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BREAKTHROUGH PARAMETERS OF SPE PROCEDURE ON C18 CARTRIDGES FOR SOME POLAR COMPOUNDS

Elena BACALUM, Medeea RADULESCU, Emilia-Elena IORGULESCU and Victor DAVID*

University of Bucharest, Faculty of Chemistry, Department of Analytical Chemistry, Sos. Panduri, no. 90, sect 5, Bucharest, 050663, Roumania

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The SPE retention of three polar compounds of pharmaceutical interest (atenolol, metoprolol, and pentoxifylline) was studied on octadecylsilicagel based sorbent. The breakthrough parameters (the hold-up, breakthrough, and retention volumes) were measured for chosen model solutes from the experimental data obtained by percolating the C18 cartridge with stock solutions for each compound. The experimental curves were fitted by means of Boltzmann's function, and the main regression parameters were used in calculating the breakthrough parameters. The breakthrough volumes for atenolol and metoprolol are lower for acidic solutions compared to neutral solutions, and it keeps a constant value for pentoxifylline. Although these solutes are multi-functional polar compounds the find-outs showed that these compounds can be enriched from aqueous solutions by retaining them on C18 sorbents. The analytical procedure allows a minimum concentration factor of 10, within the concentration interval of 0.1 – 2 ppm. The measurements were performed by UV absorption at wavelengths specific to each studied compound.

INTRODUCTION

Solid-phase extraction (SPE) is an alternative procedure to liquid-liquid extraction that is mainly used for retaining specific compounds from aqueous solutions, and it is based on adsorption of analytes on solid materials.[1] The major adsorption parameters characterizing a SPE procedure are efficiency and breakthrough volume,[2,3] which may influence some of the main analytical parameters of a determination process (selectivity, concentration ratio, and sample capacity). From this point of view, one of the most important parameters is the breakthrough volume, which influences the maximum volume of aqueous sample that can be loaded into a cartridge (adsorption capacity).

A large number of sorbents are used in analytical SPE procedures, most of them deriving from silica matrix.[4-6] Among them octadecyl based silica are still of large interest in using them as retaining materials for a wide variety of organic compounds from aqueous samples.[7-9] Although their intrinsic property relies on hydrophobic surface, these materials can be successfully utilized in isolation and enrichment of low hydrophobic or even polar compounds.[10-14] The physical characteristics of the sorbents, such as their surface area, particle size, pore size, or pore volume are also crucial properties in different applications with analytical purposes.[15]

The extraction ability of sorbents in the SPE bed depends also on: (i) the bed capacity; (ii) the loaded volume of sample, (iii) the nature and volumes of conditioning solvents and eluents.[16] The aim of this paper is to investigate the retention behavior of three polar organic compounds chosen as model solutes (atenolol, metoprolol, and pentoxifylline) on C18 sorbent and to estimate some breakthrough volume parameters and the cartridge capacity for certain analytical enrichment purposes.

* Corresponding author: Vict_David@yahoo.com

EXPERIMENTAL

Materials and methods: Two of the studied compounds were β-blockers: atenolol, and metoprolol, and the third studied compound was pentoxifylline (used in the treatment of cerebrovascular and peripheral vascular diseases), which were kindly offered by LaborMed Pharma S.A. Their structures are shown in Fig. 1.

Methanol was HPLC (gradient) grade from Merck (Darmstadt, Germany). Water (resistivity minimum 18.2 MΩ and TOC maximum 30 ppb) was produced within the laboratory with a TKA Lab HP 6UV/UF instrument.

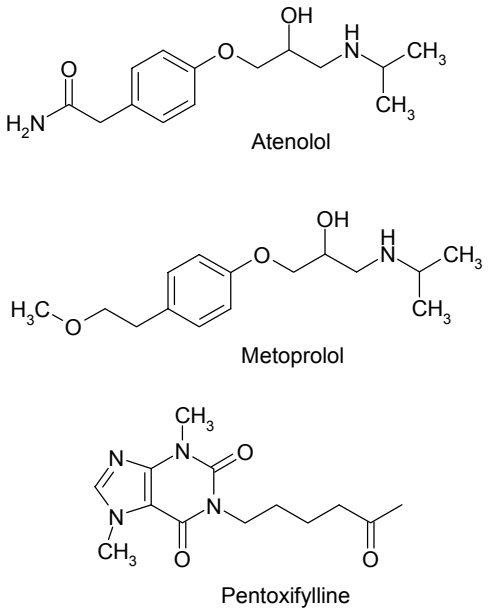

Fig. 1 – Chemical structures of studied compounds.

Preparation of solutions: Three 100 ppm stock solutions of atenolol, metoprolol and pentoxifylline were prepared either in water or methanol. Working solutions were prepared by successive dilutions of the stock solution for obtaining eight calibration standards, ranging from 1 to 25 ppm.

Equipments: A manual SPE system (Alltech) was used for this study. The cartridge used (sample loading of 4 mL) contained C18 reversed-phase chemical modified sorbent (Ultra Clean) with the following characteristics: sorbent mass 200 mg, having 5.6% C and surface area 498 m^2/g, average pore size: 60 A and particle size 50 μm. Cartridges were loaded on a vacuum manifold, to which a pressure of 15 mmHg was applied for all samples. The vacuum value is of high importance because of its influence on the flow value.

The absorption spectra were recorded with a Jasko V-530 double beam spectrophotometer, in 1 cm quartz cells. The λ_{max} values of studied compounds were determined by taking scans of the standard solutions in the UV region (200-350 nm). The absorbances were measured at 225 nm for both β-blockers, and 274 nm for pentoxifylline. Calibration curve data were achieved in the range 1-25 ppm for each of the studied compounds, either in water, or methanol as solution solvents. The main regression parameters for these compounds in the two solution solvents used in this study are given in Table 1. All measurements were performed at room temperature.

Table 1

Calibration equations for studied compounds in methanol and water.
(A – absorbance; C – concentration; r^2 – determination coefficient)

Compound	methanol		water	
	Equation	r^2	Equation	r^2
Atenolol	A = 0.0460·C – 0.0110	0.9928	A = 0.0439·C + 0.0163	0.9986
Metoprolol	A = 0.0464·C + 0.0002	0.9961	A = 0.0293·C + 0.0280	0.9998
Pentoxifylline	A = 0.0645·C + 0.0088	0.9972	A = 0.0743·C - 0.0027	0.9998

Off-line SPE procedure: The sample 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 is 2.5 ml. Each eluate was collected separate and the analyte concentration was spectrometrically measured and the values were plotted by means of Origin program.

RESULTS AND DISCUSSION

The three studied compounds are characterized by a low hydrophobicity character, given by octanol/water partition constant (log K_{ow}). Calculation of this parameter by means of

fragment methodology[17,18] leads to the following theoretical values, which can be compared to the experimental values (Table 2). This low hydrophobic character results in a low or moderate retention in reversed-phase liquid chromatography using either C8 or C18 stationary phases.[19,20]

Table 2

Hydrophibicity parameters for studied compounds

Compound	CAS number	log K_{ow} (experimental)	log K_{ow} (calculated)
Atenolol	29122-68-7	0.16	-0.03
Metoprolol	37350-58-6	1.88	1.69
Pentoxifylline	6493-05-6	0.56	0.29

The retention study of these model compounds on C18 sorbent was achieved according to the standard SPE procedure. Consequently, sample processing in SPE involved four distinct steps:

Conditioning of sorbent before use with the appropriate solvent (methanol – MeOH) in order to improve the reproducibility of analyte retention. Next, the conditioning solvent is rinsed from the sorbent with the same solvent as the sample solvent.

Retention step when the analyte will be retained by the sorbent and thus preconcentrated. The flow rate should be small enough for maximal retention.

Optional washing step with a weak solvent to displace unwanted matrix components from the sorbent material without displacing the analytes.

Desorption step of analytes from sorbent with a small volume of an appropriate solvent for further chemical analysis.

The entire experimental procedure used in this study is schematically described in Fig. 2.

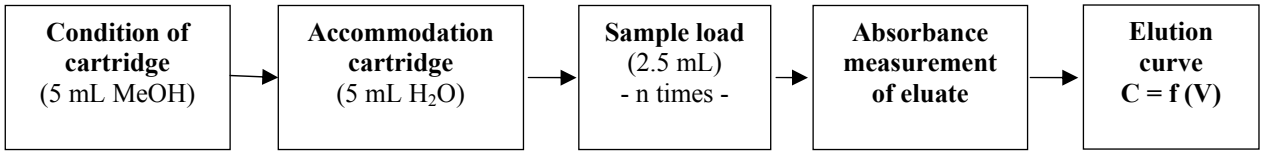

Fig. 2 – Schematic diagram of the SPE used procedure (V being the cumulative volume of loaded solution).

Breakthrough volume (V_B) can be defined as the volume giving an assumed breakthrough level (the ratio of the outlet to inlet concentration).[16] The breakthrough volume depends on kinetic parameters of sorbent and on retention parameters as well as on the flow rate of the sample. Usually, the breakthrough curve has a sigmoid shape (as can be seen in the following figures) and the retention parameters can be estimated from its characteristic points as following:

V_B – the volume of the mobile phase, that corresponds to 1% of maximum concentration of the analyte in the effluent;

V_M – the hold-up volume, the volume of the mobile phase that corresponds to 99% of the maximum concentration in the effluent;

V_R – the retention volume of the model solute, which corresponds to the inflection point of the curve, where the solute adsorption is in equilibrium with desorption from sorbent surface.

The best mathematical function used to fit the experimental data for a breakthrough curve is the Boltzmann's equation, written in the form used by Origin program:

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

where Y represents the analyte concentration in effluent, x is the volume of percolation using a single cartridge, A_1 and A_2 are two regression parameters, and x_0 is the inflexion point where Y becomes $(A_1 + A_2)/2$. The maximum value of Y is A_2, obtained for $x \to \infty$, while the minimum value of Y is approximately A_1, obtained for $x \to 0$. By means of these parameters we can calculate the characteristic points of the breakthrough curve as following: $Y = \frac{99}{100} \cdot A_2$ (for hold-up volume, V_M); $Y = \frac{1}{100} \cdot A_2$ (for breakthrough volume, V_B), and $V_R = x_0$ (for retention volume). The direct estimated parameter is however V_R, where the analyte concentration in effluent is $(A_1 + A_2)/2$.

Estimation of V_M and V_B from Boltzmann's equation can be achieved by setting the above two

conditions to the value of Y, and solving this equation, which leads to the following formula of breakthrough parameters:

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

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

The experimental breakthrough curves for studied compounds are given in Fig. 3 and Fig. 4, which are fitted by Boltzmann's function. As can be seen from Fig. 3 both β-blockers showed similar breakthrough curves, with closed breakthrough parameters. The values of these parameters estimated from experimental and regression parameters, using eq. (2) and (3), are given in the Table 3. However, a significant difference can be seen from the calculated and observed values of V_B for these measurements, and for this reason the best way of estimating V_B is from initial plateau, before sorbent breakthrough.

Table 3

Breakthrough parameters resulted from percolating of C18 cartridge with 25 μg/mL individual solutions

Compound	V_B (mL)		V_M (mL)	V_R (mL)	N	k
	Calculated	Observed				
Atenolol	12.5	20	57.7	29.2	40	0.97
Metoprolol	12.8	17.5	42.7	25.1	44	0.70
Pentoxifylline	84.2	95	121.1	103.4	606	0.17

By means of breakthrough volumes the adsorption capacity of SPE sorbent was calculated for atenolol (10.4 μmol/g sorbent), for metoprolol (9.4 μmol/g sorbent), and pentoxifylline (37.1 μmol/g sorbent). These differences between adsorption capacity towards β-blockers and pentoxifylline may be explained by possible adsorption of several molecules of pentoxifylline (at least three) on the adsorption sites of sorbent due to polar-polar interactions between the first pentoxifylline adsorbed on the sorbent surface and other pentoxifylline molecules. So far, such possible event has not been reported by literature, the only explanation for sorption property of modified silica surface being given by hydrophobic interactions between solute and hydrocarbonaceous chains (C18) covering its silica.

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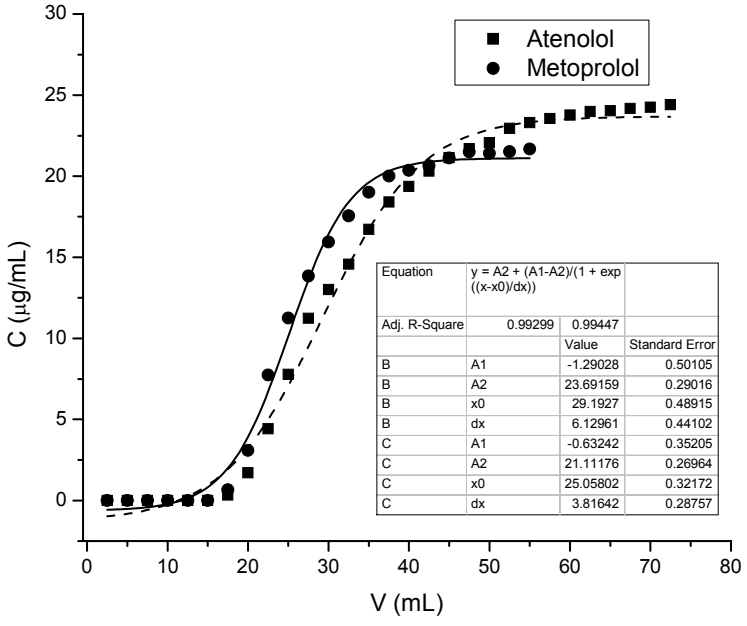

Fig. 3 – Dependence of atenolol and metoprolol concentrations on successive 2.5 mL volume loading of aqueous samples and modeling by Boltzmann's function.

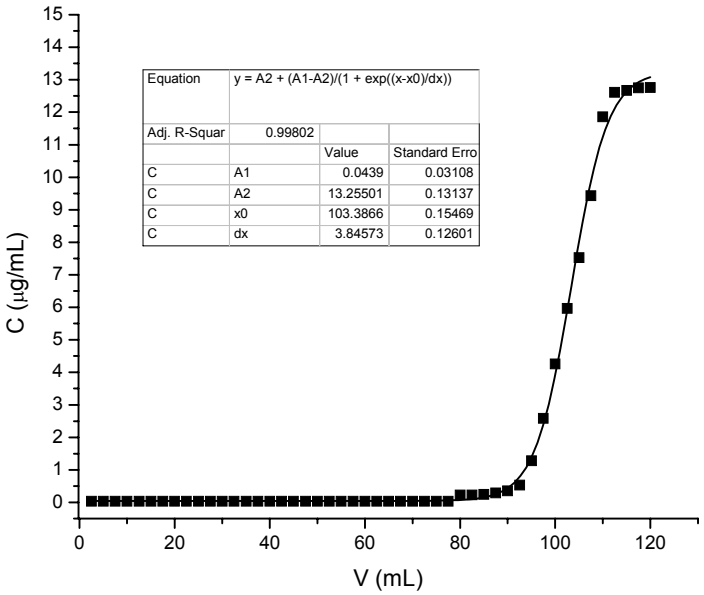

Fig. 4 – Dependence of pentoxifylline concentration on successive 2.5 mL volume loading of aqueous samples and modeling by a Boltzmann's function.

The number of the theoretical plate (N) corresponds to the efficiency of the SPE column and can be calculated from the breakthrough curve, using the relationship proposed by Werkhoven-Goewie:[15,16,23,24]

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

The retention factor (k) assigned to SPE process can be proved to depend on breakthrough parameters according to the simple relationship:[15]

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

The values of k and N, calculated by means of the above relationships are given in Table 3. Taking into consideration that the length of sorbent bed is 1 cm, that means the efficiency of SPE process is fairly closed to the efficiency obtained in LC separations

The similarity between SPE and liquid-chromatography (LC) retentions is generally accepted by literature. Consequently, the pH of loaded sample may play an important role in analyte retaining, just as is explained by partition model in LC retention process.[21] Two of the studied compounds (atenolol and metoprolol) contain secondary amino group, which can be protonated and thus the breakthrough parameters are likely to be influenced. The breakthrough curves for both forms of metoprolol, for instance, show a similar shape, but a different hold-up volume, as can be seen from Fig. 5. The sorbent breakthrough takes place for acidic solution volume of approximately of 10 mL. The breakthrough curves for pentoxifylline in acidic or neutral solutions were almost identical, showing that in this particular case the protonation of N atom(s) does not occur, the basicity of pentoxifylline being very low ($pK_b < 10$).[22]

Analytical application: This study revealed the possibility of retaining these polar compounds on very hydrophobic sorbents, which can be an alternative to the cartridges with a strong cationic resin sorbent,[25] or liquid-liquid extraction.[26] Analytical procedures for enrichment of these compounds from aqueous solutions were studied according to the schema given in Fig. 6. For this purpose six aqueous solutions at different concentration levels (0.1 - 2 ppm) were used. After conditioning of sorbent, the SPE cartridge was loaded with 20 mL sample, and then the compounds were eluted with the aid of 2 mL of methanol, followed by UV absorbance measurement.

The three graphs for these analytical procedures are depicted in Fig. 7, whose pretty good correlation coefficients can be observed. The sensitivity of calibration function for metoprolol was unexpectedly lower than that obtained for atenolol. Although these two compounds exhibited similar behavior in what is the adsorption process on C18 sorbent, it is likely that the desorption of metoprolol to be slower than for atenolol, and thus it influences the recovery value of this analyte.

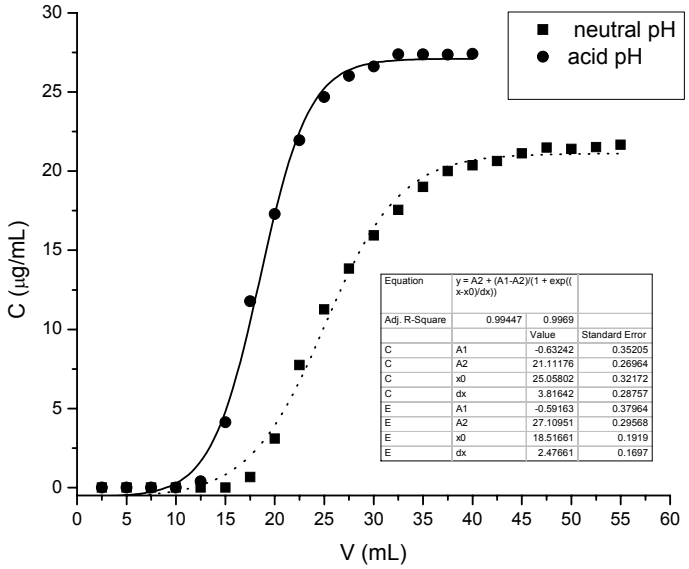

Fig. 5 – Comparison of the breakthrough curve for metoprolol between acid and neutral solution samples applied to SPE procedure.

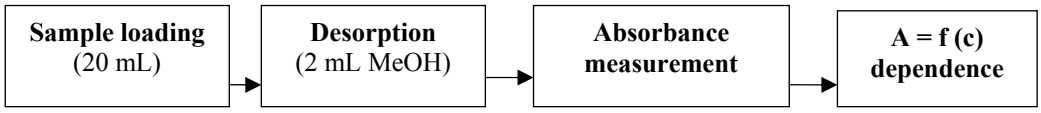

Fig. 6 – Schematic diagram of calibration procedure.

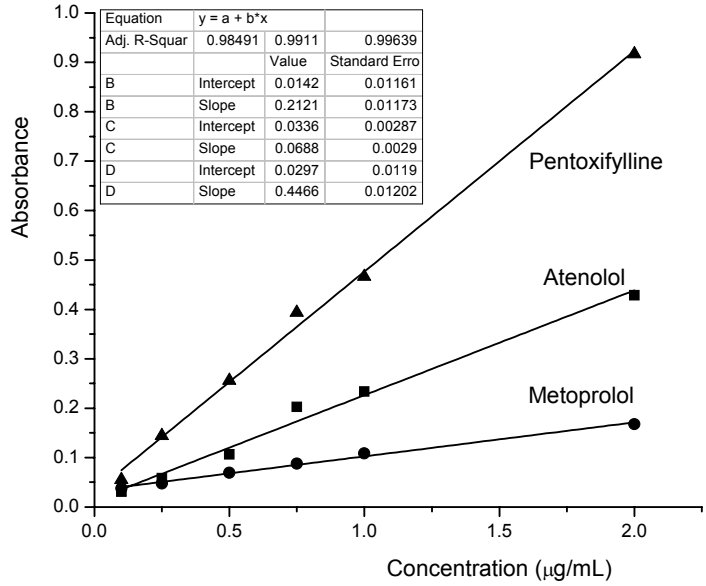

Fig. 7 – Calibration curves for the three studied compounds based on 10-fold enrichment on SPE procedure.

CONCLUSIONS

This SPE study performed on C18 sorbent showed expected breakthrough curves for three polar compounds, which may be useful in analytical applications. These curves were fitted by Boltzmann's function, whose regression parameters could be used in estimating the retention and hold-up volumes, and with a less probability the breakthrough volume, which can be however estimated from the real observations. Some retention parameters, such as efficiency and

retention factor, were relatively closed to the reversed-phase liquid chromatography with which this analytical technique is phenomenologically compared.

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