) applied to forensic or clinical investigations [1]. In undirected examinations, where no information about an overdosed drug (drugs) is available, a general isolation system for potentially toxic compounds is required. This should be a compromise between extraction yield of drugs and degree of interfering substances removal from a biological matrix. Various physicochemical properties of the possible detected compounds like dissociation constants (pK_a) or partition coefficients ($\log P$) affect the ability to extract these substances from biological material into an appropriately chosen medium. Therefore, there is no possibility to isolate and analyze such a variety of compounds (e.g. medicaments) in a one step. It is commonly accepted that the drugs are isolated into two groups: acidic/neutral compounds (fraction A) and basic compounds (fraction B) prior screening chromatographic analysis. There are two main methodologies: liquid–liquid extraction (LLE) and solid-phase extraction (SPE) which are routinely used for isolation of medicaments in biological samples screened by chromatographic methods. In STA, the traditional sample preparation realized with LLE technique has been used for a long time, often combined with such sample pretreatment procedures such as conjugate hydrolysis, digestion and protein removal [1,2]. Although the LLE technique is suitable in numerous cases of screening drug analysis, it also possess some disadvantages like emulsion creation or the use of large amounts of toxic solvents. The more contemporary extraction technique – SPE – has overcome some drawbacks of the LLE technique [3], however it requires rather expensive columns. Therefore, another methodology as cloud-point extraction (CPE) seems to be worth consideration for preparation of biological samples subjected to drug screening by a HPLC method. The CPE technique may be characterized as simple, cheap, environmentally benign and relatively fast.

In CPE, separation of two phases, i.e. the surfactant-rich phase with the isolated analytes and the aqueous supernatant phase with the surfactant close to critical micelle concentration, is caused mainly by the influence of temperature [4,5]. The compounds characterized by an appropriate hydrophobicity (most medicaments demonstrate hydrophobic properties) affect to a micelle core. According to the rule, the more hydrophobic character of a

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compound, the greater strength of its affinity to a hydrophobic micelle core. Surfactants (substances which at appropriate concentrations create micelles) may be divided into three groups: (1) ionic, (2) non-ionic and (3) zwitterionic. Surfactants have already been applied to cloud-point extraction of one [6–12], two [13] or three [14] medicaments from a body fluid (usually plasma, serum or urine). Lately, two validated CPE procedures combined with HPLC/DAD method for analysis of acidic/neutral medicaments [15] and basic medicaments [16] present in human plasma, have also been reported.

This study was focused on the development of a sequential extraction system with use of the CPE technique for basic and acidic/neutral medicaments, present in human plasma samples, which were analyzed by screening HPLC/DAD method. To our best knowledge, it is first time when the CPE technique is proposed as a complex preparation method for a biological material screened for medicaments. Eight model drugs: paracetamol, promazine, chlorpromazine, amitriptyline, salicyclic acid, opipramol, alprazolam and carbamazepine characterized by a wide range of partition coefficients (log *P*) were selected for this study. A non-ionic surfactant Triton X-114 was chosen as the extraction medium. Structures and some physicochemical properties of the examined medicaments were shown in Table 1.

2. Experimental

2.1. Apparatus and chromatographic conditions

A chromatographic system, Merck-Hitachi LaChrom, consisting of an L-7100 pump and an L-7455 programmable diode array detector DAD (Darmastadt, Germany) was used.

Examinations of separation conditions for the tested drugs were performed on a column Nucleosil C8 (250 mm × 4.6 mm i.d., 5 μm) supplied by Merck (Germany). The used column was thermostated at 40 °C.

Chromatographic analyses were curried out using gradient conditions with the mobile phase consisting of phase A: 0.002 M aqueous orthophosphoric acid (pH c.a. 3) and phase B: acetonitrile. The gradient profile was as follows: 0 min: 100% phase A, 0–30 min: 30% phase A and 70% phase B and 30–33 min: 100% phase A. The flow rate of the mobile phase was 1 mL min^{-1}. The drugs were detected by UV-light absorption at λ 254 and 210 nm.

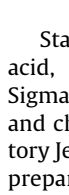
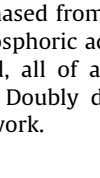
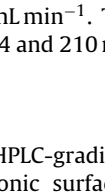
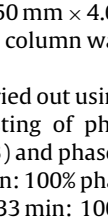
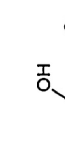
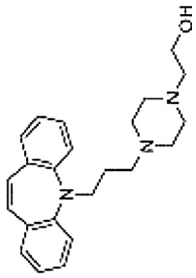
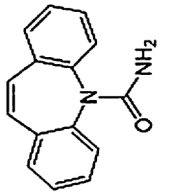
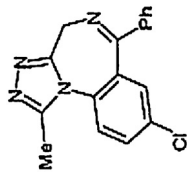
2.2. Reagents

Acetonitrile and methanol, both of HPLC-gradient grade, were supplied by Merck (Germany). Non-ionic surfactant Triton X-114 and solid Rhodamine B were purchased from Sigma–Aldrich (Germany). The reagents: 85% orthophosphoric acid, 30% sodium hydroxide and 36% hydrochloride acid, all of analytical grade, were purchased from POCH (Poland). Doubly deionized water (<1.0 μS/cm) was used throughout the work.

2.3. Examined drugs and materials

Standard substances of paracetamol, amitriptyline, salicyclic acid, alprazolam and carbamazepine were purchased from Sigma–Aldrich (Germany), and standards of opipramol, promazine and chlorpromazine were obtained from the pharmaceutical factory Jelfa (Poland). A stock solution of each drug (10 mg mL^{-1}) was prepared in methanol and stored in a refrigerator (4 °C). Working drug solutions were prepared by appropriate dilution of the stock drug solutions with a mixture of phase A and phase B (1:1, v/v). In order to prepare control samples, human plasma obtained from the local blood bank (Krakow, Poland) was spiked with water diluted standard drugs.

Table 1
Structures, dissociation constants and participation coefficients of the examined drugs. respectively.

Drug	Basic drugs				Acidic/neutral drugs			
	Parac	Proma	Amitri	Clomi	Salicacid	Opipra	Carba	Alpraz
Structural formula								
pK_a (25 °C)	9.5	9.4	9.4	–[a]	3.0 3.14	3.8	7.0	2.4
Log *P*	0.5	2.5	4.9	5.2	2.3[b]	3.4[b]	2.45[b]	2.12[b]

Parac, paracetamol; Proma, promazine; Amitri, amitriptyline; Clomi, clomipramine; Salicacid, salicylic acid; Opipra, opipramol; Carba, carbamazepine; Alpraz, alprazolam.
[a] Lack of literature data.
[b] Participation coefficient (octanol/buffer 7.4), in the rest of drugs tested log *P* was given for octanol/water.