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Short communication

The use of the new SPE methods for isolation of some tricyclic antidepressant drugs from human serum

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Abstract

Solid-phase extraction (SPE) methods were applied to isolation of amitriptyline (AMI), imipramine (IMI) and chlorprothixene (CPX) from blood human serum. SPE was carried out using the octadecyl (C_{18}) column for isolation of AMI and cyclohexyl (CH) columns in the case of IMI and CPX. The spiked serum samples were used to examine the recoveries of these drugs from C_{18} and CH sorbent materials. The volume of serum sample was 500 μl. The recoveries of SPE method using CH cartridge were $100.3 \pm 1.63\%$ ($n = 7$), $99.7 \pm 2.3\%$ ($n = 9$) for IMI and CPX, respectively. The recovery of AMI from C_{18} cartridge was $99.5 \pm 1.5\%$ ($n = 8$). Finally, after SPE sample clean-up step the antidepressant drugs were assayed by the own extractive-spectrophotometric methods.

Keywords: Solid-phase extraction; Amitriptyline; Imipramine; Chlorprothixene; Extractive-spectrophotometric determination

1. Introduction

Amitriptyline (AMI), imipramine (IMI) and chlorprothixene (CPX), belong to the group of tricyclic antidepressant drug (TCA). They differ from each other in the chemical structure of tricyclic skeleton and substitution of nitrogen or number of bonds for a carbon in the aromatic ring. From chemical point of view they are weak organic basis so the differences in a structure of this drugs do not influence the choice of the technique of sample purification. Analysis of serum for tricyclic amines drugs requires extensive sample preparation and purification to isolate the components of interest from sample matrix because most analytical instruments, especially all chromatographic methods of determination, cannot handle the matrix directly [1].

For a long time, a liquid–liquid extraction (LLE) technique has been a traditional way to prepare sample to analysis, often combined with sample pretreatment procedures such as conjugate hydrolysis, digestion and protein removal [2].

On the ground of the review of literatures it can be stated that classical standard procedure for the extraction of AMI and IMI from serum or plasma samples is based on a LLE after alkalisation by sodium hydroxide, ammonia, borate buffer or

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sodium carbonate [3–9]. In some methods the back-extraction step of AMI and IMI from the organic phase is used [10,11]. Sometimes methods, which are based on a three-step LLE of AMI and CPX from serum or plasma are used [12–14].

The classical LLE technique is often used in isolation of these drugs from biological samples, in spite of its disadvantages, e.g. matrix interferences, temporary emulsion formation by agitating two immiscible liquids, such as water and organic solvent, use of large volumes of hazardous solvent and relatively large volume of sample. The excess of organic solvent very often must be dried prior final measurement. This operation is often tedious and time-consuming [2,15].

The number of solid-phase extraction (SPE) methods for the isolation of AMI, IMI and CPX from serum or plasma samples [16–20] is very limited.

The aim of the presented study is to replace many traditional sample work up LLE methods by the determination of amitriptyline (AMI), imipramine (IMI) and chlorprothixene (CPX) in serum samples with SPE procedures. For that reason, here is described the new SPE techniques for the isolation of three basic drugs: AMI, IMI and CPX from human serum without evaporation and deproteinisation steps. The contents of isolated analytes was directly determined by the own extractive-spectrophotometric methods [21,22].

2. Experimental

2.1. Reagents and chemicals

AMI, IMI, CPX (all as hydrochloride salts) were purchased from Sigma Chemical Co. and pyrocatechol violet was obtained from Lachema (Brno, Chechoslovakia). The solution of reagents were maintained at room temperature in an amber coloured bottle. All other chemicals used were of analytical grade.

2.2. Apparatus

There were used J.T. Baker 3 ml solid-phase extraction columns packed with reversed phase cyclohexylsilane (C_6H_{11}) and octadecylsilane (C_{18}) bonded to silica gel (40 μm APD, 60 Å). Extraction was done by SPE-12G, System J.T. Baker. A Specol-10 Spectrophotometer (Carl Zeiss, Jena, Germany) was used for absorbance measurement.

2.3. Biological samples

Blood samples to be used for determination of AMI, IMI and CPX were collected as steady-state concentration samples. After blood coagulation, the samples were centrifuged at $1500 \times g$ for 10 min. The serum was separated and stored at −4 °C until analysis.

3. Extraction procedure

3.1. SPE isolation of amitriptyline from spiked serum samples

Blank serum (0.5 ml) samples were spiked with 1.0 ml 2.0×10^{-4} mol/l of amitriptyline and buffered with 0.5 ml of the acetate buffer (pH 4.0; 0.1 M). The C_{18} extraction columns were activated subsequently once with 2.0 ml of methanol, 2.0 ml of water, 1.0 ml of acetate buffer and twice with 1.0 ml portion of methanol–water mixture (1:1, v/v). The column must not become dry before sample application. Afterwards the buffered serum sample was slowly applied onto the conditioned column. The sample was passed slowly through the column under mild vacuum (−200 mmHg). The column was then washed with 0.5 ml of chloroform–acetone mixture (1:1, v/v) to elute interferences from the cartridge and dried completely after the washing. The elution of AMI was achieved by rinse with seven 1 ml portions of chloroform–*n*-butanol mixture (2:1, v/v).

The elutes were collected into a glass tube and transferred to a 50 ml separatory funnel. Then, an extractive-spectophotometric determination of AMI by the use of pyrocatechol violet was carried out. For this purpose to the separatory funnel containing 7.0 ml of elute were added subsequently 4.5 ml 5.0×10^{-4} mol/l of pyrocatechol violet, 2.5 ml 1.0×10^{-2} mol/l of H_2SO_4. The total volume of aqueous phase was adjusted to 10 ml with

deionized distilled water. The solution was extracted for 1 min with 7.0 ml of chloroform–*n*-butanol mixture (2:1, v/v). Then, two phases were allowed to separate and the organic phase was transferred into 10 ml calibrated test tubes and extraction was repeated with 3.0 ml portion of chloroform–*n*-butanol (2:1, v/v) solution. The extracts were joined and diluted to the mark with the same mixture of chloroform-*n*-butanol (2:1, v/v). The absorbance was measured at λ 445 nm against the reagents blank.

3.2. SPE isolation of imipramine from spiked serum samples

Serum (0.5 ml) was mixed with 1.0 ml 5.0×10^{-4} mol/l of IMI and 0.5 ml of acetate buffer (pH 4.0; 0.1 mol/l). Then, the whole buffered aqueous sample was slowly applied onto the cyclohexyl (CH) extraction column, which was earlier conditioned once with 2.0 ml of methanol, 1.0 ml of water and twice with 1.0 ml of acetate buffer (pH 4.0; 0.1 mol/l). Before the elution step, the column was washed with 0.5 ml of dichloromethane followed by 0.1 ml of acetonitrile. The adsorbed analyte was eluted by seven 1.0 ml portions of chloroform–*n*-butanol mixture (2:1, v/v). The elutes were collected into a glass tube and transferred to a 50 ml separatory funnel. Determination of IMI was carried out by extractive-spectrophotometric method according to the method described in [21].

3.3. SPE procedure of chlorprothixene isolation from spiked serum samples

Blank serum (0.5 ml) samples were spiked with 1.0 ml 5.0×10^{-4} mol/l of CPX and mixed with 0.5 ml 10% CH_3OH in 0.25 mol/l HCl. The CH extraction column was conditioned subsequently by 2.0 ml of methanol, 1.0 ml of water, 0.5 ml of 10% CH_3OH in 0.25 mol/l HCl and 1.0 ml of water. After loaded the whole spiked serum sample onto the activated column, the cartridge was then washed with 1.0 ml of acetonitrile. Afterwards, the column was dried completely before eluting step. Elution was done with seven 1.0 ml portions of chloroform–*n*-butanol mixture (5:1, v/v) to a glass tube and then transferred to a 50 ml separatory funnel. Finally, the extract was subjected to an extractive-spectrophotometric determination according to the procedures described in [22].

3.4. Stability of AMI, IMT and CPX in serum

Analogical studies of isolation of AMI, IMI and CPX from serum were made within 24 h since analysed drugs into biological sample were added. For the two series of samples (investigated directly and within 24 h) the absolute extraction recoveries of studied drugs from serum were compared.

4. Results and discussion

The SPE procedure described in this paper allows the selective isolation of AMI, IMI and CPX from blood human serum.

In this work were tested different type of SPE sorbent materials, using the optimum conditions for C_{18} and CH columns. The results obtained on silica gel SPE columns did not allow to use they to isolation of three studied drugs from serum. The SPE recoveries were very low in the range 54.0–69.0%.

The C_{18} cartridges gave the best results only for AMI. In the IMI and CPX instance, the SPE recoveries on C_{18} columns were relatively low in the range 35.0–80.0%. The best results for IMI and CPX were obtained on a CH column. In every instance methanol was used as an activating solvent. Retention of basic drugs on C_{18} and CH columns is mainly due to cation-exchange processes with charged residual silanol groups when the medium pH is acidic enough to maintain the drug in its charged form [17]. Therefore, acetate buffer was used (pH 4.0; 0.1 mol/l) in AMI and IMI case and 0.25 mol/l hydrochloride acid for retention of CPX.

In order to select an appropriate solvents to elute interferences from C_{18} and CH columns, the following solvents were checked: acetonitrile, dichloromethane, water, acetone, acetate buffer and different mixtures of these solvents. The correct selection of the washing solvent was the most important for obtaining the good recoveries of

studied drugs by SPE procedure. The best results were achieved when 0.5 ml of chloroform–acetone mixture (1:1, v/v) in AMI instance, 0.5 ml of CH_2Cl_2 followed by 0.1 ml of acetonitrile for IMI and 1.0 ml of acetonitrile for CPX were used.

The aim of this study was the simplification of the procedure by elemination of evaporation step of eluent. Thus, for this purpose different volumes of organic solvents (chloroform–*n*-butanol) used for the spectrophotometric determination of AMI, IMI and CPX were checked [21,22]. The optimal volume of solvent found was depended on the type of sorbent materials and kind of researched drug. For AMI and IMI, 7.0 ml mixture of chloroform–*n*-butanol was used (2:1, v/v). For elution of CPX the same volume of chloroform–*n*-butanol mixture in proportion 5:1, v/v was used. The SPE conditions were given in Table 1, for the compounds studied in this paper. In every instance, the selected elution solvents provided the cleanest samples, which could be directly analysed by the own extractive-spectrophotometric methods [21,22].

In Table 2 the absolute recovery of elaborated SPE methods are shown. The absolute recovery values of the analytes were calculated by comparison of the actual content of the drugs in the extracts with those theoretically expected. In every instance, the recoveries of the drugs from C_{18} and CH bonded silica columns (Baker) were surprisingly high, uniform and stable in the range 96–103%.

Above mentioned values of recoveries were obtained for samples investigated directly and within 24 h since the drugs were added to serum. After this time, decreasing of concentration of analysed drugs was observed.

In order to obtain a new SPE procedure for isolation of studied drugs from serum the spiked serum samples were used. The preparation procedures of serum samples usually includes the deproteinisation step with organic solvents (e.g. methanol or ethanol alcohols), inorganic salts or strong acids [23]. It was observed that the deproteinisation operation is the source of serious error, probably due to co-precipitation and/or adsorption of studied compounds on protein precipitate. The introduction of this step into elaborated SPE

Table 1
SPE conditions of isolation of drugs from human blood serum sample

Drug sample	SPE column type	Column conditioning	Elution method of interferences	Elution method of analyte
0.5 ml serum, 1.0 ml 2.5×10^{-4} mol/l AMI, 0.5 ml acetate buffer (pH 4.0; 0.1 mol/l)	C_{18}	2.0 ml methanol, 2.0 ml water, 1.0 ml acetate buffer (pH 4.0; 0.1 mol/l), 2×1.0 ml methanol–water mixture (1:1, v/v)	0.5 ml chloroform–acetone mixture (1:1, v/v)	7×1.0 ml chloroform–*n*-butanol (2:1, v/v)
0.5 ml serum, 1.0 ml 5.0×10^{-4} mol/l IMI, 0.5 ml acetate buffer (pH 4.0; 0.1 mol/l)	CH	2.0 ml methanol, 1.0 ml water, 2×1.0 ml acetate buffer (pH 4.0; 0.1 mol/l)	0.5 ml CH_2Cl_2, 0.1 ml acetonitrile	7×1.0 ml chloroform–*n*-butanol (2:1, v/v)
0.5 ml serum, 1.0 ml 5.0×10^{-4} mol/l CPX, 0.5 ml 10% methanol in 0.25 mol/l HCl	CH	2.0 ml methanol, 1.0 ml water, 0.5 ml 10% methanol in 0.25 mol/l HCl	1.0 ml acetonitrile	7×1.0 ml chloroform–*n*-butanol (5:1, v/v)

Table 2
The absolute recoveries of known amount of analysed drugs

Drug	Concentration added (μg/ml)	Absolute recovery
AMI	7.847	100.37
		97.76
		100.75
		97.39
		101.12
		98.51
		98.89
		100.93
IMI	15.845	99.56
		102.09
		102.09
		101.1
		97.68
		99.1
		100.66
CPX	17.615	102.1
		96.61
		100.94
		99.3
		97.89
		99.76
		100.35
		97.08
		103.39

procedures resulted in very poor recoveries of examined drugs (about 30%), therefore, this operation was abandoned. The use of chloroform–acetone mixture (1:1, v/v) and organic solvents: dichloromethane, acetonitrile to elution of interferences has removed effectively almost all components of serum and for this reason, the sorbent materials were not plugged. It caused that the SPE columns may be used for a long time.

As a conclusion it can be stated that the SPE methods reported here are simple and rapid to carry out and do not require the deproteinisation and evaporation steps, so the elute can be directly assayed by extractive-spectrophotometric methods. The connection of solid phase extraction procedures of sample preparations with spectrophotometric methods of determination of drugs caused: high analyte recovery, reduce time of analysis and use of toxic organic solvents and neglected multi-step procedures (evaporation, deprotonisation) that are prone to losing analytes.

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