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Use of a novel cation-exchange restricted-access material for automated sample clean-up prior to the determination of basic drugs in plasma by liquid chromatography

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Abstract

A new kind of silica-based restricted-access material (RAM) has been tested in pre-columns for the on-line solid-phase extraction (SPE) of basic drugs from directly injected plasma samples before their quantitative analysis by reversed-phase liquid chromatography (LC), using the column switching technique. The outer surface of the porous RAM particles contains hydrophilic diol groups while sulphonic acid groups are bound to the internal surface, which gives the sorbent the properties of a strong cation exchanger towards low molecular mass compounds. Macromolecules such as proteins have no access to the internal surface of the pre-column due to their exclusion from the pores and are then flushed directly out. The retention capability of this novel packing material has been tested for some hydrophilic basic drugs, such as atropine, fenoterol, ipratropium, procaine, sotalol and terbutaline, used as model compounds. The influence of the composition of the washing liquid on the retention of the analytes in the pre-column has been investigated. The elution profiles of the different compounds and the plasma matrix as well as the time needed for the transfer of the analytes from the pre-column to the analytical column were determined in order to deduce the most suitable conditions for the clean-up step and develop on-line methods for the LC determination of these compounds in plasma. The cationic exchange sorbent was also compared to another RAM, namely RP-18 ADS (alkyl diol silica) sorbent with respect to retention capability towards basic analytes.

Keywords: Cation-exchange restricted-access material; Column-switching; Sample preparation; Stationary phases, LC; Atropine; Fenoterol; Ipratropium; Procaine; Sotalol; Terbutaline

1. Introduction

Methods for the quantitative analysis of drugs in plasma most often involve nowadays the use of reversed-phase liquid chromatography (LC). The majority of bioanalytical procedures include one or more sample preparation steps in order to eliminate proteins and other matrix macromolecules, isolate the analyte(s) from potentially interfering low molecular mass sample components and increase the analyte concentration.

Column-switching systems in which a pre-column is coupled to the analytical LC column via a switching valve has been used successfully for the

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quantitative analysis of drugs and metabolites in biological media and especially in plasma. In these column-switching systems, the use of restricted-access material (RAM) in the pre-column is an approach which permits the direct injection of protein-rich samples, such as plasma. A family of restricted access sorbents, namely alkyl diol silica (ADS), belonging to the group of internal surface reversed-phase (ISRP) supports, was developed by Boos et al. a few years ago [1,2]. Low molecular mass compounds such as drugs can have access to the internal surface of the sorbent, on which either butyryl (C_4), capryloyl (C_8) or stearoyl (C_{18}) moieties are bonded. These compounds are retained mainly by hydrophobic interactions while macromolecules like proteins are excluded and eluted directly from the pre-column. The access restriction is obtained by use of silica particles (25 μm) with an appropriate pore diameter (6 nm). Moreover, the adsorption and denaturation of proteins is prevented by hydrophilic and electroneutral diol groups present on the external surface of the particles. These restricted access supports have been applied successively for the clean-up of biological samples in column-switching systems [3–14].

Nevertheless, due to the main retention mechanism of the ADS material, on-line LC methods based on this technique for sample clean-up could present a lack of selectivity, especially in the case of analytes characterised by an absorption maximum at low wavelengths in UV and/or by low molar absorptivities. Moreover, this kind of material could not retain sufficiently the hydrophilic drugs due to too weak interactions with the inner surface of the sorbent.

Therefore, a novel silica-based RAM, namely XDS (exchange diol silica) sorbent, has been recently developed. The pore diameter of the silica particles is also about 6 nm, yielding a molecular mass cut-off of ~15 kDa. Due to this physical diffusion barrier, macromolecules, such as proteins, have no access to the inner surface, to which sulphonic acid groups are bonded via short chain alkyl spacers. Consequently, this sorbent presents the properties of a strong cation exchanger towards low molecular mass compounds, such as basic drugs. Moreover, some hydrophilic diol groups are also bound to the external surface of the silica particles, which prevent the adsorption of proteins.

To our knowledge, only one application based on the coupling of this kind of RAM to LC in a column-switching system has been reported. Such a system was used for the analysis of neuropeptide Y and its metabolites in plasma [15].

The aim of the present work is to test the retention capability of this cation-exchange restricted-access material for some hydrophilic basic drugs, such as atropine, fenoterol, ipratropium, procaine, sotalol and terbutaline, used as model compounds. As far as we know no automated methods based on the coupling of pre-columns packed with restricted-access material to LC have been reported for the determination of these drugs in plasma.

The primary goal is to investigate the influence of the composition of the washing liquid on the retention of the analytes on the cationic exchange sorbent. Different factors, such as the nature and the concentration of the competing ion, the pH and the organic modifier content of the washing liquid were studied. The elution profiles of the different compounds and the plasma matrix as well as the time needed for the transfer of the analytes from the pre-column to the LC column were determined in order to deduce the most suitable times for the rotation of the switching valve. The second objective consists of deducing generic conditions for sample clean-up and developing fully automated methods for the LC determination of the compounds of interest in plasma. The cationic exchange sorbent was also compared to another RAM, namely RP-18 ADS sorbent, with respect to the retention capability towards the basic drugs selected.

2. Experimental

2.1. Chemicals and reagents

Atropine sulphate, fenoterol hydrobromide, procaine hydrochloride, ipratropium bromide, sotalol hydrochloride and terbutaline sulphate were obtained from Sigma (St. Louis, MO, USA) and were used without further purification. Potassium dihydrogenphosphate dihydrate, potassium hydroxide, phosphoric acid (85%), perchloric acid, sodium perchlorate, potassium perchlorate, lithium perchlorate and magnesium perchlorate were purchased from Merck (Darmstadt, Germany) and were of analytical grade.

1-Butanesulphonic acid and 1-octanesulphonic acid sodium salts as well as a solution of tetrabutylammonium hydroxide (40%) were supplied by Sigma. Methanol and acetonitrile were LiChrosolv LC gradient grade solvents purchased from Merck. The water used in all experiments was purified by means of a Milli-Q system (Millipore Corporation, Bedford, MA, USA). Human plasma samples were obtained from the Blood Transfusion Centre of Liège (Belgium).

The pre-columns were pre-packed with LiChrospher 60 XDS (SO_3/Diol) (supplied as research samples) (particle size, 25 μm) and LiChrospher 60 RP-18 ADS (particle size, 25 μm) from Merck.

The analytical column pre-packed with LiChrospher 60 RP-Select B (particle size, 5 μm) was obtained from Merck.

2.2. Apparatus

The LC-integrated sample clean-up system was composed of a model 422 LC pump from Kontron Instruments (Schlieren, Switzerland) (pump 1) and the following units from Merck-Hitachi: a model L-6200 A pump (pump 2), a model AS-2000 A autosampler equipped with a 100-μl injection loop and a model L-4250 UV–Vis detector. A schematic representation of this column-switching system was shown elsewhere [8].

The LiChroCART pre-column (25×4 mm, I.D.) packed with restricted-access material from Merck was fitted to a Valco model VICI AG six-port switching valve (Valco Europe, Schenkon, Switzerland). A replaceable in-line filter (2–5 μm, sieve) contained in a holder (Merck) was also installed between the sample injector and the pre-column.

The analytical column was a LiChroCART column (250×4 mm, I.D.) from Merck and was thermostated at 25±0.1 °C in a model L-5025 programmable column oven (Merck).

The different modules were connected through an interface (D-6000, Merck) with an IBM compatible computer (PC-AT; CPU type Pentium) on which the D-7000 HPLC manager software was loaded for the control of the analytical system and data collection. The model 422 pump from Kontron (pump 1) was controlled manually.

2.3. Chromatographic conditions

The chromatographic separations were performed in the isocratic mode at 25 °C using a constant flow-rate of 1.0 ml/min. The composition of the different LC mobile phases is presented in Table 1. Prior to use, the latter were degassed for 15 min in an ultrasonic bath. The UV detection was performed at 220 nm, except for sotalol and fenoterol that were monitored photometrically at 230 nm.

2.4. Standard solutions

2.4.1. Stock solutions

Stock solutions of each analyte were prepared in methanol at a concentration of 1.0 mg/ml and were stored in a refrigerator at 4 °C when not in use.

Table 1
Composition of the different mobile phases for the LC separation of the compounds

Compound	Organic modifier (O.M.)	O.M. (%)	Buffer pH	Ion pairing (I.P.) agent	Conc. of the I.P. agent (m*M*)
Sotalol	MeOH	20	7.0	$O.S.^-$	1
Fenoterol	MeOH	20	7.0	–	–
Terbutaline	ACN	18	3.0	$B.S.^-$	0.5
Atropine	ACN	20	3.0	$B.S.^-$	0.5
Ipratropium	ACN	20	3.0	$B.S.^-$	0.5
Procaine[a]	ACN	20	7.0	–	–

Mobile phase, 50 m*M* phosphate buffer–O.M. (v/v) containing or not octanesulfonate ($O.S.^-$) or butanesulfonate ($B.S.^-$). ACN, acetonitrile; MeOH, methanol.

[a] The mobile phase buffer contained tetrabutylammonium hydroxide at a concentration of 5 m*M* and the pH of the solution was adjusted to 7.0 after the addition of this agent.

2.4.2. Intermediate solutions

Intermediate solutions of each compound were prepared by diluting the stock solutions with water to obtain a concentration of 50 μg/ml. These intermediate solutions were stored in a refrigerator at 4 °C and were found to remain stable for at least 1 week. They were then diluted with water or drug-free human plasma to reach final concentrations of 5 μg/ml and 0.1 μg/ml for each analyte. The latter solutions were prepared daily.

2.5. Automated sample preparation

After thawing at ambient temperature and centrifugation of the plasma sample at 3900 *g* for 10 min, an aliquot (1.0 ml) was introduced into a vial (1.5 ml) located in the appropriate rack of the autosampler. All other sample handling operations were then executed automatically.

Unless stated otherwise, the automatic sequence was performed in the following way.

- Sample application and washing step (flow-rate, 1.0 ml/min): 100 μl of plasma sample were injected onto the XDS pre-column with a washing liquid delivered by pump 1 and consisting of a mixture of 2 m*M* lithium perchlorate and methanol (97:3, v/v). The pre-column was then washed with this solution for 10 min. For the clean-up of samples containing atropine, fenoterol, ipratropium or procaine, the solution of lithium perchlorate was first adjusted to pH 3.0 with 1 *M* perchloric acid. During the washing step, the analytical column was re-equilibrated with the LC mobile phase delivered by pump 2 at a flow-rate of 1.0 ml/min.
- Transfer (flow-rate, 1.0 ml/min): by rotation of the switching valve, the analytes were then eluted in the back-flush mode with the LC mobile phase and transferred to the analytical column.
- Reconditioning (flow-rate, 1.0 ml/min): 2 min later, the switching valve was returned to its initial position after complete transfer of the analytes, allowing the XDS pre-column to be re-equilibrated with the washing liquid for 5 min before the handling of the next sample. Simultaneously, the chromatographic separation was started.

3. Results and discussion

3.1. Study of the retention properties of the XDS sorbent

In order to study the retention properties of the XDS sorbent, some hydrophilic basic drugs (pK_a values comprised between 7.5 and 9.8) were selected as model compounds. As can be seen in Fig. 1, fenoterol, sotalol and terbutaline contain a secondary amino group, while atropine possesses a tertiary amino group and ipratropium is a quaternary ammonium. As for procaine, it contains a primary aromatic amino group as well as a tertiary amino group.

Since the sorbent is made of cation exchange material, the retention of these cationic analytes can be expected to be mainly due to electrostatic interactions with the sulphonic acid groups bonded on the inner surface of this sorbent.

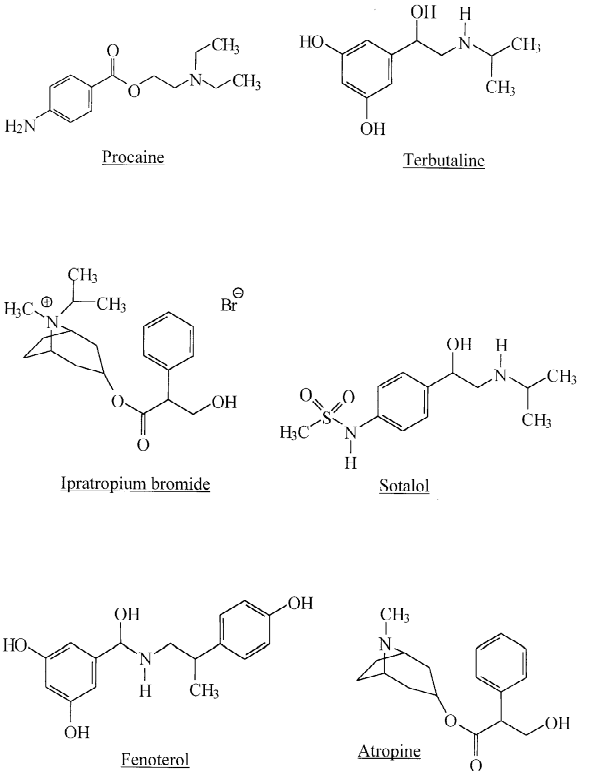

Fig. 1. Structures of procaine, terbutaline, ipratropium, sotalol, fenoterol and atropine.

The study of the retention capability of this kind of material consisted of determining the elution profiles of each compound with different washing liquids by monitoring the UV absorbance of aqueous solutions of the different analytes injected on the pre-column connected directly to the UV detector set at 230 nm. At each experiment, the retention factors (k) and the breakthrough volumes (V_b), corresponding to the beginning of the elution of the analyte, were determined for each compound of interest. This breakthrough volume corresponds to that observed in frontal analysis when a solution of an analyte having an initial absorbance A_0 is percolated through a pre-column. The breakthrough volume (V_b) is equal to the volume from which a peak or a breakthrough curve can be observed and it is usually defined at 1% of the initial absorbance [16]. Instead of determining breakthrough volumes experimentally, it is possible to calculate them from the following expression [16]:

$$V_b = (1 + k')(1 - 2.3/\sqrt{N})V_M \quad (1)$$

where V_M is the void volume of the pre-column and N is the number of theoretical plates.

Indeed, the measurement of breakthrough volumes is sometimes difficult, especially when the analyte is strongly retained on the sorbent or it exhibits poor UV properties.

Table 2 shows the influence of the concentration of sodium perchlorate used as washing liquid on the breakthrough volume and the retention factor of the compounds. The different breakthrough volumes were measured experimentally and were comparable to those obtained by using Eq. (1). As expected, an increase of the ionic strength of the washing liquid gave rise to a decrease in the breakthrough volumes and the retention factors of the analytes, due to competition effects with the sodium ion. As can be seen in this table, procaine had the strongest affinity for the cationic exchange sorbent, probably due to a somewhat higher positive charge under these conditions. Besides, the breakthrough volumes for sotalol and terbutaline were comparable, as well as those for atropine and fenoterol. From these results, it can be concluded that other interactions than electrostatic interactions with the sulphonic acid groups of the sorbent influenced the retention of the tested compounds.

During the development of bioanalytical methods based on the on-line coupling of a pre-column packed with restricted access material to LC, a small amount of organic modifier, such as methanol, acetonitrile or 2-propanol, is usually added to the washing liquid in order to achieve the release of the drug to be analysed from the binding sites of the plasma proteins and to enhance sample clean-up [8,17].

Consequently, the retention of the analytes on the cationic exchange sorbent was tested with a washing liquid consisting of mixtures of 5 m*M* sodium perchlorate with methanol or acetonitrile. The percentage of the organic solvent in the washing liquid was varied from 0 to 12%. The results showed a significant decrease in retention only when the percentage of methanol was higher than 3%. More-

Table 2
Influence of the concentration of sodium perchlorate used as washing liquid on the breakthrough volumes (V_b) and the retention factors (k) of the analytes

Compound	Concentration of sodium perchlorate (m*M*)							
	2		5		10		20	
	V_b (ml)	k	V_b (ml)	k	V_b (ml)	k	V_b (ml)	k
Sotalol	19	108	7.4	36	3.1	16	1.5	8.9
Terbutaline	ND	ND	8.4	40	3.1	16	1.8	11
Fenoterol	ND	ND	18	79	6.9	40	2.5	20
Atropine	ND	ND	21	87	10	41	6.3	25
Ipratropium	ND	ND	30	111	13	53	8.2	35
Procaine	ND	ND	59	113	35	59	8.4	41

Washing liquid, solution of sodium perchlorate; flow-rate, 1.0 ml/min; sample, aqueous solution of each analyte (conc., 50 μg/ml); detection, UV at 230 nm; other conditions, see Experimental. ND, not determined.

over, the loss in retention was more pronounced with acetonitrile than with methanol. A content of 3% (v/v) of methanol in the washing liquid was thus considered as adequate.

The next step consisted of comparing the retention capability of the XDS sorbent using as washing liquids several solutions of sodium perchlorate at different concentrations, adjusted or not to pH 3.0 with 1 *M* perchloric acid. Fig. 2A and B illustrate the relationship of the inverse of the retention factor ($1/k$) for sotalol and terbutaline as a function of the concentration of sodium perchlorate varying from 5 to 25 m*M*. As mentioned above, a decrease in retention was observed for both compounds with increasing the concentration of sodium perchlorate, due to competition effects from sodium ion. Nevertheless, Fig. 2 shows some differences according to the pH of the washing liquid. At pH 3.0, the decrease in retention seems to be linear. A regression line with a coefficient of determination close to 1 could be fitted on the values of $1/k$. On the other hand, when using a simple solution of perchlorate as washing liquid, the relationship between the inverse of k and the concentration of sodium perchlorate presented a curvature. The retention of both compounds was higher than that observed at pH 3.0 and the difference in retention was more pronounced in the higher concentration range of sodium perchlorate. These results demonstrated that, when the washing liquid was not adjusted, the two compounds were retained due to electrostatic interactions not only with the sulphonic acid groups of the support, but also with residual silanol groups present on the silica surface. On the other hand, at pH 3.0, the effects of the residual silanol groups were much lower, since they were not ionised in this pH range. Consequently, the retention of both compounds was mainly due to interactions with only one kind of anionic group, which explained the linear relationship observed.

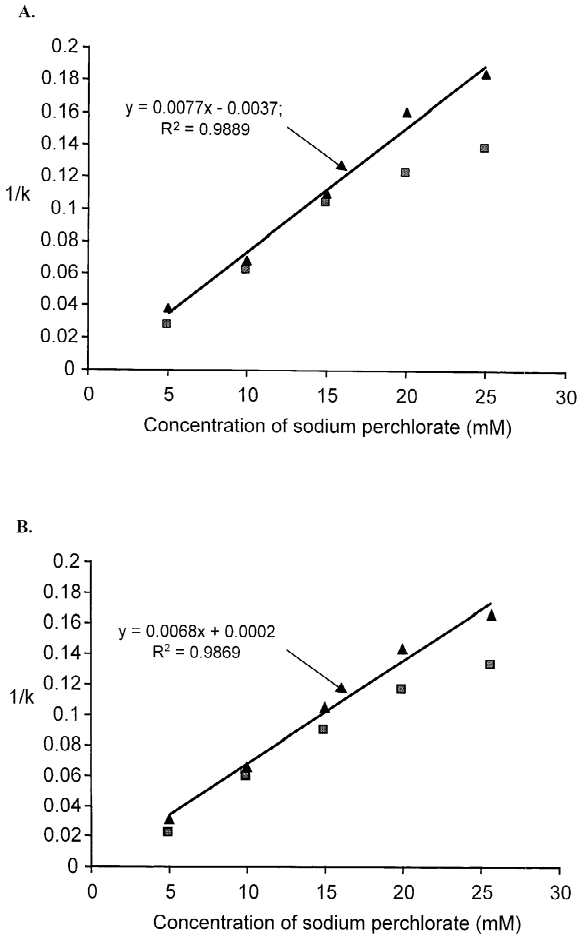

Fig. 2. Retention capability of the XDS sorbent towards sotalol and terbutaline using acidic and neutral solutions of sodium perchlorate as washing liquid. (A) Sotalol. (B) Terbutaline. Washing liquid, ■ solution of sodium perchlorate containing 3% (v/v) of methanol; ▲ solution of sodium perchlorate containing 3% (v/v) of methanol and adjusted to pH 3.0 with 1 *M* perchloric acid; flow-rate, 1.0 ml/min; sample, aqueous solution of sotalol or terbutaline (conc., 50 μg/ml); detection, UV at 230 nm; other conditions, see Experimental.

The nature of the co-ion could also influence the retention of the analytes on the XDS sorbent. Therefore, solutions of lithium, potassium and magnesium perchlorate were tested as washing liquids in order to evaluate their influence on the breakthrough volume and the retention factor of sotalol. As shown in Table 3, a lower breakthrough volume was obtained in the presence of magnesium ions, due to a stronger competition effect from this co-ion, compared to that observed from potassium, sodium or lithium ions. As expected, the size and the charge of the co-ions had a significant effect on retention. The order of the elution power of these inorganic co-ions was in agreement with data reported in a previous paper reporting the use of cation-exchange extraction cartridges [18]. Since the highest retention for sotalol (the least retained compound) was obtained with

Table 3
Influence of the nature of the co-ion present in the washing liquid on the breakthrough volume (V_b) and the retention factor (k) of sotalol

Co-ion of the washing liquid	Sotalol	
	V_b (ml)	k
Lithium	20	106
Sodium	17	81
Potassium	15	76
Magnesium	0.7	5.3

Washing liquid, solution of 2 m*M* lithium, sodium, potassium or magnesium perchlorate containing 3% of methanol (v/v); flow-rate, 1.0 ml/min; sample, aqueous solution of sotalol (conc., 50 μg/ml); detection, UV at 230 nm; other conditions, see Experimental.

2 m*M* lithium perchlorate containing 3% of methanol (v/v), this solution was finally selected as washing liquid.

Table 4 presents the breakthrough volumes and the retention factors of the different compounds of interest by using this washing liquid adjusted or not to pH 3.0 with perchloric acid. The retention of the analytes was higher when the washing liquid was not acidified. Indeed, the retention factors for sotalol and terbutaline were multiplied by a factor of about 2. The strongest affinity of the compounds for the XDS sorbent at neutral pH was certainly related to the combination of electrostatic interactions with sulphonic acid groups and residual silanol groups.

Table 4
Retention capability of the XDS sorbent towards the analytes using acidic and neutral solutions of lithium perchlorate as washing liquid

Compound	pH of the washing liquid			
	3.0		Not adjusted	
	V_b (ml)	k	V_b (ml)	k
Sotalol	13	53	23	110
Terbutaline	13	54	20	98
Fenoterol	25	127	>120	>200
Atropine	30	139	>120	>200
Ipratropium	43	185	>120	>200
Procaine	>120	>200	>120	>200

Washing liquid, 2 m*M* lithium perchlorate–methanol (97:3, v/v) adjusted or not to pH 3.0 with 1 *M* perchloric acid; flow-rate, 1.0 ml/min; sample, aqueous solution of each analyte (conc., 50 μg/ml); detection, UV at 230 nm; other conditions, see Experimental. V_b, breakthrough volume; k, retention factor.

3.2. Selection of generic conditions for automated sample preparation method

The next step consisted of determining the elution profile of the biological matrix. One hundred microlitres of a blank plasma sample were injected onto the pre-column connected to a UV detector set at 280 nm, using the selected washing liquid (a mixture of 2 m*M* lithium perchlorate and methanol (97:3, v/v)). The flow-rate of the washing liquid was obviously the same as that used for the determination of the elution profiles of the analytes (1.0 ml/min). As shown in Fig. 3, the UV absorbance was monitored and the fractionation step was considered complete when the detector signal reached the baseline. The time for a complete elimination of the sample matrix (T_M) was 10 min and corresponded to the first time for the rotation of the switching valve. No significant differences were observed between the elution profiles obtained by using the acidic washing liquid or the neutral washing liquid. During the clean-up step, the analytes had to be extracted and enriched on the cationic sorbent. For sotalol and terbutaline, the washing liquid was not acidified since the retention was not sufficient at pH 3.0. On the other hand, for the four other drugs, the pH of the washing liquid was adjusted to 3.0 in order to avoid a too strong retention on the sorbent and thus to facilitate their elution from the pre-column in the back-flush mode as well as their transfer to the analytical column by means of the LC mobile phase.

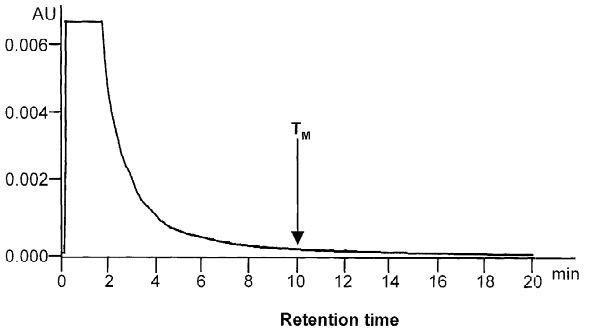

Fig. 3. Typical elution profile of a blank plasma sample. Washing liquid, 2 m*M* lithium perchlorate–methanol (97:3, v/v); flow-rate, 1.0 ml/min; injection volume, 100 μl; detection, UV at 280 nm; other conditions, see Experimental. T_M represents the time for the complete elution of the sample matrix.

The period of time needed to transfer the compounds of interest quantitatively from the pre-column to the LC column was then determined. Since the ionic strength and the percentage of organic modifier in the different LC mobile phases were much higher than in the washing liquid, the analytes were desorbed from the XDS pre-column in less than 2 min using a flow-rate of 1.0 ml/min. Under these conditions, peak compression could be expected at the top of the analytical column. A time period of 2 min was selected to transfer the compounds in a narrow elution band. Twelve min after sample application, the switching valve was returned to its initial position, allowing the XDS pre-column to be re-equilibrated with the washing liquid. The next sample application was carried out 5 min later and a new cycle could be started.

Figs. 4 and 5 illustrate typical chromatograms obtained by the analysis of a plasma sample spiked with fenoterol and atropine at concentrations of 5 μg/ml and 0.1 μg/ml, respectively, and a blank plasma sample obtained from six different sources of the same matrix. As can be seen in these figures, no interfering endogenous components of plasma were observed at the retention times of the analytes. The solvent front was also reduced even in the high-sensitivity range (Fig. 5). Consequently, the on-line coupling of a cationic exchange RAM to a chromatographic column constitutes a novel approach to enhance method selectivity for basic compounds, especially when UV detection is performed at low wavelengths.

Moreover, as can be seen in Table 5, satisfactory results were observed for repeatability and extraction efficiency. Indeed, the relative standard deviations obtained after the analysis of six plasma samples spiked with different compounds of interest were comprised between about 2 and 4.5%. As for extraction efficiency, the mean absolute recoveries were about 90%. Then, in order to demonstrate that the extraction efficiency was relatively constant over a concentration range, the absolute recoveries for sotalol were determined at three concentration levels. The mean recoveries were 94.2±1.5, 96.6±1.2 and 98.2±0.9% ($n=3$) at 10, 100 and 500 ng/ml, respectively. Such results show the constancy of the extraction efficiency over a relatively large concentration range.

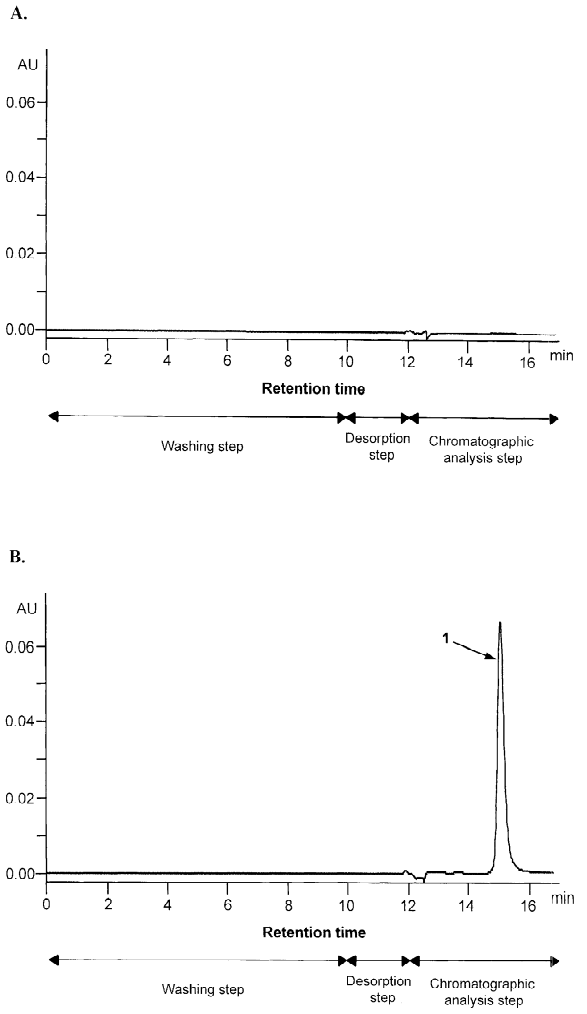

Fig. 4. Typical chromatograms obtained after on-line coupling of the cationic exchange pre-column to LC for the automated determination of fenoterol in plasma. (A) Chromatogram of a blank plasma sample. (B) Chromatogram of a plasma sample spiked with fenoterol (concentration, 5 μg/ml). Operating conditions given in Experimental. Peak 1, fenoterol.

3.3. Comparison of the retention capabilities of the XDS and RP-18 ADS pre-columns

The cationic exchange sorbent was then compared to another RAM, namely RP-18 ADS (alkyl diol silica) sorbent with respect to the retention capability towards the basic drugs selected as model compounds, using acidic and neutral washing liquids. As shown in Table 6, except for fenoterol which is a

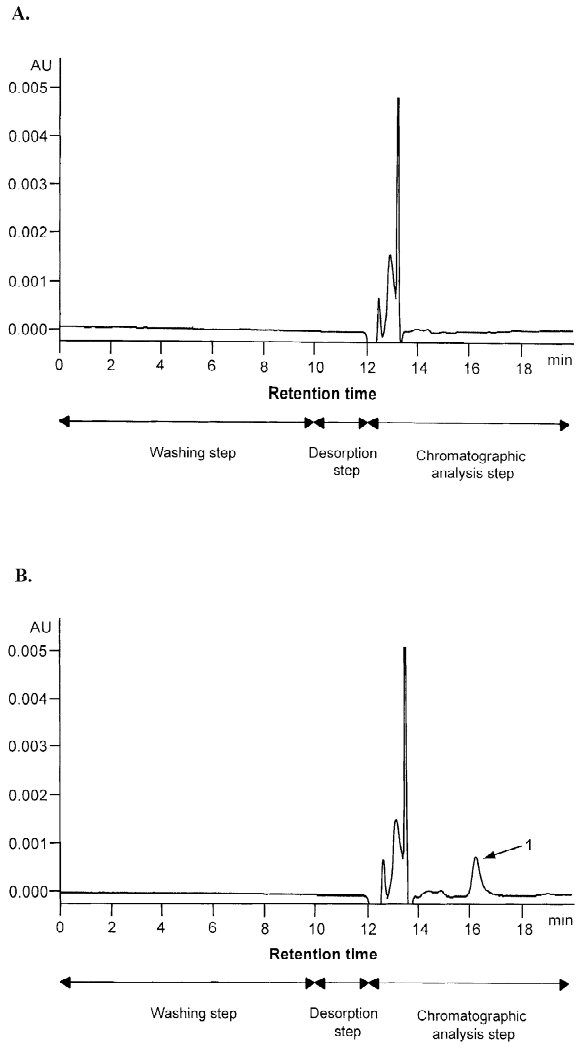

Fig. 5. Typical chromatograms obtained after on-line coupling of the cationic exchange pre-column to LC for the automated determination of atropine in plasma. (A) Chromatogram of a blank plasma sample. (B) Chromatogram of a plasma sample spiked with atropine (concentration, 100 ng/ml). Operating conditions given in Experimental. Peak 1, atropine.

more hydrophobic compound, the breakthrough volumes obtained with XDS material were larger than those observed with ADS sorbent, irrespective of the washing liquid pH. The retention factors were also higher. However, the retention factors of sotalol and terbutaline were comparable with the two sorbents when the washing liquid was neutral, but the peaks obtained with ADS material were broader and consequently these two compounds started to be eluted earlier from this material. The results presented in Table 6 also demonstrated the excellent retention capability of XDS sorbent for atropine and ipratropium.

Table 5
Repeatability and extraction efficiency

Compound	Repeatability (RSD, %; $n=6$)	Recovery (%; mean±SD; $n=3$)
Sotalol	2.0	95.6±1.2
Terbutaline	3.0	90.4±2.0
Atropine	4.1	89.5±1.8
Ipratropium	4.3	85.3±2.1
Fenoterol	1.5	93.2±1.1

Concentration level, 0.1 μg/ml, except for fenoterol (5 μg/ml). Other conditions, see Experimental.

The stability of the XDS pre-column was comparable to that of the ADS pre-columns. In order to maintain an optimal lifetime, it is important to centrifuge the samples, to add an organic modifier to the washing liquid used for the fractionation step and to install a replaceable in-line filter between the sample injector and the pre-column protecting the sieves and tubing from blocking. Under the proposed experimental conditions, the pre-column was shown to be functional for over 800 injections of 100 μl of plasma. Back-pressure was always lower than 6 bars. The lifetime of the XDS pre-column was equivalent to about 80 ml of human plasma.

4. Conclusions

The use of a recently developed cation-exchange restricted-access material, namely XDS sorbent, packed in pre-columns was studied for on-line sample clean-up prior to the determination of basic drugs in plasma by liquid chromatography. The retention capability of this sorbent was investigated using some hydrophilic basic compounds, such as atropine, fenoterol, ipratropium, procaine, sotalol and terbutaline, as model compounds.

In this study, the breakthrough volumes corresponding to the beginning of elution of the analytes from the pre-column were decreased with increasing the concentration, size and charge of the co-ion as well as the content of organic modifier in the

Table 6
Comparison of the retention capability of the RP-18 ADS and XDS sorbents towards basic drugs

Compound	Sorbent/pH of the washing liquid							
	ADS/ not adjusted		XDS/ not adjusted		ADS/ pH 3.0		XDS/ pH 3.0	
	V_b (ml)	k	V_b (ml)	k	V_b (ml)	k	V_b (ml)	k
Sotalol	3.2	45	7.8	36	0.8	12	6.5	28
Terbutaline	2.5	38	8.3	40	0.3	15	6.5	32
Fenoterol	>120	>120	18	79	13	69	13	58
Atropine	0.3	2.9	21	87	0.2	1.8	15	60
Ipratropium	0.5	1.7	30	120	0.2	1.7	24	88
Procaine	10	49	27	124	0.5	4.2	39	180

Sorbent, LiChrospher 60 RP-18 ADS or 60 XDS (SO_3/Diol) packed in LiChroCART pre-columns (25×4 mm; I.D.); washing liquid, 5 m*M* sodium perchlorate–methanol (97:3, v/v) adjusted or not to pH 3.0 with 1 *M* perchloric acid; flow-rate, 1.0 ml/min; sample, aqueous solution of each analyte (conc., 50 μg/ml); detection, UV at 230 nm; other conditions, see Experimental. V_b, breakthrough volume; k, retention factor.

washing liquid. The influence of the washing liquid pH on the retention of the analytes was also investigated. Moreover, the contribution of both sulphonic acid groups and residual silanol groups was demonstrated for the retention of the compounds at neutral pH.

From the elution profiles of the analytes and the plasma matrix, the times for the rotation of the switching valve were then determined in order to ensure good sample clean-up and complete transfer of the compounds to the LC column. Generic conditions for the clean-up step were also deduced. By coupling the cation exchange pre-column to the analytical column for the on-line determination of fenoterol and atropine in plasma, method selectivity was demonstrated towards endogenous components of plasma. Moreover, the comparison of the XDS sorbent to another RAM, namely RP-18 ADS material, demonstrated its higher retention capability for hydrophilic basic drugs.

Consequently, the coupling of this new kind of sorbent to LC seems to be a promising approach for automated sample clean-up and fully integrated on-line solid-phase extraction of basic drugs from directly injected biological fluids, such as plasma. In order to demonstrate the reliability of the developed methods, the complete validation of the method based on this sample preparation technique for the LC determination of sotalol in plasma is in progress and the full results will be presented.

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