



## RETENTION MECHANISMS APPLIED IN SOLID PHASE EXTRACTION FOR SOME POLAR COMPOUNDS

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**abstract:** Two basic mechanisms were applied for studying some polar compounds of pharmaceutical interest (captopril, atenolol, and metformin) by solid phase extraction, using an octadecilsilica based adsorbent. The retention of these compounds was achieved under reversed-phase or ion-pair mechanisms using cartridges containing two different sorbent loadings. The breakthrough parameters (hold-up, breakthrough, and retention volumes) were measured for chosen model solutes from the experimental data obtained by percolating the C18 cartridges with stock solutions for each compound. The experimental curves were fitted by means of Boltzmann's function, and the regression parameters were used in calculating the breakthrough parameters.

**key words:** solid phase extraction; retention; breakthrough; C18; Boltzmann's function.

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### Introduction

Solid phase extraction is a method largely used for isolation and concentration of analytes by transfer to an immobilized solid-phase, due to its advantages over liquid-liquid extraction [1-3]. Different types of sorbents can be used in SPE, such as: silica based sorbents, carbon based sorbents, polymer based sorbents and sorbents based on molecular recognition [4]. The sorbent chosen in an analytical procedure is a key factor due to its influence on several parameters, such as selectivity, or breakthrough parameters [5]. The solutes are retained as a result of van der Waals, electrostatic interactions, and hydrogen bond. One of the most important parameters that influence the extraction process is the breakthrough volume and its control is desirable for development of a new SPE method. Many drugs are ionic or ionizable compounds, which are difficult to be extracted from aqueous media. In this case, methods employing sorbents based on normal phase, or ion pair mechanisms can be methods of choice [6].

Ion-pair (IP) reagents have been used in liquid chromatography for their ability of increasing retention of polar compounds and changing selectivity of reversed-phase

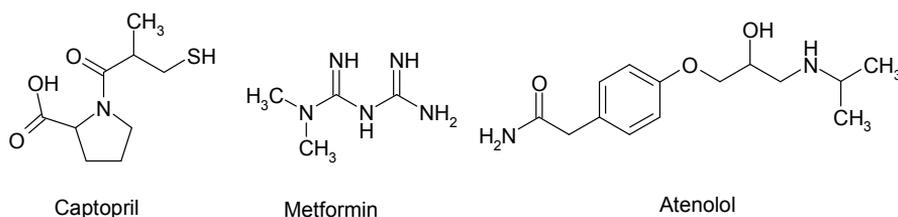
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columns. Ion-pair reagents contain a long aliphatic chain and a polar group (acidic or basic). The hydrophobic chain of the reagent interacts with the hydrophobic surface of stationary phase, while the polar moiety of the IP reagent interacts with the acidic or basic moiety of the solutes, forming an ion-pair [7-9]. The aim of this paper is to investigate the retention mechanism for three polar compounds, which have either acid or basic groups, on octadecyl bonded silica sorbents and calculation of retention parameters that characterize this process. One of them is highly polar compound (metformin), and in this case an ion pair mechanism was applied in order to enhance its retention on hydrophobic sorbent (C18). These compounds have been extensively studied in biological samples by means of different analytical techniques [10-13], but their enrichment by SPE was rarely applied due to the lack of analytical data concerning the adsorption properties of silica based sorbents.

## Experimental

The compounds studied were: atenolol, captopril and metformin chloride (further referred in the paper as metformin), which were kindly offered by LaborMed Pharma S.A. Their structures are shown in Figure 1. Hexan-1-sulfonic acid sodium salt and heptan-1-sulfonic acid sodium salt were purchased from Merck (Darmstadt, Germany). Water (resistivity minimum 18.2 M $\Omega$  and TOC maximum 30 ppb) was produced with a TKA Lab HP 6UV/UF instrument in the laboratory. Metformin, a disubstituted biguanide (1,1-dimethylbiguanide), is a drug prescribed for diabetes type 2. Captopril is used in the treatment of hypertension and heart failure. It contains a sulphhydryl group which can binds to albumin and other proteins from plasma. By slight oxidation it forms a disulphides dimer [14]. Atenolol is known as a  $\beta$ -blocker and used in the treatment of cardiovascular diseases.



**Fig. 1** Chemical structure of the studied compounds.

Three 100 ppm stock solutions of atenolol, captopril and metformin were prepared in water. Working solutions were further prepared by successive dilutions of the stock solution for obtaining six calibration standards, from 1 to 25 ppm.

An off-line SPE system (Alltech) was used for this study. The cartridges used contained octadecyl silica bonded phase sorbent (C18) with the characteristics presented in Table 1. The solute was introduced into the cartridge in aliquots. The sorbent was conditioned earlier with 5 mL methanol and 5 mL water. The volume of the individual aliquot added manually was 2.5 mL.

The absorption spectra were recorded with a Jasco V-530 double beam spectrometer, in 1 cm quartz cells. The absorbance of studied compounds were measured at  $\lambda_{\max} = 233$  nm

for metformin,  $\lambda_{\max} = 205$  nm for captopril and  $\lambda_{\max} = 225$  nm for atenolol. Calibration curve data were obtained in the range 1-25 ppm for each of the studied compounds in water.

**Table 1** *The sorbents characteristics.*

Cartridge type	Sorbent mass	Sample loading	Surface area	Particle size	Average pore size	Carbon loading
Ultra Clean C18	200 mg	4 mL	498 m <sup>2</sup> /g	50 $\mu$ m	60 Å	5.6%
Bond-Elut C18	2 g	12 mL	494 m <sup>2</sup> /g	57 $\mu$ m	71 Å	18.34%

## Results and discussion

One of the major parameter describing the interaction between silica based sorbents and organic compounds are their hydrophobicity. From this point of view, the studied compounds are characterized by a low hydrophobicity parameter, estimated by the theoretical value of the octanol/water partition constant (denoted by  $\log K_{ow}$ ). The  $\log K_{ow}$  values and information regarding their acidic properties are given in Table 2.

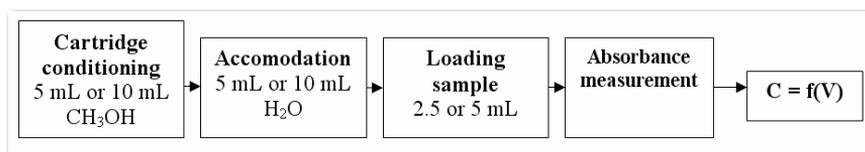
**Table 2** *Octanol/water partition constant and acidity constant of the studied compound.*

Compound	Molecular weight	$\log K_{ow}$ [15]	pK <sub>a</sub> [16]
Atenolol	266.34	-0.03	13.1
Captopril	217.28	0.84	?
Metformin	129.17	-2.64	12.4

Sample processing in SPE involved four major steps [17]:

- 1) Conditioning of sorbent to remove the impurities and improve the analyte retention by solvating the functional groups with a proper solvent. Then, the sorbent is rinsed with the sample solvent is used to remove the conditioning solvent.
- 2) Retention of analyte by the sorbent, involving different interactions. The flow-rate should be chosen to assure a maximal retention.
- 3) Washing is optional; the sorbent is rinsed with a weak solvent to remove the interfering compounds without eluting the analytes.
- 4) Desorption of analytes from the sorbent with a strong solvent for further chromatographic or spectrometric analysis. In the overall process flow-rate value is important owing to its influence on retention and desorption steps.

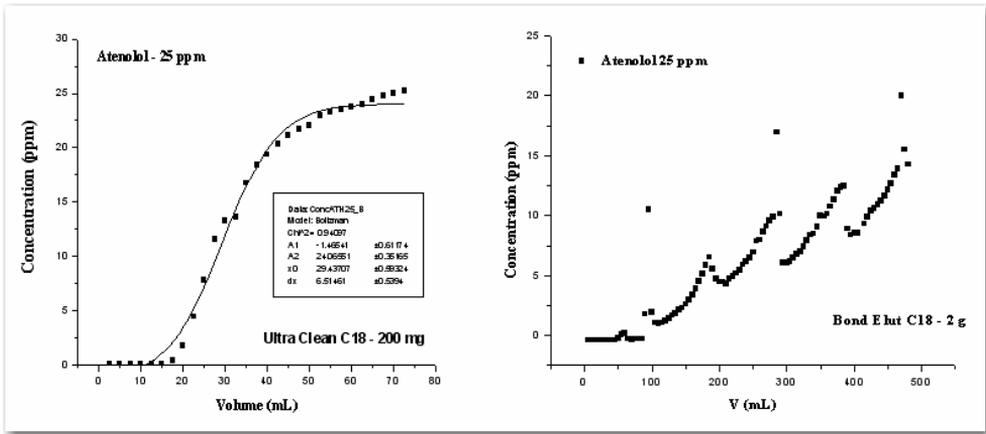
The procedure for solid phase extraction of atenolol and captopril on Ultra Clean C18 and Bond Elut C18 cartridges is presented in the diagram below.



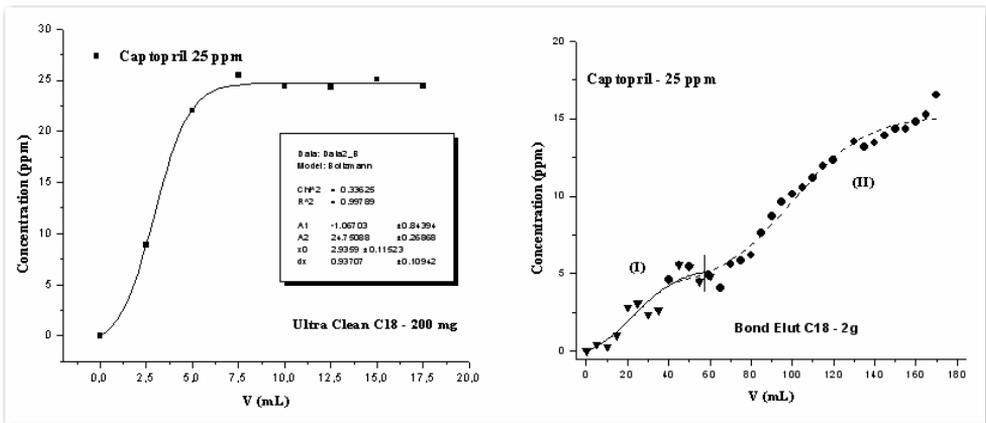
**Fig. 2** *Schematic diagram of the SPE used procedure for elution curve,  $C = f(V)$ .*

The retention parameters can be estimated from three characteristic points of sigmoid shape breakthrough curve (as can be seen in the following figures) [18]:

- $V_B$  – the breakthrough volume, which corresponds to 1% of maximum concentration of the analyte in the effluent; it indicates the volume that can be loaded on the sorbent with no loose of the solutes.
- $V_R$  – the retention volume, or inflection point of the curve, where the solute adsorption is in equilibrium with desorption from sorbent surface.
- $V_M$  – the hold-up volume, which corresponds to the volume at which analyte concentration entering and exiting the cartridge is almost the same.



**Fig. 3** Dependence of atenolol concentrations on successive 2.5 mL volume loading of aqueous samples and modeling by Boltzmann's function.



**Fig. 4** Dependence of captopril concentration on successive 2.5 mL volume loading of aqueous samples and modeling by Boltzmann's function.

The experimental breakthrough curves obtained for studied compounds given in Fig. 3 and Fig. 4 can be fitted by Boltzmann's function (eq. 1) and from them the estimation of the  $V_B$ ,  $V_R$ ,  $V_M$  values can be achieved using the formulas presented elsewhere [19]:

$$Y = A_2 + \frac{A_1 - A_2}{1 + e^{\frac{x-x_0}{dx}}} \quad (1)$$

$$V_B = x_0 + (dx) \cdot \ln \left[ \frac{100}{99} \left( 1 - \frac{A_1}{A_2} \right) - 1 \right] \quad (2)$$

$$V_M = x_0 + (dx) \cdot \ln \left( 99 - 100 \cdot \frac{A_1}{A_2} \right) \quad (3)$$

Atenolol and captopril are retained on C18 adsorbent as a result of interactions with the hydrophobic chains and free silanol groups from its matrix. A significant difference between the adsorption capacities for atenolol and captopril were observed for the two types of used cartridges containing different adsorbent mass. The atypical shape of atenolol breakthrough curve obtained for Bond Elut C18 sorbent could be explained only by change of interactions between analyte and the sorbent.

In case of captopril, two profiles were for the breakthrough curve, which may be explained by two distinct adsorption processes occurring in SPE process of this compound due to the higher content of residual silanol groups in Bond Elut cartridge than in Ultra Clean cartridge (Fig. 4). Data processing of the breakthrough curves leads to the main parameters given in Table 3.

**Table 3** Breakthrough parameters for captopril.

Compound	Cartridge type	$V_B$	$V_R$	$V_M$	$k$	$N$
	Ultra Clean C18	0.18	2.93	7.27	1.49	5
Captopril	Bond Elut C18 (curve I)	1.66	22.87	79.45	2.47	5
	Bond Elut (curve II)	–	99.77	177.64	0.78	–

The breakthrough parameters were estimated from experimental curves and their regression parameters. The number of theoretical plate ( $N$ ) can be calculated using the relationship proposed by Werkhoven-Goewie [20,21]:

$$V_B = V_R \times \frac{\sqrt{N} - 2}{\sqrt{N}} \quad (4)$$

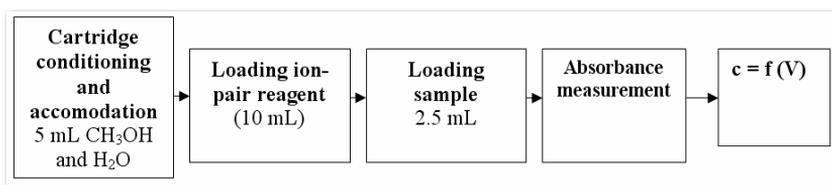
and the formula for the retention factor ( $k$ ), which have influence on recovery, is [22-24]:

$$V_M = V_R (1 + k) \quad (5)$$

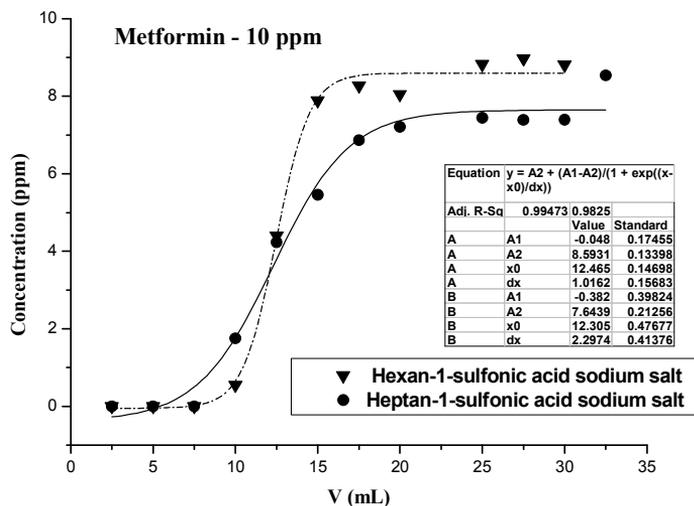
In case of metformin, it was taken into account its high polarity and the possibility of ion-pairing with different reagents, such as alkylsulphonates [25]. Thus, the literature reports a method for the determination of metformin in plasma using 2 mM sodium dodecylsulphate as ion pairing reagent for SPE [26]. Another method has been described for the

determination of metformin in human plasma based on solid-phase extraction on a C18 cartridge and methanol + 3% acetic acid elution followed by CE-UV analysis [27].

In this work we used hexan-1-sulfonic sodium salt and heptan-1-sulfonic sodium salt as ion-pairing reagents for metformin extraction, in order to view whether the length of hydrocarbonaceous chain from ion pairing reagent may influence the extraction parameters. For this purpose, the following procedure for solid phase extraction of metformin using these ion pair reagents is presented in the diagram given below. This time the SPE procedure was based on two distinct adsorption processes: 1) the retaining of ion pairing reagent onto the surface of the SPE sorbent; 2) the adsorption of analyte onto the new adsorption surface owing to the strong polar interactions between metformin molecules and ionic groups from the ion pairing reagent adsorbed onto the surface of C18.



**Fig. 5** Schematic diagram of the SPE used procedure for ion pairing mechanism.



**Fig. 6** Dependence of metformin concentrations on successive 2.5 mL volumes loading of aqueous samples and their modeling by Boltzmann's function.

In Fig. 6 the breakthrough curves for both reagents used are comparatively presented. The breakthrough parameters for the two curves are given in Table 4, where one can observe that the two experimental curves are characterized by a same retention volume ( $V_R$ ), which means that the ion pairing reagents used in these experiments do not influence significantly the retention process of studied compound.

**Table 4** Breakthrough parameters for calculated for metformin.

Compound	Ion-pair reagent	$V_B$	$V_R$	$V_M$	$k$	$N$
Metformin	$C_6H_{13}SO_3Na$	8.25	12.47	17.14	0.38	35
	$C_7H_{15}SO_3Na$	5.87	12.31	22.98	0.87	15

## Conclusions

The SPE study on octadecyl silica sorbents showed that polar compounds can be retained by different mechanisms (reversed phase or ion pair), and the breakthrough parameters for this process can be calculated from the experimental dependence between analyte concentration from eluate and the sample volume applied to the cartridge. Low hydrophobicity of two of the studied compounds showed that SPE can be applied to their enrichment from aqueous samples. When the analyte is strongly polar, an ion pairing mechanism should be applied, using a reagent containing a hydrocarbonaceous chain in their molecule, such as sodium salts of alkylsulphonates.

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