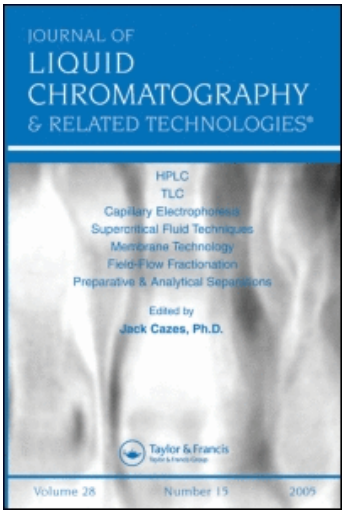

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COMPARISON OF VARIOUS SORBENTS FOR THE ENRICHMENT OF SAMPLES OF ALIPHATIC AMINES USING SOLID-PHASE EXTRACTION PRIOR TO THE DETERMINATION BY HPLC WITH FLUORIMETRIC DETECTION

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ABSTRACT

Possibilities of the determination of lower aliphatic amines by high performance liquid chromatography (HPLC) with fluorimetric detection after derivatization with o-phthaldialdehyde (OPA) and sample enrichment using solid-phase extraction were investigated. Various materials including octadecyl silica and strong and weak cation exchangers with organic matrices were compared as the sorbents for enrichment of aqueous samples of amines. The best results were achieved using the weak cation exchanger Spheron C 1000 as the sorbent and methanolic perchloric acid as the desorption liquid in the enrichment step. The recovery, linearity and detection limits of the method were determined. The concentration limits of determination of the amines are in the range of nmole.l^{-1}, with the enrichment factor of 240 in the preconcentration step.

INTRODUCTION

Aliphatic amines are organic bases often found in environmental and biological samples. As lower primary and secondary amines can form cancerogenic nitrosamines in presence of nitrites or other nitrosation agents (1), the need for a sensitive analytical method for their determination is obvious.

High performance liquid chromatography (HPLC) is well suited for the analysis of aliphatic amines, but its applications in trace analysis of these compounds are connected with problems originating from the low sensitivity of the detection using standard HPLC methods. Because the aliphatic amines do not absorb significantly in the UV region, it is not possible to use UV detection in their analysis. Fortunately, there is a plethora of derivatization agents making it possible to prepare fluorescent derivatives of aliphatic amines, such as N,N-dimethyl-5-aminonaphthalene-1-sulphochloride (dansylchloride) (2,3,4), N,N-dibutyl-5-aminonaphthalene-1-sulphochloride (bansylchloride) (5), 4-phenylspiro[furan-2-(3H),1'-phthalan]-3,3'-dione (fluorescamine) (2), 1,2-naphtoylbenzimidazole-6-sulphochloride (1,2-NBI-6-SO_2Cl) (6), acridone and acridine derivatives (5), 9-fluorenylmethylformate (9-FMOC) (7), 9-fluorenylmethyl chloroformate (9-FMOC-Cl) (8,9), 4-fluoro-7-nitrobenzo-2-oxa-1,3-diazole (NBD-F and NBD-Cl) (10,11,12), o-carboxybenzoic acid (OPT) (11), 1,2-naphthoquinone-4-sulphonate (13), 3-(2-furoyl)-quinoline-2-carbaldehyde (FQCA) (14), o-phthaldialdehyde (OPA) (2,10,11,15,16) and other. These derivatives make it not only possible to use sensitive fluorimetric detection, but also to increase the selectivity of determination and to suppress the effects of possible interfering compounds present in real samples. Pre-column derivatization often improves

chromatographic behaviour of the amines in common HPLC systems (enhances the selectivity of separation, decreases peak tailing, etc.).

Even with sensitive fluorimetric detection, sample enrichment is often necessary to achieve low detection limits required in trace analysis. Preconcentration of various compounds in aqueous samples is usually accomplished by liquid extraction or by adsorption of sample compounds on a suitable sorbent packed in a small column or in a cartridge. The latter technique, so-called "liquid-solid extraction" (LSE) or "solid-phase extraction" (SPE), has become increasingly popular because it avoids using harmful solvents and tedious manual operations usually connected with liquid-liquid extraction procedures.

Nonpolar sorbents, either organic polymers or alkyl modified silicas, are used most frequently for the enrichment of organic compounds, where the affinity of the nonpolar parts of organic molecules to the nonpolar (usually hydrocarboneous) surface of the adsorbent is the driving force of adsorption, much like in reversed-phase HPLC systems (17,18,19,20). However, compounds with small polar molecules are often only slightly retained by nonpolar sorbents. Ionized or ionizable compounds can be pre-concentrated on the columns or cartridges packed with ion exchangers. For example, phenoxyacid herbicides and other carboxylic acids in water can be enriched on anion exchangers (21,22).

Styrene-divinylbenzene cation exchange resins have been used both for off-line (23,24) and on-line (25) preconcentration of amines in aqueous samples prior to their chromatographic analysis. In addition to ion exchangers, unmodified silica was also used for enrichment of amines in aqueous samples (26) and in the air (27), followed by in-situ derivatization and HPLC analysis.

The main objective of the present work was to compare octadecyl silica and various cation exchangers with hydrophilic matrices as potential sorbents for sample enrichment of aliphatic amines in aqueous samples prior to the derivatization and separation by HPLC with fluorimetric detection.

MATERIALS AND METHODS

Apparatus

HPLC was performed using either an HP 1090M liquid chromatograph equiped with an HP 1046A fluorimetric detector and an HP 9000/310 data station (Hewlett-Packard, Avondale, PA, U.S.A.) or a liquid chromatograph comprised of an HP 1046A fluorimetric detector and an HPP 4001 high-pressure pump, an LCI 30 manual sample injector with a 20 µl sample loop, a CI 100 integrator and a TZ 4221 line recorder (all from Laboratory Instrument Works, Prague, Czech Republic). A glass cartridge column, 150x3 mm I.D., packed with Separon SGX C18, 7 µm, (Tessek, Prague) was used in both instruments.

A Specord M 400 UV-VIS spectrophotometer (Carl Zeiss, Jena, Germany) was used to analyze the fractions of the eluate in the determination of breakthrough volumes by frontal analysis and in the recovery tests. A Dorcus vacuum manifold (Tessek, Prague) was used for off-line solid-phase extraction experiments.

Chemicals

Methanol and ethanol, both UV spectroscopic grade, sodium hydroxide, sodium tetraborate (decahydrate) and

sodium hydrogencarbonate were obtained from Lachema, Brno, Czech Republic; o-phthaldialdehyde (OPA), 2-mercaptoethanol, n-ethylamine, n-propylamine, n-butylamine, n-pentylamine, n-hexylamine and n-heptylamine were all from Fluka, Buchs, Switzerland; perchloric acid was from Carlo Erba, Milano, Italy.
A Mili-Q apparatus (Millipore, Bedford, MA, U.S.A.) was employed to pretreat water used for preparation of all the solutions and of the mobile phase. All the other chemicals were used as obtained, without further purification.

Sorbents

Separon SGX C18 (60 μm), Separon HEMA-BIO CM (60 μm) and Separon HEMA-BIO SB (60 μm) were obtained from Tessek, Prague, Czech Republic, as the Silica-cart and HEMA-cart systems consisting each of a pre-packed 1 ml polypropylene cartridge (medical grade Tatren PD 140) packed with the individual sorbent.

Spheron C 1000, Spheron SB 1000, both 25-40 μm, were purchased as bulk materials from Lachema, Brno and were packed in the laboratory into the empty polypropylene cartridges of the same size as the packed Silica-cart and Hema-cart cartridges.

Separon SGX C18 (60 μm) is octadecyl silica of the same type as the packing used in reversed-phase HPLC, with larger particle size.

Both Separon HEMA and Spheron cation exchangers are based on the hydrophilic organic matrix, a macroporous co-polymer of 2-hydroxyethyl methacrylate and ethylene dimethacrylate. The Separon HEMA-BIO matrix was subject to additional chemical treatment, increasing the content of the hydroxyl groups in the matrix and making its

surface more hydrophilic than that of the untreated Spheron matrix.

Spheron C 1000 and Separon HEMA-BIO CM are both weak cation exchangers containing carboxylic groups, with cation-exchange capacities of 2 ± 0,25 mmole.g^{-1}.

Spheron SB 1000 and Separon HEMA-BIO SB are strong cation exchangers with sulphobutylic exchange groups and cation-exchange capacities of approximately 1.5 mmole.g^{-1}.

Derivatization Procedure (2)

Solution A: 50 mg of o-phthaldialdehyde dissolved in 5 ml of ethanol

Solution B: 22.5 µl of 2-mercaptoethanol dissolved in 5 ml of ethanol

Solution C: borate buffer, pH=10.5

The OPA derivatization reagent was prepared by mixing 1.5 ml of A with 1.5 ml of B and 90 ml of C. The mixed reagent was kept in a dark bottle in the refrigerator and was stabile for 2 days. After this period, fresh reagent was prepared. The derivatization was performed in a 10 ml volumetric flask by adding 3 ml of the OPA reagent to the solution of amine(s). The mixture was kept at ambient temperature for 5 minutes and then diluted to final volume with methanol.

Determination of the breakthrough curves and of the recovery in the sorption and desorption procedures.

All ion exchange cartridges were washed with water, 5 ml of 0.1 M perchloric acid and again with water to neutral pH before the use to make sure that the cation exchangers are in the H^+ form.

Separon SGX C 18 was washed with 5 ml of methanol and with water to remove possible organic impurities from the cartridge.

The breakthrough curves were determined by frontal analysis in which the stock solution containing 10^{-3} *mole.l*$^{-1}$ *n-butylamine in water* was passed through the cartridge fixed in the Dorcus vacuum manifold. The flow rate was kept between 5-10 ml.min^{-1} during the sorption procedure, fractions of the effluent were collected and the amine in the fractions was derivatized with o-phthaldialdehyde as described above.

The concentrations of n-butylamine were measured using a Specord M 400 UV-VIS spectrophotometer at the wavelength of 335 nm, corresponding to the absorption maximum of the OPA derivative. The determination was based on the calibration curve constructed for the concentration range 3.10^{-6} to 2.10^{-4} mole.l^{-1} of n-butylamine in water. The calibration curve was linear with correlation coefficient of 0.9998. The experimental absorbance values were corrected for the absorbance of the blank solution containing only the derivatization reagent in water.

Methanol acidified to pH=3 with perchloric acid was used for desorption of n-butylamine from the Separon SGX C18 Silica-cart cartridge while 0.1 - 1.0 M perchloric acid in water or in aqueous methanol served as the desorption liquid from the cation exchange cartridges.

A Separon SGX C18 Silica-cart cartridge was used to test the possibilities of in-situ derivatization during the sample enrichment step. The cartridge was pre-conditioned by washing with 5 ml of the mixture of the solutions A and B (OPA with mercaptoethanol). The aqueous sample of n-butylamine was brought to pH=10.5 necessary for the derivatization reaction by addition of the borate buffer immediately before the enrichment.

Each fraction of the effluent was divided into two portions. The first was analyzed spectrophotometrically without any further treatment while the other was mixed with the OPA reagent before the analysis, as described above. Pure ethanol was used for desorption and the fractions of the desorbate were collected and analyzed in the same way as the effluent in the sorption step.

HPLC Procedure

The method was first tested for the analysis of aqueous samples containing 10^{-9} - 10^{-8} mole.l^{-1} n-butylamine, using the liquid chromatograph built from the individual parts, including an HP 1046A fluorimetric detector. 500 ml volume of each sample was passed through a cartridge packed with Spheron C 1000 weak cation exchanger in the Dorcus vacuum manifold. After the enrichment step, the amine was desorbed from the cartridge by elution with 3 ml 1 M perchloric acid in 50% methanol; the desorbate was neutralized with 1 M sodium hydroxide and derivatized as described above.

Two artificial samples containing 10^{-7} mole.l^{-1} and 10^{-9} mole.l^{-1}, respectively, of each ethyl-, n-propyl-, n-butyl-, n-pentyl-, n-hexyl- and n-heptylamine in water were prepared. The first sample was derivatized without any pre-treatment and the second sample was subject to the same enrichment procedure as the samples containing only n-butylamine. Both samples were analyzed by HPLC using the HP 1090M liquid chromatograph with the HP 1046A fluorimetric detector.

The chromatographic conditions in the two instrumental setups were identical: isocratic elution with methanol-water 70:30 as the mobile phase at 0.5 ml.min^{-1}. The excitation and the emision wavelengths were

set to yield maximum sensitivity of detection: λ_{ex}=223 nm and λ_{em}=435 nm. Sample volume of 20 µl was injected into the liquid chromatograph in each experiment.

RESULTS AND DISCUSSION

Tests of the Sorbents for Solid-Phase Extraction

1. Octadecyl silica

The retention volume of n-butylamine was evaluated from the inflexion point on the breakthrough curve (plot of the concentration of n-butylamine in the effluent vs. time elapsed from the start of the frontal analysis) and the breakthrough volume was determined from the intersection point of the tangent (at the inflexion point) with the baseline.

The breakthrough curve of 10^{-3} M n-butylamine in water on a Separon SGX C18 cartridge is shown in Fig.1a. The profile of the breakthrough curve is atypical, with the breakthrough volume at 25 ml followed by a slow continuous increase of the concentration of n-butylamine up to 120 ml, where a steep increase in concentration occurs. It is not clear if this behavior can be attributed to the presence of the residual silanol groups in the sorbent or to another effect.

Methanol brought with $HClO_4$ to pH=3 was used for the elution of n-butylamine sorbed on the cartridge and 98% of the sorbed compound was eluted in 6 ml of the eluate (desorption curve 1 in Fig. 2). The desorption with acidified methanol would be suitable for the recovery of n-butylamine, but the shape of the breakthrough curve indicates that only very low sample volumes can be

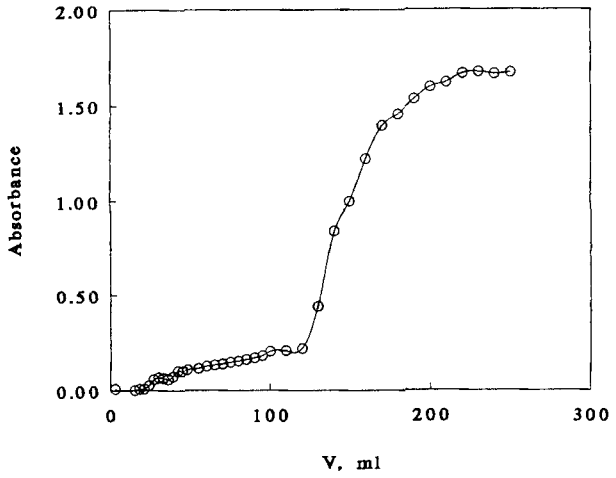

FIGURE 1a

Breakthrough curve of 10^{-3} M n-butylamine in water on a Separon SGX C18 cartridge.
V - volume of the effluent; Absorbance measured in fractions after derivatization with OPA at 335 nm.

employed in this system, resulting in inacceptably low enrichment factors, which precludes efficient use of this sorbent for the enrichment of aqueous samples of alkylamines.

The octadecyl silica material was tested as possible support for in-situ derivatization during the solid-phase extraction step. For this purpose, the Separon SGX C18 cartridge was first conditioned with the OPA reagent, as described in Experimental and the aqueous solution of n-butylamine was buffered to pH=10.5 before the sorption. Although the high pH is not compatible with continuous use of silica based columns, it can be applied in the work with a cartridge intended for a single use.

To investigate the process of in-situ derivatization in detail, the fractions of the effluent

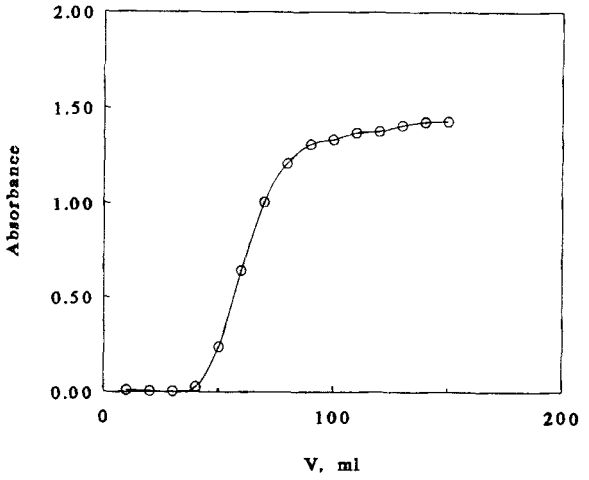

FIGURE 1b

Breakthrough curve of 10^{-3} M n-butylamine in water (buffered to pH 10.5) on a Separon SGX C18 cartridge conditioned with the OPA reagent.
V - volume of the effluent; Absorbance measured in fractions after derivatization at 335 nm.

from the cartridge during the sorption and desorption processes were divided into two portions, the first of which was measured photometrically without any treatment and the other was subject to the derivatization procedure with OPA prior to the photometric determination (see Experimental). Untreated portions of the fractions of the effluent collected during the sorption process did not show significant absorbance. However, the absorbance increased in the portions subject to the derivatization procedure and the resulting breakthrough curve had almost symmetrical profile and the breakthrough volume of 44 ml (Fig. 1b).

Pure ethanol was used as the desorption liquid for n-butylamine retained on the cartridge preconditioned

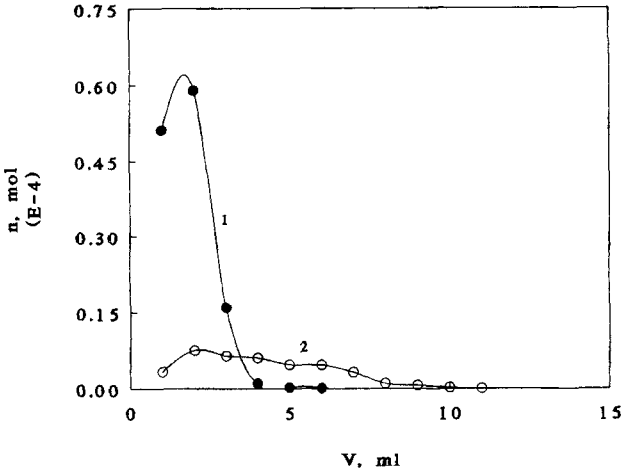

FIGURE 2

Desorption curves of n-butylamine after sorption on a Separon SGX C18 cartridge. 1. Desorption with methanol acidified with $HClO_4$ to pH=3 from unconditioned cartridge; 2. Desorption with ethanol from the cartridge conditioned with the OPA reagent;
V - volume of the desorbate; n - mass of the amine in fractions.

with OPA. No significant absorbance was found in the untreated fractions of the desorbate, in contrast to the portions subject to the derivatization procedure, where the desorption curve shown in Fig. 2 (curve 2) was obtained. This desorption curve was shallow and only approximately 70% of the derivative was recovered in the desorption step.

These experiments indicate that the pre-conditioning of the C18 cartridge with the OPA reagent results in an improved profile of the breakthrough curve with respect to the unconditioned cartridge, possibly because the reagent blocks the unreacted silanol groups in the sorbent, but that real in-situ derivatization of

n-butylamine hardly occurs under the conditions employed.

2. Cation exchangers

The main driving force of the sorption of alkylamines on cation exchangers in the H^+ form are ion-exchange interactions between the protonized form of the amine and the exchange groups of the exchanger. Strong cation exchangers posses $-SO_3^-$ groups, which are dissociated over the wide range of pH values from 1 to 14. On the other hand, carboxylic groups of weak cation exchangers are dissociated only in alkaline solutions and do not show ion-exchange properties in acidic solutions.

In addition to the ion-exchange mechanism, interactions with the matrix of the cation exchanger may contribute to the sorption of amines. Ion-exchangers based on styrene-divinylbenzene copolymers often show strong hydrophobic interactions with various organic compounds. In the alkylamine series, these interactions can be expected to increase with increasing length of the alkyl in the amine, so that the sorption capacities, breakthrough volume and recovery can be significantly influenced by the structure of the amine.

These effects are likely to be of minor importance for the sorption of amines on polymeric ion-exchangers with hydrophilic matrices, such as Spheron or Separon HEMA-BIO materials based on ethyleneglycol-methacrylate copolymers. The two types of matrices differ in additional hydrophilization introducing hydroxyl groups into the structure of the HEMA-BIO matrix.

The experimental breakthrough curves of 10^{-3} M n-butylamine on the cartridges packed with the cation exchangers tested and with unconditioned Separon SGX C18

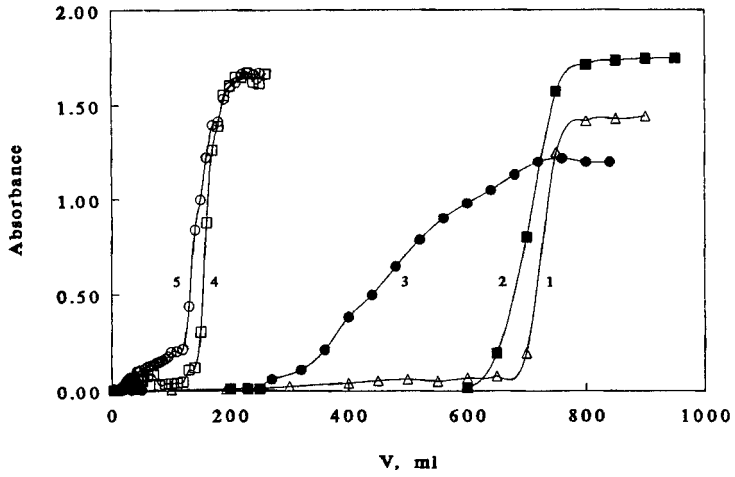

FIGURE 3

Breakthrough curves of 10^{-3} M n-butylamine in water on cartridges packed with Spheron C 1000 (1), Spheron SB 1000 (2), Separon HEMA-BIO SB (3), Separon HEMA-BIO CM (4) and Separon SGX C18 (5).
V - volume of the effluent; Absorbance measured in fractions after derivatization at 335 nm.

are compared in Fig.3. The exchangers with the hydrophilized Separon HEMA-BIO matrix provide significantly lower breakthrough volumes than the Spheron materials. The profile of the breakthrough curve on the weak cation exchanger HEMA-BIO CM is similar to that observed for the octadecyl silica SGX C18 material, possibly because of simultaneous effects of the sorption on the carboxylic ion-exchange groups and on the lipophilic hydroxy groups in the matrix of the exchanger. This may be also the reason for the gradual slope of the breakthrough curve observed on the strong cation exchanger Separon HEMA-BIO SB, where the first breakthrough is observed at 270 ml, but the equilibrium is not achieved before 700 ml of the effluent have

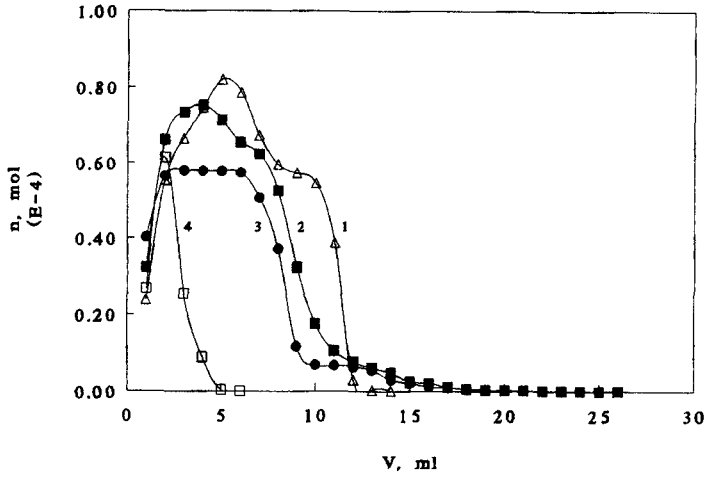

FIGURE 4

Desorption curves of n-butylamine from cartridges packed with Spheron C 1000 (1), Spheron SB 1000 (2), Separon HEMA-BIO SB (3) and Separon HEMA-BIO CM (4) with 0.1 M $HClO_4$ in water as the desorption liquid.
V - volume of the desorbate; n - mass of the amine in fractions.

passed through the cartridge. The Spheron cation exchangers with the matrix not subject to additional hydrophilization showed much sharper breakthrough curves at significantly higher breakthrough volumes (640 ml for Spheron SB and 730 ml for Spheron C 1000). These results indicate that the materials with untreated matrix are more suitable for the enrichment of n-butylamine than hydrophilized HEMA-BIO cation exchangers.

Recoveries from ion exchangers saturated with n-butylamine in the sorption step to the full ion exchange capacities were compared in another set of experiments. Fig. 4 shows desorption curves of n-butylamine obtained with 0.1 M perchloric acid in

water as the desorption liquid. Strong cation exchangers show tailing desorption curves, which means that larger volumes of the desorption liquid should be used in comparison to weak cation exchangers. This indicates that carboxylic cation exchangers are more suitable for the enrichment of amines than cation exchangers with sulfonic ion-exchange groups. The elution from the Separon HEMA-BIO CM cartridge can be accomplished in 5 ml of the desorption liquid whereas 14 ml are necessary for the Spheron C 1000 cartridge. However, because of much higher breakthrough volume (730 ml) on the latter cation exchanger, the maximum enrichment factor for this material is higher than for other sorbents studied and the recovery of 92% is satisfactory and better than with the Separon HEMA-BIO CM and Spheron SB 1000 cation exchangers (Table 1). After comparison of the breakthrough volumes, retention volumes, enrichment factors and recoveries under comparable conditions, Spheron C 1000 was selected as the sorbent most suitable for the enrichment of aliphatic amines.

Composition of the Desorption Liquid

To achieve the elution of an ionized solute from an ion-exchanger it is necessary to employ mobile phase containing ions that compete with the solute ions for the ion exchange functional groups. The elution is enhanced with increasing concentration of the competing ions in the desorption liquid. In solutions of strong acids, H_3O^+ ions participate in these competitive interactions. When a dilute acid is used to elute solutes sorbed on a weak cation exchanger, the desorption effect is further enhanced by suppression of the dissociation of weak cation exchange (carboxylic)

TABLE 1

Characteristics of the Sorption and Desorption of n-Butylamine on Cartridges Packed with Various Sorbents

Separon SGX C18 (1), Separon SGX C18 conditioned with OPA (2), Separon HEMA-BIO SB (3), Separon HEMA-BIO CM (4), Spheron SB 1000 (5), Spheron C 1000 (6)
Desorption liquid: methanol acidified to pH = 3 (1), ethanol (2), 0.1 M perchloric acid (3-6)

V_D in ml, volume of the desorption liquid necessary for quantitative elution
V_R in ml, retention volume evaluated from the inflexion point on the breakthrough curve
V_B in ml, breakthrough volume
n_s in mmole, adsorbed amount of the amine, calculated from V_R and concentration of the amine in the sample (1 mmole.l^{-1})
n_e in mmole, recovered amount of the amine in the desorption step
R in %, recovery
f_e enrichment factor $f_e = V_B/V_D$

Cartridge	1	2	3	4	5	6
V_D [ml]	6.0	11.0	24.0	6.0	25.0	14.0
V_R [ml]	130	55	440	155	705	770
V_B [ml]	25	44	275	140	640	727
n_s [mmole]	0.130	0.055	0.440	0.155	0.705	0.77
n_e [mmole]	0.127	0.038	0.468	0.123	0.586	0.708
R [%]	97.7	69.1	106.4	79.4	83.10	91.9
f_e	4.2	4.0	11.5	23.3	24.9	52

groups of the exchanger at low pH, which decreases its ion-exchange capacity.

As 14 ml of 0.1 M $HClO_4$ necessary to achieve quantitative recovery of n-butylamine sorbed on the Spheron C 1000 cartridge is too large a volume for practical enrichment of the samples of amines, we investigated possibilities of decreasing this volume by increasing the concentration of perchloric acid and by adding methanol to the desorption liquid. When 0.33 M $HClO_4$ in 50% methanol was used, the volume necessary for quantitative desorption was decreased to 6 ml (Table 2). Further increasing the concentration of perchloric acid

TABLE 2

The Effect of the Composition of the Desorption Liquid on the Recovery and Enrichment Factor of n-Butylamine for the Spheron C 1000 Cartridge

1: 0.1 M $HClO_4$ in water; 2: 0.33 M $HClO_4$ in 50% methanol; 3: 1.0 M $HClO_4$ in 50% methanol.
Symbols as in TABLE 1.
f_e calculated using V_B = 730 ± 50 ml

desorption liquid	1	2	3
V_D [ml]	14.0	6.0	3.0
n_s [mmole]	0.72	0.82	0.77
n_e [mmole]	0.66	0.83	0.74
R [%]	91.7	101.2	96.1
f_e	52.1 ± 3.6	121 ± 8.3	243 ± 16.7

to 1.0 M reduced of the necessary volume of the desorption liquid to 3 ml, with resulting enrichment factor of 240 (Table 2), which we found satisfactory for practical enrichment purposes. Fig.5 compares the volumes of various desorption liquids necessary to accomplish quantitative recovery of n-butylamine from the Spheron C 1000 cartridge when the amine is sorbed to the full saturation capacity of the exchanger.

HPLC Determination of Amines after Sample Enrichment by SPE

The breakthrough volume of 10^{-3} M n-butylamine on the Spheron C 1000 cartridge was 730 ml and significantly higher breakthrough volumes can be expected for lower concentrations of alkylamines. Therefore we used "safe" volume of 500 ml of aqueous samples of amines and 3 ml of 1 M perchloric acid in 50% methanol as the desorption liquid in the enrichment step before the HPLC analysis in all the experiments. The enrichment factor related to 10 ml of the sample after the derivatization procedure was 50.

To verify the HPLC method, 20 µl samples of n-butylamine in the concentration range of 10^{-4} to 9.10^{-7} M were analyzed by HPLC with fluorimetric detection at the highest settings of the detector sensitivity (PMT GAIN = 15 and 16). The minimum detectable concentration corresponding to the peak height equal three times the baseline noise was 7.10^{-9} M, which is comparable with the data published by Mellbin and Smith (11). The calibration curve was linear in the concentration range tested, with correlation coefficient of 0.9997.

This calibration curve was used to test the HPLC method of determination of n-butylamine after the

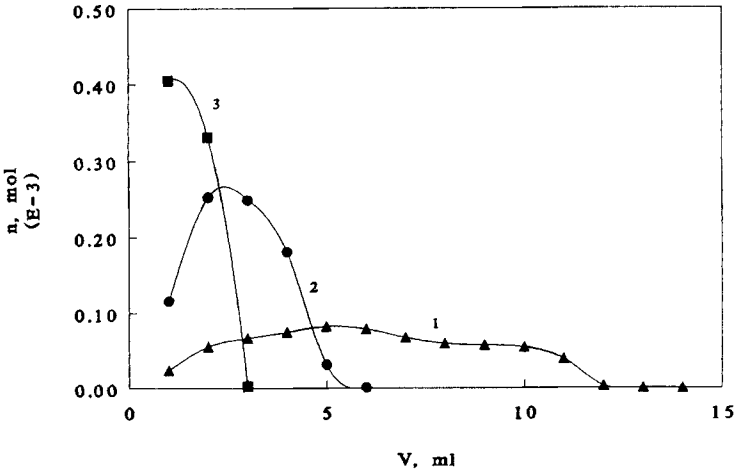

FIGURE 5

Desorption curves of n-butylamine from a cartridge packed with Spheron C 1000 using 0.1 M $HClO_4$ in water (1), 0.33 M $HClO_4$ in 50% methanol (2) and 1.0 M $HClO_4$ in 50% methanol (3) as the desorption liquid.
V - volume desorbate; n - mass of the amine in fractions.

enrichment step. The recovery of the method for n-butylamine in the concentration range from 1.10^{-9} to 1.10^{-8} M was 98-102% (Table 3). The limit of detection calculated from the baseline noise corresponds to $1.4.10^{-10}$ M. Because of the small peak of the impurity found in the experiments with blank sample, probably originating from the chemicals, practical limits of determination correspond to approximately 1.10^{-9} M, i.e., 75 ppt.

The method was tested on an artificial mixture of six alkylamines. 20 µl of a sample containing ethyl-, n-propyl-, n-butyl-, n-pentyl-, n-hexyl and n-heptylamine derivatives with OPA in concentrations $2.6.10^{-7}$ - $3.8.10^{-7}$ M was injected directly into the 1090M HP liquid chromatograph with fluorimetric detector. The

TABLE 3

Enrichment of Aqueous Solution of n-Butylamine on a Spheron C 1000 Cartridge

Sample volume 500 ml; desorption with 3 ml 1.0 M $HClO_4$ in 50% methanol (enrichment factor f_e=50 after the derivatization step, final volume of derivatized sample = 10 ml)

c_0 in nmole.l^{-1}, concentration of the amine in the original sample
c_1 in nmole.l^{-1}, expected concentration of the amine in the sample after enrichment and derivatization
c_2 in nmole.l^{-1} - experimentally found concentration of the amine in the enriched sample by HPLC
S sensitivity settings of the fluorimetric detector (PMT GAIN)
R in %, recovery of the method

c_0	c_0, ppb	c_1	c_2	S	R
11.0	0.80	550	564	15	102.5
4.6	0.34	230	231	15	100.5
1.2	0.09	60	58.9	16	98.2
0.0	0.03	-	18.6	16	-

resulting chromatogram (Fig.6) was compared with the separation of an aqueous sample containing the amines in concentrations $5.2.10^{-9}$ - $7.6.10^{-9}$ M, after enrichment from 500 ml to the final volume of 10 ml after derivatization with OPA, as described above (Fig.7).

The amounts of the amines in 20 µl samples should be theoretically equal in the two experiments (5.2-7.8 pmoles of the individual amines). Theoretical (c_1) and

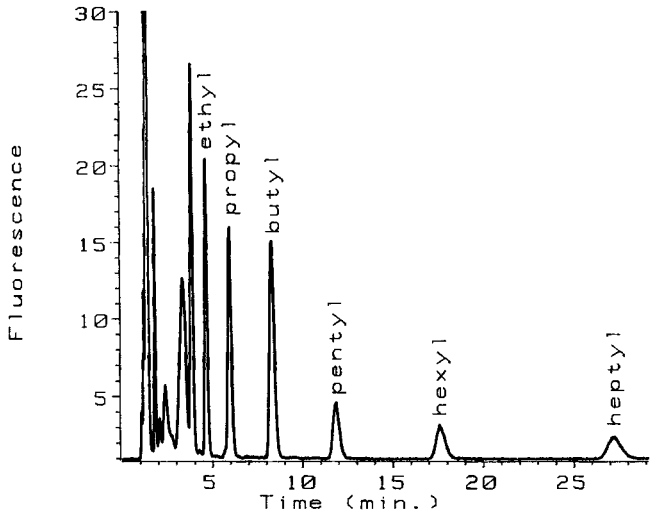

FIGURE 6

Chromatographic separation of 20 µl of a mixture of n-alkylamines ($2.6.10^{-7}$-$3.8.10^{-7}$ M each) after derivatization with OPA and fluorimetric detection. Column: Separon SGX C18, 7 µm (150x3 mm); mobile phase: methanol in water, 70:30, 0.5 ml.min^{-1}. λ_{ex}=223 nm; λ_{em}=435 nm.

experimentally found (c_i) concentrations of the individual amines, recoveries and minimum detectable concentrations equivalent to the signal threefold the baseline noise are given in Table 4.

The recoveries were between 90 and 114%, which is sufficient for practical applications. The detection limits for n-butylamine are approximately three times lower than those found for the instrumental setup with the CI 100 integrator, which can be attributed to better performance of the data station (and possibly, of the pump) in the 1090M liquid chromatograph in comparison with the modular instrument built from the individual parts. The practical limits of determination, however,

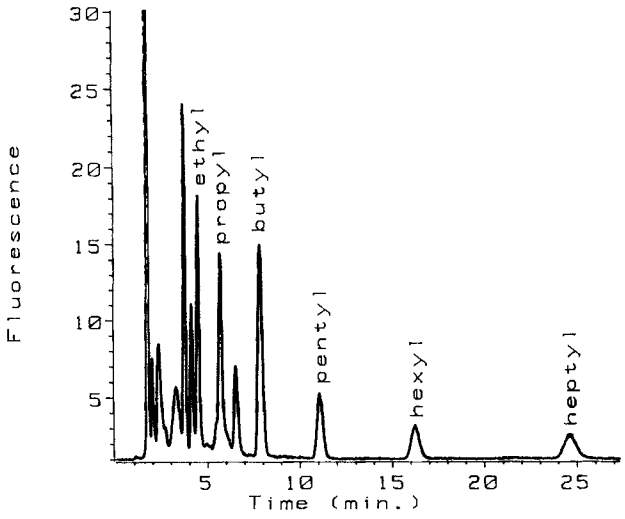

FIGURE 7

Chromatographic separation of a mixture of n-alkylamines ($5.2.10^{-9}$–$7.6.10^{-9}$ M each) after enrichment of 500 ml of the sample on a Spheron C 1000 cartridge and derivatization with OPA (volume of the sample after derivatization = 10 ml; 20 µl injected). Other conditions in Fig.6

are likely to be limited by impurities present in the sample, desorption liquid, mobile phase, or derivatizing reagent rather than by the baseline noise. The detection limits increased with increasing molecular weight of the amine because of decreasing peak height of more retained solutes. The detection limits of more strongly retained compounds could probably be slightly improved by using gradient elution.

CONCLUSIONS

Octadecyl silica Separon SGX C18 is not suitable sorbent for enrichment of aqueous samples of alkylamines

TABLE 4

Enrichment of Aqueous Solution of a Mixture of n-Alkylamines on a Spheron C 1000 Cartridge

MDC in nmole.l^{-1}, minimum detectable concentration other symbols as in TABLE 3.

AMINE	c_0	c_0,ppb	c_1	c_2	R	MDC
ETHYL	7.50	0.38	375	337	89.8	2.4
PROPYL	7.20	0.42	360	372	103.4	3.0
BUTYL	6.0	0.43	300	292	97.3	2.7
PENTYL	5.24	0.46	262	297	113.4	8.7
HEXYL	5.44	0.55	272	266	97.9	15
HEPTYL	5.66	0.65	283	310	109.6	24

by solid-phase extraction, as the breakthrough volumes are too low for a satisfactory enrichment factor. The attempts to use this material as the support for in-situ derivatization during the enrichment step were also unsuccessful. The breakthrough volumes were significantly increased when polymeric cation exchangers with hydrophilic matrix in the H^+ form were used as the sorbents for solid-phase extraction. Untreated cation exchangers show larger breakthrough volumes than the exchangers with the matrix subject to additional hydrophilization procedure .

Weak cation exchangers show steeper desorption curves and lower desorption volumes than the strong

cation exchangers tested. Based on the results of the sorption-desorption tests, weak cation exchanger Spheron C 1000 with carboxylic acid ion-exchange groups was selected as the best sorbent for enrichment of aqueous samples of alkylamines by solid-phase extraction. With aqueous-methanolic perchloric acid as the desorption liquid, the enrichment factor of 50 can readily be achieved in the sample pre-treatment step, including adsorption, desorption and derivatization of amines with o-phthaldialdehyde reagent.

The solid-phase extraction enrichment procedure was combined with reversed-phase HPLC and fluorimetric detection for determination of aliphatic amines in aqueous samples. The method was linear over the concentration range of at least two orders of magnitude and limits of determination below 100 ppt of the amine in the original sample could be achieved, with recoveries from 90 to 115%.

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