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Application of solid-phase extraction to the isolation and determination of paracetamol and its metabolites*

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Abstract: A series of clean-up columns for solid-phase extraction (SPE) were packed with various C_{18} phases of different physico-chemical surface properties. These SPE columns were used for the isolation and determination of paracetamol and its metabolites from biological samples. The packing with a monomeric structure of chemically bonded phase showed the best recovery of tested substances in urine.

Keywords: *Solid-phase extraction; chemically bonded phase; acetaminophenoles; glucuronide and glutathione-derived conjugates.*

Introduction

Paracetamol (*p*-acetaminophen) is a widely used antipyretic–analgesic drug. The isolation and determination of its metabolites from biological fluids is of clinical importance to evaluate non-invasively the hepatic drug metabolizing system [1]. In addition, *in vitro* assays of paracetamol metabolism encountered difficulties resulting from high concentration of enzyme substrates and relatively low concentration of formed metabolites.

Among the metabolites excreted in the urine are the glucuronides representing more than 50% of the given dose [2]. Furthermore, the formation of glutathione-derived conjugates (cysteine and mercapturic acid conjugates) is considered as an important detoxification pathway eradicating the formed reactive intermediates from the body [3].

Solid-phase extraction (SPE) is a successful tool used for isolation and purification of some drugs and their metabolites from biological samples, followed by various analytical methods such as HPLC [4, 5]. Under certain conditions for HPLC analysis with electrochemical detection, purification of the sample from other biological substances may be necessary for better detection [6].

Improved reproducibility and high recovery levels have been obtained with packing materials having chemically bonded phases (CBP) [7, 8]. In a previous study, it has been shown that coverage density, structure of siliceous support and CBP have an important influence on the recovery rate of isolated substances from biological materials [8].

In this work, we present a clinical application for the clean-up SPE columns packed with various C_{18} phases of different surface physico-chemical characters in the determination of paracetamol and its metabolites in urine.

Experimental

Chemically bonded phases (CBP) were prepared on the basis of silica gel (SG-100) with wide pores manufactured in the Polymer Institute of the Slovak Academy of Sciences (Bratislava, Czechoslovakia) [9]. The characteristics of this bare material are listed in Table 1.

The chemical modification of bare surface was made according to the method described by Buszewski [7, 8] using the monofunctional (MC_{18}) and trifunctional (TC_{18}) octadecylchlorosilanes (Wacker GmbH, Munich, FRG).

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Table 1
Physico-chemical characteristics of the packing materials used for SPE columns

Column number	Type of packing	Fraction (μm)	S_{BET}	Porosity V_P	D	Coverage P_c	α_{RP}
0	SG-100	45–60	196	2.10	23.0	Bare	
1	SG-100/MC_{18}					18.45	2.99
2	SG-100/TC_{18}					13.22	3.08
3	SG-100/TC_{18}/EC					17.40	4.03

where: S_{BET}, specific surface area ($m^2 g^{-1}$); V_P, pore volume ($cm^3 g^{-1}$); D, mean pore diameter (nm); P_c, percent of carbon (%); α_{RP}, concentration of alkylsilyl groups on the support surface ($\mu mol\ m^{-2}$).

Trimethylchlorosilane (Merck, Darmstadt, FRG) was used as an end-capping (EC) reagent. All chemicals were analytical-grade purity and were purchased from Merck. The SPE columns were prepared by packing 2-ml plastic extraction tubes with the appropriate materials to a bed height of 2 cm.

A urine sample was obtained from a healthy volunteer 10 h after administration of 1 g of paracetamol orally. 500 μl of 1:10 diluted urine were loaded on prewetted SPE columns. Then, the columns were washed with 1 ml of water followed by 1 ml of 3% acetonitrile in 1% acetic acid (pH 2.8). Analysis of loaded urine and collected SPE eluted fractions for paracetamol and its different metabolites, namely, the glucuronide, cysteine and mercapturate conjugates was carried out by HPLC as previously described [1].

The physico-chemical characterization of SPE packings before and after chemical modification was performed as described previously [10, 11].

Results and Discussion

Table 1 lists the parameters characterizing the prepared material packings for SPE columns. Comparison of the carbon content values (P_c) obtained by chemical modification of the surface of these packings revealed that using monofunctional silane modifiers gave a higher P_c value. It is consistent with CP/MAS NMR data [8, 11]. Differences in α_{RP} values are caused by the different molecular weights of the modifiers used and the specific surface area of silica gel with wide pores (Table 1), which was used as a support for CBP [9]. On the basis of data obtained from CP/MAS NMR spectroscopy, the identification of the formed structure of CBP was confirmed. In addition, the influence of this structure on the isolation mechanism and recovery rates of determined substances was explained [7, 8].

In Table 2 are presented the recovery rates (RR) for isolated substances (paracetamol and its metabolites) using prepared SPE columns and the percent of sorbed substances on the modified surface of packing bed (SP). The extraction process was measured by HPLC analysis using UV detection for determination of paracetamol glucuronide (Gluc.). Other isolated urinary compounds: paracetamol (Parac.), cysteine (Cyst.) and mercapturic acid (Mercap.) conjugates were determined by electrochemical detection. Representative chromatograms are shown in Fig. 1a and 1b.

Analysis of data obtained for packings 1 and 2 (Table 1) revealed that the concentration of alkylsilyl groups on the support surface, α_{RP}, for packing 2 is somewhat higher than for packing 1. Packing 1 has a monomeric chemically-bonded C_{18} phase, whilst packing 2 is a polyuric-bonded C_{18} phase. The sorption percentages (SP values, Table 2) were close to 100% for both packings, so that differences in

Table 2
Sorption percentage (SP) and recovery rates (RR) of paracetamol and its metabolites in urine*

Column number	Gluc. SP	Gluc. RR	Parac. SP	Parac. RR	Cyst. SP	Cyst. RR	Mercap. SP	Mercap. RR
1	92 ± 2	100 ± 2	100 ± 2	25 ± 1	100 ± 1	46 ± 6	100 ± 4	51 ± 9
2	93 ± 1	98 ± 8	100 ± 2	1 ± 0.2	100 ± 2	15 ± 3	100 ± 2	5 ± 0.1
3	12 ± 1	110 ± 2	52 ± 1	42 ± 2	15 ± 0.3	120 ± 3	10 ± 0.2	110 ± 4

*Values are means ±SD of sorption percentages (SP) and recovery rates (RR) for paracetamol (Parac.) and its conjugates with glucuronic acid (Gluc.), cysteine (Cyst.) and mercapturic acid (Mercap.). SP, percent of loaded substances in urine sample retained on SPE columns before elution. RR were calculated as percentage of SP.

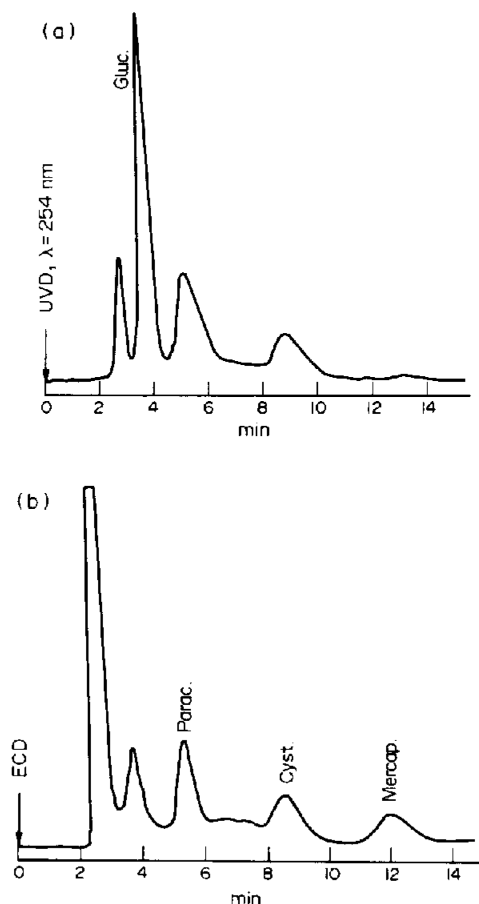

Figure 1
Representative chromatograms of urine sample containing paracetamol and its metabolites: (a) UV detector response at 254 nm used for the determination of paracetamol glucuronide (Gluc.); (b) electrochemical detector response used for the quantitation of paracetamol (Parac.) and its conjugates with cysteine (Cyst.) and mercapturic acid (Mercap.). RP–HPLC analysis conditions were as described previously [1].

recovery rates (RR values, Table 2) may be due to differences in accessibility of residual silanol groups. End-capping of initial packing material 2 (packing 3) resulted in higher α_{RP} values (of about 1 μmol m^{-2}, Table 1) indicating an effective blockage of residual silanol groups by the end-capping process. This secondary silanization of packing 2 with TC_{18} phase gave lower sorption capacities for the tested substances, ranging between 10–52% (Table 2). With this packing (packing 3), a hydrophobic film of CBP was formed of long C_{18} chains and small methylsilyl groups [—Si $(CH_3)_3$]. This resulted in blockage of a large number of silanol groups and in a preferential interaction between long C_{18} chains and trimethylsilyl groups [12, 13]. Accordingly, a considerable part of the biological sample went through the SPE column bed [8], and a lower sorption capacity in this case was observed.

Considering the RR values (Table 2) after desorption of previously sorbed compounds (e.g. Gluc.), it shows that by using packing material with monomeric structure of chemically-bonded C_{18} phase (packing 1, Table 1) a 100% recovery has been obtained. The same result has been obtained also for this compound during extraction on packing 2 (Table 2). Different RR values were observed during desorption of the remaining three substances. With packing 2, the influence of residual silanol groups in formed polymeric strucure of CBP is more evident (Table 2) [8, 12]. It indicated that during desorption of sorbed substances on packing material 2, the use of eluent with stronger elutropic power may be necessary (e.g. higher concentration of organic compound — acetonitrile). However, the packing material 2 could be used in studies of glucuronidation where the interest is focused on isolation and quantitation of the glucuronide conjugate possibly present in relatively low concentration, as is the case in *in vitro* incubation conditions. On the other hand, when the simultaneous isolation and determination of paracetamol and its metabolites are required, the use of packing 1 would be appropriate.

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