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Selectivity and efficiency of different reversed-phase and mixed-mode sorbents to preconcentrate and isolate aroma molecules

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ABSTRACT

A comparison of the ability of different sorbent systems, including mixed-mode resins and reversed-phase sorbents, to extract and isolate volatile molecules from hydroalcoholic medium has been carried out by means of the determination of liquid–solid distribution coefficients. Eighteen volatile compounds covering a wide range of physicochemical properties (acids, bases and neutrals) and chemical functionalities, and thirteen different sorbents have been tested. LiChrolut EN and Isolute ENV (both polymeric with high surface area) showed the highest retention capability for nearly all analytes at all pHs tested. Exceptions were 2,3,5-trimethylpyrazine, most efficiently extracted with Strata XC at acidic pH, and indole best retained with Oasis MCX and Strata XC at any pH. Although nearly all basic compounds were most selectively extracted with cationic mixed-mode resins at acid pHs and 3-mercaptohexyl acetate and m-cresol show maxima α at pH 10 with Oasis MAX, the α values obtained have been relatively low, which suggests that retention is not particularly driven by ionic forces. The study has also shown that selectivity depends on the pH, the exact kind of mixed-mode sorbent and on the polarity of the analyte. High selectivity towards ionogenic compounds can be obtained by combining retention in mixed mode, a rinsing with a non-polar solvent and further elution with a solvent containing a neutralizing agent. However, not all the ionogenic molecules seem to be retained in ionic mode in the conditions tested and the complete elution of some analytes can be difficult, which suggests that analyte-specific isolation procedures should be analyzed case by case.

1. Introduction

Solid-phase extraction (SPE) involves a liquid–solid partition where the extraction phase is a solid sorbent. This technique has been used extensively in the last few years in different laboratory areas such as environment, pharmaceutical, clinical or food industry and different papers and reviews have been published about the development and optimization of SPE-based isolation strategies [1–6]. SPE is used for isolation, trace determination, sample concentration or sample clean up. Many benefits of SPE methods have been commonly cited including its robustness, potential for automation, capacity for providing clean extracts, selective isolations and even a fractionation of the different sample components. For these reasons, SPE is a powerful preconcentration technique which can be easily adapted for routine analysis. Methods based on this technique have been developed in several articles [7–12]. Moreover, some examples of the use of SPE in food analysis were given in a review by Buldini et al. [13].

The most critical factor to develop an efficient SPE is to choose the appropriated sorbent. According to Fritz [14] a successful SPE system must achieve two requirements: a high and reproducible percentage of compounds must be kept in sorbent and after they must be eluted completely. The selection of the correct sorbent must take into account the sample nature (aqueous/organic) and the properties of the target compounds (polar/non-polar; neutrals or weak or strong acids or bases). According to solute and sorbent characteristics, the potential retention mechanisms are different: normal phase, reversed phase, ionic interactions, mixed mode or immunoextraction. Three major sorbent groups can be distinguished: inorganic oxides, and low and high selectivity organic-polymeric sorbents [15].

Sorbents based on new-generation porous polymers are included in the second group and in general, they are styrene-divinylbenzene copolymers of high retention ability because of their large specific surface areas (700–1200 $m^2\ g^{-1}$). Mixed-mode sorbents are classified in the third group and they are often used in clinical and pharmaceutical laboratories for the isolation of drugs containing ionized functional groups in biological fluids [16–19]. The mixed-mode sorbents combine lipophilic retention with ionic interactions (cationic and anionic), which makes it possible to isolate acids, basic and neutral analytes in the same experiment. For

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example, Oasis MCX combines the properties of the Oasis HLB sorbents with the characteristics of a strong cation exchanger. The additional sulfonic acid groups ($-SO_3H$) and the appropriate combination of washing and elution steps cause improvement in selectivity and hence in sensitivity for the determination of basic components of plasma, urine or whole blood [5].

This study tries to assess the potentiality of neutral polymeric and mixed-mode resins for the selective isolation of small volatile molecules with acid or basic properties such as amines, pyrazines, phenols, organic acids and mercaptans by means of the calculation of liquid–solid distribution coefficients, K_{SL}. Some small volatile molecules containing those functionalities have remarkable bioactive properties, such as low odor detection thresholds or even a high toxicity [20–24]. The low mass of the ionic fragments obtained by mass spectrometry of these molecules and the low concentration at which they should be determined make advisable the development of highly selective isolation schemes for their analytical determination. The assumption is that the correct use of mixed-mode resins would provide much more selective isolation schemes for these families of compounds in comparison with those achieved with neutral polymeric resins of high active surface, such as LiChrolut EN.

Selected analytes are trace aromatic compounds present in wine and potential interferences, such as isoamyl alcohol, β-phenylethanol and diethyl succinate. Experiences in this paper are made in hydroalcoholic medium, which is similar to real wine, to simplify this study and to provide more general information about the behavior of analytes and interferences in a SPE system.

The main aims of this research are, first, to compare the isolation and extraction abilities of different adsorbent systems in hydroalcoholic medium and second, to provide data that might allow the selection of one of them according to quantitative criteria.

2. Materials and methods

2.1. Reagents, samples and standards

Ethanol and hexane LiChrosolv quality, dichloromethane, methanol SupraSolv quality and diethylether (99.7%) were from Merck (Darmstadt, Germany). Pure water was obtained from a Mili-Q purification system (Millipore, Bedford, MA, USA). Formic acid (95%), ethyl acetate (99.7%) and triethylamine (99.5%) were supplied by Sigma–Aldrich (St. Louis, MO, USA). L(+)-Tartaric acid (99.5%), hydrochloric acid (37%), sodium hydrogen carbonate (99,7%) and potassium hydrogen phthalate (99%) were from Panreac. Sodium hydroxide (99%) was from Scharlau and potassium chloride (99%) from Fluka.

Hydroalcoholic solutions: 13% (v/v) in ethanol, 5 g L^{-1} tartaric acid and pH adjusted to 3.5 with sodium hydroxide 1 M, pH buffers, pH 2: 50 mL of potassium chloride 0.2 M, 13 mL of hydrochloric acid 0.2 M; pH 3.5: 100 mL of potassium hydrogen phthalate 0.1 M, 16.4 mL of hydrochloric acid 0.1 M; pH 10: 100 mL of sodium hydrogen carbonate 0.05 M, 21.4 mL of sodium hydroxide 0.1 M.

Bond Elut ENV, Bond Elut LMS, Bond Elut PPL, Bond Elut Plexa, Bond Elut Certify and Bond Elut Certify II resins were obtained from Varian (Palo Alto, CA, USA). LiChrolut EN was from Merk (Darmstadt, Germany), Isolute ENV+ from IST (MID Glamorgan, U.K.), Supelclean ENVI-Chromp and Discovery DSC-MCAX from Supelco (Bellefonte, PA, USA), Oasis MCX and Oasis MAX from Waters (Milford, MA, USA) and Strata XC from Phenomenex (Torrance, CA, USA). Resins characteristics are shown in Table 1.

The following chemical standards were used:

Basic molecules: 2-acetylpyrazine (Lancaster 98%), methyl anthranilate (Fluka purum ≥98%), 2-isobutyl-3-methoxypyrazine

Table 1
Characteristic of the sorbents considered in the study.

Code	Polymeric resins							Mixed-mode resins					
								Cationic				Anionic	
	Bond Elut LMS	Bond Elut ENV	Bond Elut PPL	Bond Elut Plexa	Supelclean ENVI-Chromp	Isolute ENV	LiChrolut EN	Strata XC	Oasis MCX	Discovery DSC-MCAX	Bond Elut Certify	Oasis MAX	Bond Elut Certify II
	BE LMS	BE ENV	BE PPL	BE Plexa	S-EnviC	Isolute	LiChrolut	Strata XC	O-MCX	DSC-MCAX	BE Certify	O-MAX	BE Certify II
Particle size (μm)	125	75	125	40–120	80–160	90	40–120	28–34	30	50	47–60	60	40–120
Pore size (Å)	450	300	150	50–250	110–175	800	0.83[a]	75–91	73–89	70	57–87	73–89	60
Surface area (m^2 g^{-1})	500	500	600	1200	800-950	1000–1100	1200–1500	705–825	727–889	480	460–520	727–889	n.d.
Pore volume (mL g^{-1})	1.3	1.3	1	0.75	n.d.	n.d.	0.75	1.57–1.87	1.18–1.44	0.9	n.d.	1.18–1.44	n.d.
Functional group	SDB	SDB	SDB	SDB-OH	SDB	SDB-OH	SDB-EVB	SCX/SDB	SCX/VPDB	C8/SCX	C8/SCX	SAX/VPDB	C8/SAX

SDB: styrene/divinylbenzene copolymer, VPDB: vinylpyrrolidone/divinylbenzene copolymer, EVB: ethylvinylbenzene, OH: hydroxy; C8: octyl group; SCX: cation-exchange sulfonic groups; SAX: anion-exchange amine groups. n.d.: no data.

[a] Pore volume (cm^3/g).

(Aldrich 95%), o-aminoacetophenone (Aldrich 99%), indole (Aldrich 99%), 2,3,5-trimethylpyrazine (Aldrich 99%).

Neutral molecules: γ-octalactone (SAFC 97%), 3-nonen-2-one (Alfa Aesar 86%), cis-2-hexenol (Lancaster 98%), linalool (Fluka 98.5%), ethyl phenylacetate (Fluka Puriss), isoamyl alcohol (Sigma 98%), β-phenylethanol (Fluka 99%), diethyl succinate (Fluka 99%).

Acidic molecules: 4-ethylguaiacol (Lancaster 98%), hexanoic acid (Fluka Puriss), m-cresol (Aldrich 99%), 3-mercaptohexyl acetate (Oxford Chemicals ≥99%).

4-Hydroxy-4-methyl-2-pentanone (99%), used as internal standard, was purchased from Aldrich, Spain (Madrid).

Physicochemical parameters for the chemicals are shown in Table 2.

2.2. Retention process: determination of distribution coefficient of solid–liquid systems (K_{SL})

Solid sorbents were previously washed with methanol and dried under vacuum. An exact weight of the sorbent (around 50 mg) was placed inside 25 mL glass tubes, with 20 mL of hydroalcoholic solution (buffered at pH 2, 3.5 or 10) containing 0.625 mg L^{-1} of the selected volatile compounds. The pHs were selected including natural pH of wine (3.5) and pH conditions in which basic compounds must be protonated (2) and acidic analytes are presented in their deprotonated structure (10). The closed tubes were shaken softly (44 rpm) for 840 min in an automatic shaker (Heidolph, Promax 1020) to ensure that the equilibrium is reached. All the experiments were carried out with the same phase ratio (1/400) and in duplicate on different days.

After this period of time, volatiles remaining in the aqueous phase were extracted by solid-phase extraction (SPE) with LiChrolut EN 200 mg cartridges, previously conditioned with 4 mL dichloromethane, 4 mL methanol and 4 mL of a 13% (v/v) ethanol aqueous solution. Analytes were recovered by elution with 1.0 mL of dichloromethane/methanol (1%, v/v). Twenty microliters of the internal standard solution (4-hydroxy-4-methyl-2-pentanone 500 mg L^{-1} in dichloromethane) was added to the extract. One microliter of this extract was then injected in the GC-FID system. The areas obtained were normalized by those of the internal standard and were interpolated in the calibration graphs. These graphs were built by the analysis of standard solutions in dichloromethane/methanol (1%, v/v) containing known amounts of the same volatile compounds. The results were corrected by the corresponding recoveries shown in Table 3.

2.3. Elution process

Fifty mg SPE cartridges of Strata XC and Oasis MAX have been prepared representing mixed-mode cation exchangers and mixed-mode anion exchangers, respectively. Two milliliters of hydroalcoholic solution (13%, v/v), previously spiked with the volatile compounds (6.25 mg L^{-1}), is loaded onto these cartridges. The solutions have been buffered at pH 2 and 10 according to the resins used. The proportion of analyte (%) not retained in the mixed-mode resin was determined by further extracting the loading solution in a LiChrolut EN 200 mg cartridge.

Analytes retained in the mixed-mode sorbent were then recovered by sequential elution with 1 or 2 mL of different solvents, typically a first non-polar solvent such as dichloromethane and a second solvent system containing a neutralizing agent, such as dichloromethane with triethylamine (10 g L^{-1}) or with formic acid (10%, v/v). The corresponding extracts were spiked with 20 μl of the internal standard solution (4-hydroxy-4-methyl-2-pentanone 500 mg L^{-1} in dichloromethane) and were further analyzed by GC-FID. All the determinations were carried out replicately on different days.

2.4. Estimation of retention and selectivity parameters

Distribution coefficients (K_{SL}) were calculated following the formula:

$$K_{SL} = \frac{W_{retained}/W_{resin}}{W_{nonretained}/W_{liquid}}$$

K_{SL}: solid–liquid distribution coefficient, $W_{retained}$ (mg): weight of retained analyte, determined by subtracting the nonretained mass from the initial mass, W_{resin} (mg): exact weight of resin in the tube, $W_{nonretained}$ (mg): weight of analyte remaining in the liquid phase, calculated by direct extraction with LiChrolut EN 200 mg and further interpolation in the calibration graph, W_{liquid} (mg): weight of liquid phase.

Three different pH conditions were essayed (pH: 2, 3.5 and 10).

The selectivity coefficients (α) have been estimated dividing each K_{SL} by the geometric average value of K_{SL}:

$$\alpha = \frac{(K_{SL})_i}{(K_{SL})_{geometric\,average}}$$

2.5. Gas chromatography

A Carlo Erba 8000 gas chromatograph with a FID detector (flame ionization detector) was used. The column was a DB-5 from J&W (Folsom, CA, USA), 30 m × 0.32 mm i.d. with 1 μm phase thickness, and was preceded by an intermediate polarity fused silica precolumn from Supelco (Bellefonte, PA, USA), 3 m × 0.32 mm i.d. The carrier was H_2 at 3 mL min^{-1}. The chromatographic oven was initially 40 °C, which was held for 2 min, then was raised to 115 °C at 1.5 °C min^{-1} and later to 240 °C at 15 °C min^{-1}. The temperature of the injector and detector respectively were 250 °C and 270 °C. Finally, 1 μL of sample was injected in splitless mode (splitless time, 1.50 min).

2.6. Statistical analysis

Two-way analysis of variance (ANOVA) was carried out to determine the influence of the factors "resin" and "pH" on the levels of each analyte. This analysis was run with SPSS vs. 11.5 from SPSS Inc. (Chicago, IL). Principal component analysis (PCA) was carried out using Unscrambler vs. 9.5 from Camo (Norway).

3. Results and discussion

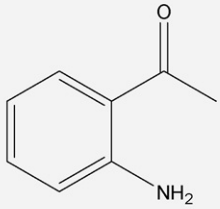
3.1. Retention properties of the sorbents

The retention ability at different pHs of 13 different analytical sorbents and 18 different aroma compounds belonging to different chemical families has been studied through the measurement of the liquid–solid distribution coefficients. Among the sorbents considered in the study, 7 reversed-phase popular sorbents, 4 mixed-mode cation exchangers and 2 mixed-mode anion exchangers have been considered. Analytes cover non-ionogenic neutral molecules of different polarity, organic acids and organic bases.

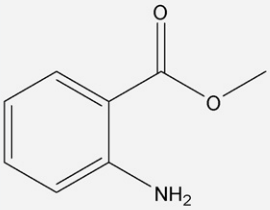
The maximum distribution coefficients (K_{SL}) obtained for each analyte and pH conditions are given in Table 4. Results show at each pH condition the sorbent giving maxima K_{SL}. As K_{SL} were estimated from the measured amount of analyte remaining in the liquid phase after the extraction, the maximum K_{SL} was set at 30,000, which roughly corresponds to the method detection limits. Such maximum value is reached for linalool, 3-mercaptohexyl acetate and 4-ethylguaiacol at pH 2 and for linalool at pH 3.5. The K_{SL} for hexanoic acid at basic pH could not be determined because at this pH this compound is purely anionic and could not be recovered. K_{SL} val-

Table 2
Analytes physicochemical parameters ($\mathrm{p}K_a$, $\log K_{ow}$ and vapour pressure).

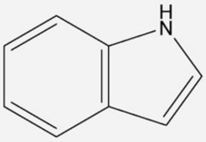
Basic compounds


o-aminoacetophenone

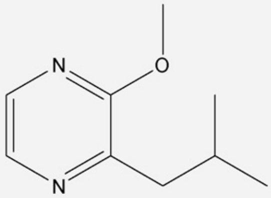
$\mathrm{p}K_a = 2.22$
$\log K_{ow} = 1.63$
Vapour pressure (n.f.)


methyl anthranilate

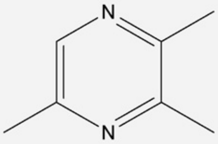
$\mathrm{p}K_a = 2.23$
$\log K_{ow} = 1.88$
Vapour pressure = 0.00989 mmHg


indol

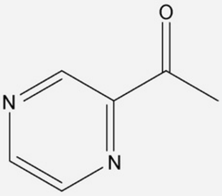
$\mathrm{p}K_a = 2.40$
$\log K_{ow} = 2.14$
Vapour pressure = 0.0122 mmHg


2-isobutyl-3-methoxypyrazine

$\mathrm{p}K_a = 0.75$
$\log K_{ow} = 2.86$
Vapour pressure = 0.035 mmHg

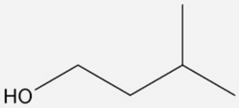

2,3,5-trimethylpyrazine

$\mathrm{p}K_a = 2.80$
$\log K_{ow} = 0.95$
Vapour pressure = 0.608 mmHg

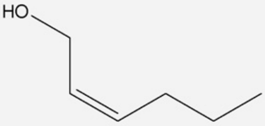

2-acetylpyrazine

$\mathrm{p}K_a$ (0.51[a])
$\log K_{ow} = 0.2$
Vapour pressure = 0.188 mmHg

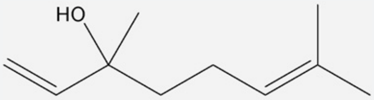
Neutral compounds


isoamyl alcohol

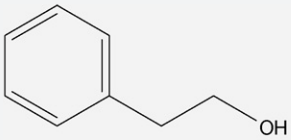
$\mathrm{p}K_a$ (n.f.)
$\log K_{ow} = 1.16$
Vapour pressure = 2.37 mmHg


cis-2-hexenol

$\mathrm{p}K_a$ (n.f.)
$\log K_{ow} = 1.61$
Vapour pressure (n.f.)

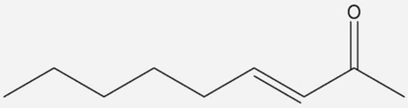
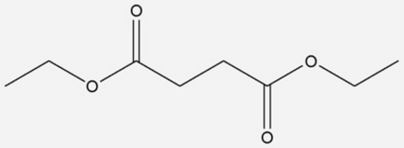
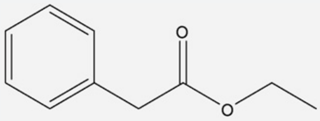
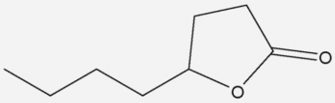
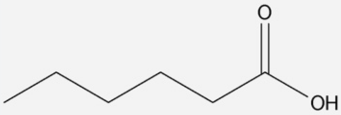
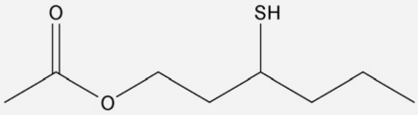
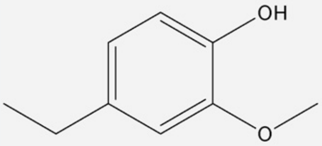
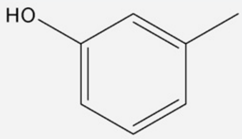

linalool

$\mathrm{p}K_a$ (n.f.)
$\log K_{ow} = 2.97$
Vapour pressure = 0.16 mmHg


b-phenylethanol

$\mathrm{p}K_a$ (n.f.)
$\log K_{ow} = 1.36$
Vapour pressure = 0.0868 mmHg

Table 2 (*Continued*)

Basic compounds	
 3-nonen-2-one pK_a (n.f.) log K_{ow} (n.f.) Vapour pressure (n.f.)	 diethylsuccinate pK_a (n.f.) log K_{ow} = 1.2 Vapour pressure = 0.0439 mmHg
 ethylphenylacetate pK_a (n.f.) log K_{ow} = 2.28 Vapour pressure = 0.062 mmHg	 g-octalactone pK_a (n.f.) log K_{ow} (n.f.) Vapour pressure (n.f.)
Acidic compounds	
 hexanoicacid pK_a = 4.85 log K_{ow} = 1.92 Vapour pressure = 0.0435 mmHg	 3-mercaptohexylacetate pK_a (n.f.) log K_{ow} (n.f.) Vapour pressure (n.f.)
 4-ethylguaiacol pK_a (9.98[a]) log K_{ow} = 2.38 Vapour pressure (n.f.)	 m-cresol pK_a = 10.09 log K_{ow} = 1.96 Vapour pressure = 0.11 mmHg

[a] pK_a values for these compounds have not been found so this table shows pK_a values from analogue analytes (pK_a from pyrazine instead of 2-acetylpyrazine and pK_a value from guaiacol instead of 4-ethylguaiacol).
n.f.: not found.

ues at pH 10 are smaller because the ionic force of these solutions was also smaller (Section 2).

Results show that in most cases the highest retention is observed for the two polymeric reversed-phase sorbents Isolute ENV and LiChrolut EN, in agreement with conclusions reached in a previous study [25]. Both resins are polystyrene-divinylbenzene copolymers of highest surface, which shows that for these relatively small molecules the size of active surface seems to be the most important criterion (see Table 1). This result was in part surprising, since perhaps naively, we had expected that basic compounds at acid pHs and acid compounds at basic pHs would have been more efficiently extracted by mixed-mode cation and anion exchangers, respectively. However, at pH 2 and 3.5, only indole and 2,3,5-trimethylpyrazine were more efficiently extracted by mixed-mode cation exchangers (indole also at pH 10), and acid compound was not more efficiently extracted by a mixed-mode anion exchanger at any pH. This suggests that ion-exchange seems to be a weak retention mode for these volatile compounds in aqueous or hydro-organic solutions and that the different non-ionic intermolecular forces are the most important mechanisms driving extraction. Probably, for non-volatile or semi-volatile compounds the results could be different and ion-exchange could be more important. As for the size of the K_{SL}, compounds can be classified into three categories: compounds reaching K_{SL} higher than 10,000, those with K_{SL} between 10,000 and 1000 and those with K_{SL} below 1000. The former are compounds very easily extracted and in any extraction cartridge it will be easy to get complete extraction for loading volumes higher than 3000 bed volumes, while the latter will reach breakthrough at less than 300 bed volumes [25]. Only isoamyl alcohol and cis-2-hexenol, the smallest molecules in the list, are included in this last group. For the rest of compounds there is a sorbent and pH condition at which retention is high enough.

A principal component analysis was carried out on the matrixes formed by the K_{SL} for all analytes and sorbents at each pH condition. Results are quite similar for the different pH conditions and

Table 3
Recovery obtained in the extraction of the target compounds with LiChrolut EN 200 mg cartridges at pH 2, 3.5 and 10. Elution was carried out with 1 mL dichloromethane/methanol 1% (v/v).

Recovery (%)	pH 2 R (%) ± DSR (%)	pH 3.5 R (%) ± DSR (%)	pH 10 R (%) ± DSR (%)
Basic compounds			
o-Aminoacetophenone	72 ± 5	100 ± 2	100 ± 1
Methyl anthranilate	71 ± 7	100 ± 6	82 ± 6
Indole	48 ± 4	100 ± 1	90 ± 6
2-Isobutyl-3-methoxypyrazine	84 ± 2	93 ± 2	89 ± 8
2,3,5-Trimethylpyrazine	84 ± 1	100 ± 1	100 ± 8
2-Acetylpyrazine	93 ± 9	100 ± 6	71 ± 2
Neutral compounds			
Isoamyl alcohol	58 ± 1	89 ± 1	88 ± 7
cis-2-Hexenol	79 ± 2	100 ± 2	82 ± 7
Linalool	85 ± 1	92 ± 1	89 ± 8
β-Phenylethanol	85 ± 5	112 ± 5	91 ± 9
3-Nonen-2-one	84 ± 1	93 ± 1	88 ± 6
Diethyl succinate	87 ± 2	100 ± 2	91 ± 9
Ethyl pheylacetate	84 ± 1	94 ± 1	89 ± 6
γ-Octalactone	86 ± 6	100 ± 6	93 ± 3
Acidic compounds			
Hexanoic acid	51 ± 17	79 ± 15	
3-Mercaptohexyl acetate	87 ± 3	100 ± 3	52 ± 6
4-Ethylguaiacol	85 ± 2	100 ± 2	82 ± 7
m-Cresol	86 ± 1	100 ± 1	71 ± 8

Recovery data from hexanoic acid could not be determined at pH 10.
All the experiments were carried out in triplicate on different days.

the PCA plot obtained at pH 2 is shown in Fig. 1 as example. As can be seen, all analytes are positively correlated to the 1st Component, which carries 87% of the variance. This means that the scores in this 1st Component are related to the general ability of the sorbents to extract the analytes. The 2nd Component, which carries just 7% of the original variance, is entirely related to the differential ability of the sorbents to extract indole. On this figure could be observed that LiChrolut EN and Isolute ENV resins, both polymer ones, are always in very positive areas of PC1 and far away from the other resins at all pH. The plot clearly shows that the general extraction ability of the sorbents studied follows the order: Isolute ENV (P5) ≈ LiChrolut EN (P6) ≫ Supelclean ENVI-Chromp (P7) ≈ Bond Elut PPL (P3) > Bond Elut ENV (P1) ≈ Bond Elut LMS (P2) > Oasis MCX (C3) ≈ Strata XC (C1) > Oasis MAX (A2) ≈ Bond Elut Plexa (P4) > Bond Elut Certify (C4) > Discovery DSC-MCAX (C2) ≈ Bond Elut Certify II (A1). The same conclusions are reached if we consider the mean K_{SL}, shown in Table 5.

The PCA plot also shows that ion exchangers have a differential behavior, but that differences are not linked to the charge (positive or negative) of the ion exchanged, but to the nature of the sorbent: polymeric or based on Silica. Polymeric mixed-mode sorbents (A2, C3 and C1 in the plot) have a specific ability to extract indole, while silica-based ion exchangers (C4, A1 and C2 in the plot) are characterized by a poor ability to extract indole.

Table 4
Maximum distribution coefficient (K_{SL}) values obtained at pH 2, 3.5 and 10.

Analyte	Resin	pH 2 K_{SL} max ± s	Resin	pH 3.5 K_{SL} max ± s	Resin	pH 10 K_{SL} max ± s
Basic compounds						
o-Aminoacetophenone	Isolute	$6.68 \times 10^3 \pm 2.26 \times 10^2$	Isolute	$6.64 \times 10^3 \pm 5.88 \times 10^2$	Isolute	$1.93 \times 10^3 \pm 9.40 \times 10^1$
Methyl anthranilate	Isolute	$5.97 \times 10^3 \pm 2.02 \times 10^2$	S-EnviC	$4.66 \times 10^3 \pm 6.22 \times 10^2$	Isolute	$8.89 \times 10^2 \pm 1.40 \times 10^1$
Indole	O-MCX	$2.00 \times 10^4 \pm 1.20 \times 10^2$	O-MCX/	$2.79 \times 10^4 \pm 3.12 \times 10^2$	O-MCX	$8.62 \times 10^3 \pm 2.38 \times 10^2$
					Strata XC	$2.77 \times 10^4 \pm 1.23 \times 10^3$
2-Isobutyl-3-methoxypyrazine	LiChrolut	$1.49 \times 10^4 \pm 7.21 \times 10^2$	Isolute	$2.78 \times 10^4 \pm 5.75 \times 10^3$	LiChrolut	$1.11 \times 10^4 \pm 1.30 \times 10^2$
2,3,5-Trimethylpyrazine	Strata XC	$1.35 \times 10^3 \pm 3.57 \times 10^2$	Strata XC	$2.63 \times 10^3 \pm 5.45 \times 10^2$	Isolute	$8.97 \times 10^2 \pm 9.70 \times 10^1$
2-Acetylpyrazine	LiChrolut	$3.86 \times 10^2 \pm 1.40 \times 10^1$	Isolute	$1.76 \times 10^3 \pm 4.86 \times 10^2$	Isolute	$9.06 \times 10^2 \pm 6.60 \times 10^1$
Neutral compounds						
Isoamyl alcohol	LiChrolut	$4.80 \times 10^2 \pm 5.60 \times 10^1$	LiChrolut	$2.49 \times 10^2 \pm 3.30 \times 10^1$	LiChrolut	$2.74 \times 10^2 \pm 5.50 \times 10^1$
cis-2-Hexenol	LiChrolut	$8.37 \times 10^2 \pm 1.75 \times 10^2$	LiChrolut	$6.22 \times 10^2 \pm 2.60 \times 10^1$	LiChrolut	$7.97 \times 10^2 \pm 9.30 \times 10^1$
Linalool	LiChrolut/Isolute	3.00×10^4	LiChrolut/Isolute	3.00×10^4	LiChrolut	$1.40 \times 10^4 \pm 2.62 \times 10^3$
β-Phenylethanol	Isolute	$1.28 \times 10^3 \pm 1.15 \times 10^2$	LiChrolut	$1.45 \times 10^3 \pm 3.90 \times 10^2$	Isolute	$8.77 \times 10^2 \pm 5.70 \times 10^1$
3-Nonen-2-one	Isolute	$2.02 \times 10^4 \pm 7.09 \times 10^2$	Isolute	$2.63 \times 10^4 \pm 2.53 \times 10^3$	Isolute	$1.13 \times 10^4 \pm 2.72 \times 10^2$
Diethyl succinate	LiChrolut	$5.94 \times 10^3 \pm 3.65 \times 10^2$	LiChrolut	$3.28 \times 10^3 \pm 7.74 \times 10^2$	LiChrolut	$3.29 \times 10^3 \pm 1.06 \times 10^2$
Ethyl phenylacetate	Isolute	$2.14 \times 10^4 \pm 6.52 \times 10^2$	Isolute	$1.91 \times 10^4 \pm 1.71 \times 10^3$	Isolute	$1.35 \times 10^4 \pm 1.82 \times 10^2$
γ-Octalactone	Isolute	$1.15 \times 10^4 \pm 3.91 \times 10^2$	LiChrolut	$1.94 \times 10^4 \pm 2.11 \times 10^2$	LiChrolut	$2.02 \times 10^3 \pm 7.70 \times 10^1$
Acidic compounds						
Hexanoic acid	Isolute	$4.83 \times 10^3 \pm 2.10 \times 10^2$	LiChrolut	$6.45 \times 10^3 \pm 4.37 \times 10^2$	n.d.	
3-Mercaptohexyl acetate	LiChrolut/Isolute	3.00×10^4	Isolute	$9.28 \times 10^3 \pm 3.06 \times 10^2$	Isolute	$6.79 \times 10^3 \pm 9.50 \times 10^1$
4-Ethylguaiacol	LiChrolut/Isolute	3.00×10^4	Isolute	$1.28 \times 10^4 \pm 3.02 \times 10^3$	Isolute	$7.02 \times 10^3 \pm 2.42 \times 10^2$
m-Cresol	Isolute	$2.31 \times 10^3 \pm 4.18 \times 10^2$	Isolute	$2.40 \times 10^3 \pm 1.49 \times 10^2$	Isolute	$3.08 \times 10^3 \pm 3.75 \times 10^2$

K_{SL} max: maximum distribution coefficient; s: standard deviation; LiChrolut: LiChrolut EN; Isolute: Isolute ENV; O-MCX: Oasis MCX; S-EnviC: Supelclean ENVI-Chromp.
All the experiments were carried out in duplicate on different days.
n.d.: not determined.

Table 5
Average (arithmetic and geometric) K_{SL} values for each resin and pH condition.

Code	Resins	pH 2		pH 3.5		pH 10	
		Arithmetic mean	Geometric mean	Arithmetic mean	Geometric mean	Arithmetic mean	Geometric mean
P1	BE ENV	3.24×10^3	1.16×10^3	2.29×10^3	1.03×10^3	1.96×10^3	8.90×10^2
P2	BE LMS	2.72×10^3	1.02×10^3	1.79×10^3	8.19×10^2	1.87×10^3	8.57×10^2
P3	BE PPL	4.80×10^3	1.70×10^3	3.20×10^3	1.42×10^3	2.77×10^3	1.22×10^3
P4	BE Plexa	1.82×10^3	8.05×10^2	1.50×10^3	8.05×10^2	1.55×10^3	8.51×10^2
P5	LiChrolut	1.13×10^4	5.09×10^3	1.05×10^4	5.07×10^3	4.92×10^3	2.64×10^3
P6	Isolute	1.13×10^4	5.09×10^3	9.86×10^3	4.56×10^3	4.89×10^3	2.63×10^3
P7	S-EnviC	5.28×10^3	1.99×10^3	4.55×10^3	2.04×10^3	3.29×10^3	1.51×10^3
C1	Strata XC	2.84×10^3	1.14×10^3	3.11×10^3	1.06×10^3	1.03×10^3	5.60×10^2
C2	DSC-MCAX	3.47×10^2	2.71×10^2	2.51×10^2	1.71×10^2	1.84×10^2	1.15×10^2
C3	O-MCX	2.92×10^3	1.19×10^3	2.55×10^3	8.02×10^2	1.08×10^3	4.98×10^2
C4	BE Certify	5.83×10^2	3.14×10^2	2.86×10^2	1.94×10^2	2.18×10^2	1.58×10^2
A1	BE Certify II	3.45×10^2	2.42×10^2	2.80×10^2	2.12×10^2	3.78×10^2	2.17×10^2
A2	O-MAX	2.03×10^3	7.18×10^2	1.20×10^3	6.16×10^2	1.16×10^3	6.17×10^2

BE: Bond Elut; LiChrolut: LiChrolut EN; Isolute: Isolute ENV; S-EnviC: Supelclean ENVI-Chromp; DSC-MCAX: Discovery DSC-MCAX; O-MCX: Oasis MCX; BE Certify: Bond Elut Certify; O-MAX: Oasis MAX.

3.2. *Retention selectivity*

Selectivity has been measured by the calculation of the selectivity parameter (α), defined as the ratio between the specific K_{SL} for a given compound, resin and pH condition and the geometric average K_{SL} for that resin and pH condition. The maximum selectivity coefficients for each analyte at the three pH conditions are given in Table 6. As can be seen, generally speaking it can be said that all basic compounds are more selectively extracted with cationic mixed-mode resins at any pH and also that the maximum selectivity for the extraction of these compounds is obtained at pH 3.5. This last observation can be explained because at this pH both resins and analytes are in ionic form. There are, however, some individual remarks worth considering. First, the maximum selectivity of indole at pH 2 is found for the mixed-mode anion exchanger Oasis MAX (although good α values were also obtained for the mixed-mode cation exchangers Oasis MCX (16.6), Strata XC (16.0) and Bond Elut Certify (14.0)). Second, something similar happens to 2-acetylpyrazine at pH 3.5, for which the maximum selectivity is obtained with an anion exchanger (although again a good α value (1.6) was also obtained for Bond Elut Certify). Third, the two other pyrazines, 2-isobutyl-3-methoxypyrazine and 2,3,5-trimethylpyrazine, at pH 3.5 (only the former) and 10, are more selectively retained with non-ionic sorbents. These observations suggest that even if K_{SL} do not reach very high values, mixed-mode sorbents are providing relatively higher retention for basic compounds, that such higher relative retention does not strictly require that the compound is in cationic form and that for the most non-polar compounds (case of 2-isobutyl-3-methoxypyrazine), non-ionic interactions become more important at pHs above 3.5. Another interesting observation is that selectivities are strongly dependent on the kind of mixed-mode cation exchanger. A strong example of this can be seen in Fig. 2a, showing the different selectivities obtained at pH 3.5 in the case of 2,3,5-trimethylpyrazine. As can be seen in the plot, differences in selectivity between the least and most selective mixed-mode cation exchanger are near a factor 5.

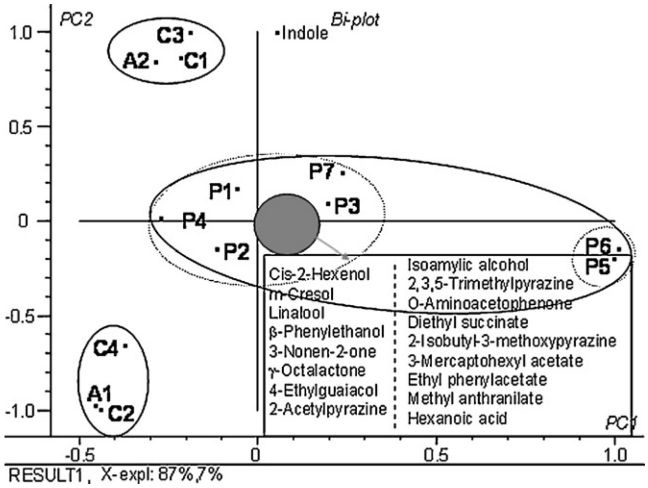


Fig. 1. Distribution coefficients (K_{SL}) PCA at pH 2.

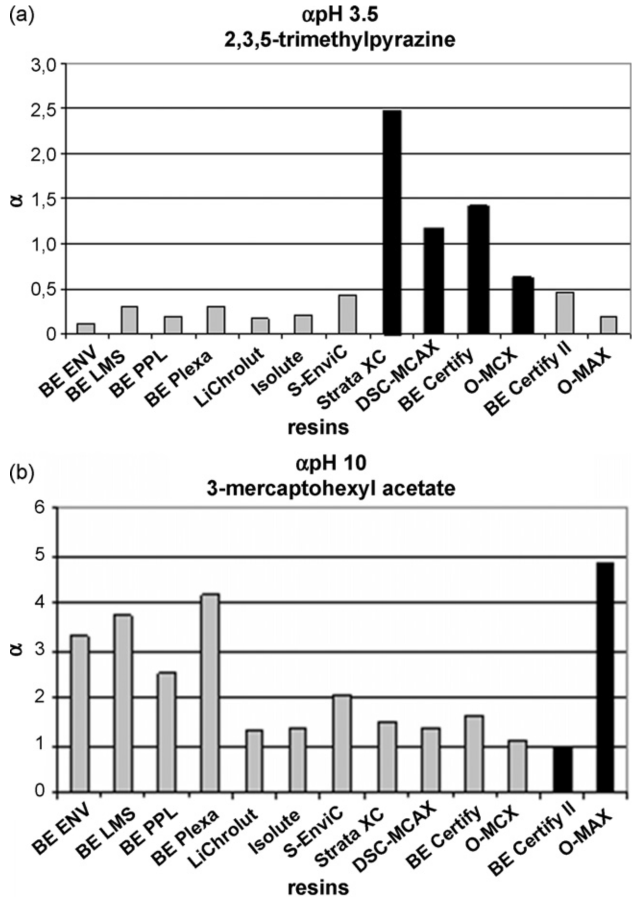


Fig. 2. (a) Selectivity coefficients (α) obtained for 2,3,5-trimethylpyrazine at pH 3.5. (b) Selectivity coefficients (α) obtained for 3-mercaptohexyl acetate at pH 10.

Table 6
Maximum selectivity parameters (α) obtained for each analyte at different pH conditions.

Analyte	pH 2		pH 3.5		pH 10		Resin	$1/\alpha_{min}$	pH
	Resin	α	Resin	α	Resin	α			
Basic compounds									
o-Aminoacetophenone	Strata XC	3.31	Strata XC	2.81	O-MCX	1.28	BE Certify	9.95	10
Methyl anthranilate	Strata XC	3.10	Strata XC	3.84	O-MCX	1.47	LiChrolut	3.20	10
Indole	O-MAX	27.9	Strata XC	34.7	O-MCX	17.3	DSC-MCAX	2.31	10
2-Isobutyl-3-methoxypyrazine	Strata XC	6.72	BE PPL	7.42	BE ENV	4.77	O-MAX	0.80	3.5
2,3,5-Trimethylpyrazine	BE Certify	1.46	Strata XC	2.47	BE Plexa	0.64	DSC-MCAX	112	10
2-Acetylpyrazine	DSC-MCAX	0.48	BE Certify II	2.00	DSC-MCAX	1.60	BE PPL	17.9	2
Neutral compounds									
Isoamylic alcohol	BE Certify II	1.26	DSC-MCAX	0.58	DSC-MCAX	1.12	Isolute	27.7	3.5
cis-2-Hexenol	DSC-MCAX	0.60	BE Certify II	0.30	DSC-MCAX	1.63	O-MCX	16.7	3.5
Linalool	S-EnviC	8.53	Isolute	6.58	LiChrolut	5.30	Strata XC	0.99	3.5
β-Phenylethanol	DSC-MCAX	0.61	BE Certify II	0.52	DSC-MCAX	1.43	DSC-MCAX	7.45	3.5
3-Nonen-2-one	BE PPL	8.49	BE PPL	8.40	BE PPL	7.73	O-MCX	0.53	3.5
Diethyl succinate	LiChrolut	1.17	DSC-MCAX	1.37	LiChrolut	1.24	Strata XC	7.28	2
Ethyl pheylacetate	Isolute	4.24	Isolute	4.19	BE PPL	7.16	BE Certify II	2.15	3.5
γ-Octalactone	Isolute	2.29	LiChrolut	3.84	LiChrolut	0.76	BE Certify II	3.39	10
Acidic compounds									
Hexanoic acid	BE Certify II	4.90	BE Certify	6.54	n.d.	BE ENV	1.66	3.5	
3-Mercaptohexyl acetate	BE ENV	13.7	BE LMS	7.55	O-MAX	9.12	BE PPL	1.02	3.5
4-Ethylguaiacol	Isolute	5.94	Isolute	2.80	BE PPL	2.72	DSC-MCAX	2.39	3.5
m-Cresol	O-MAX	1.00	O-MAX	1.10	O-MAX	1.59	DSC-MCAX	5.19	3.5

BE: Bond Elut; LiChrolut: LiChrolut EN; DSC-MCAX: Discovery DSC-MCAX; S-EnviC: Supelclean ENVI-Chromp; Isolute: Isolute ENV; O-MCX: Oasis MCX; O-MAX: Oasis MAX.

Similar observations are derived from selectivity data of acid compounds, although in this case only for m-cresol the maximum selectivity is reached with anion exchangers at all pHs. In the case of hexanoic acid, the maximum selectivity is observed for a cation exchanger at pH 3.5, and in the case of the least polar compounds (3-mercaptohexyl acetate and 4-ethylguaiacol), non-ionic sorbents provide the best results, except for 3-mercaptohexyl acetate at pH 10. The highly significant role played by the kind of exchanger in this last case is shown in Fig. 2b. As can be seen, even if the maximum selectivity is observed with a mixed-mode anion exchanger (Oasis MAX), the worst results was obtained with the other mixed-mode anion exchanger (Bond Elut Certify II), which again shows that selectivity is strongly dependent on the nature of the sorbent and that cannot be predicted beforehand.

In any case, results are somehow frustrating, although mixed-mode resins show in general highest selectivities for the extraction of ionogenic compounds, the α values obtained are relatively low. In fact, given the low number of plates of a standard SPE cartridge (between 5 and 10), selectivity values higher than 10 would be required to ensure a selective isolation of the compound during the extraction (achieved by trapping and further rinsing with a polar solvent). This condition is only fulfilled in the case of indole at any pH and is close to be fulfilled by 3-mercaptohexyl acetate at pH 10. In this last case a much better selectivity is obtained at pH 2 with a non-ionic sorbent.

The lack of retention can be also exploited to get a selective isolation (in this case interferences would be retained in the SPE cartridge and the analyte would pass unretained). This is measured in the last columns of Table 6, which gives the maximum $1/\alpha$ observed for each compound. This strategy would make it possible to get selective isolates of 2,3,5-trimethylpyrazine, 2-acetylpyrazine, isoamyl alcohol and cis-2-hexenol.

On the other hand, Discovery DSC-MCAX resin is characterized for being the less selective one. In this case α ranges from 0.5 to less than 10, which suggests that this resin would be ideal for obtaining non-selective extracts.

The general selectivity behavior of the sorbents can be seen on the PCA plot shown in Fig. 3, which roughly shows that sorbents can be classified again into three categories: reversed-phase polymeric sorbents, mixed-mode polymeric and mixed-mode silica-based sorbents. Reversed-phase polymeric sorbents are most selective for the majority of the studied compounds. Polymeric mixed-mode sorbents are more selective to indole, and silica-based mixed mode to hexanoic acid. In this case, opposite to Fig. 1, subgroups have not been observed for polymeric resins. Another difference with respect to the PCA present in Fig. 1 is that in the polymeric resins of mixed mode, the A2 anionic sorbent is not in the same position to the two cationic sorbents C1 and C3.

3.3. Elution selectivity

Even if the retention cannot be very selective, if an ionogenic compound is retained in a real mixed mode, combining hydrophobic and ion exchange modes, elution could be very selective since the compound most likely will not be eluted by neutral elution solvents. This hypothesis was tested with Strata XC and Oasis MAX mixed-mode resins. Analytes were retained in standard cartridges at pH 2 in the first case and at pH 10 in the second one. Elution was sequentially carried out first by dichloromethane and second

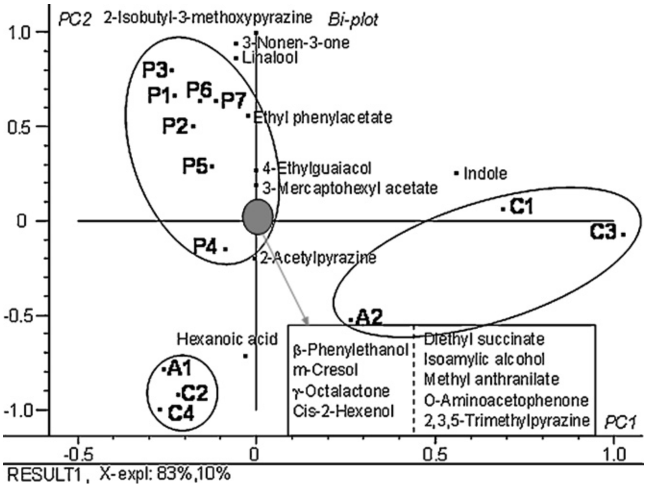


Fig. 3. Principal component plot of selectivity coefficients (α) at pH 3.5.

Table 7
Recoveries (as %) of analytes retained at pH 2 in 50 mg of Strata XC mixed-mode cation exchanger and at pH 10 in 50 mg of Oasis MAX mixed-mode anion exchanger obtained in a two-step elution sequence.

	Strata XC pH2		Oasis MAX pH10	
	1 mL DCM	1 mL DCM/triethylamine ($10\,gL^{-1}$)	1 mL DCM	1 mL DCM/formic acid 10% (v/v)
	Averages	Averages	Averages	Averages
Basic compounds				
o-Aminoacetophenone	–	18 ± 6	19 ± 9	–
Methyl anthranilate	4 ± 8	68 ± 9	74 ± 1	–
Indole	61 ± 10	–	66 ± 11	–
2-Isobutyl-3-methoxypyrazine	–	98 ± 7	73 ± 14	–
2,3,5-Trimethylpyrazine	–	5 ± 9	8 ± 15	–
2-Acetylpyrazine	–	29 ± 8	12 ± 6	–
Netutral compounds				
Isoamylic alcohol	45 ± 10	–	35 ± 12	–
cis-2-Hexenol	93 ± 15	–	84 ± 18	3 ± 1
Linalool	90 ± 15	–	88 ± 11	–
2-Phenylethanol	104 ± 5	–	94 ± 12	–
3-Nonen-2-one	92 ± 18	–	85 ± 14	–
Diethyl succinate	95 ± 12	–	96 ± 12	–
Ethyl phenylacetate	96 ± 16	–	87 ± 19	–
γ-Octalactone	105 ± 2	–	90 ± 7	–
Acidic compounds				
Hexanoic acid	12 ± 4	9 ± 3	–	51 ± 11
3-Mercaptohexyl acetate	58 ± 7	–	3 ± 5	–
4-Ethylguaiacol	97 ± 12	–	80 ± 9	–
m-Cresol	80 ± 9	20 ± 6	28 ± 4	67 ± 10

Mean of three independent experiments.

by dichloromethane enriched either with an alkali (triethylamine) or and acid (formic acid) for the cation and anion exchangers, respectively. Results are shown in Table 7. Maybe, a more polar elution solvent (e.g. methanol) would provide better recoveries, but dichloromethane was chosen for the direct insertion into the gas chromatograph.

Results in Table 7 show that 1 ml of dichloromethane elutes nearly all neutrals and also a significant part of acid compounds (except hexanoic acid), but that it is not able to elute out of the cartridge basic compounds, with the exception of indole. So, this compound at pH 2 is not protonated, in spite of its theoretical pK_a 2.4, because if indole was protonated should be retained as the other basic analytes. However, it stands out from the rest at pH 3.5 (where all basic analytes of this study present their neutral form) for its higher retention at least one magnitude order with Strata XC sorbents. Relevant data from K_{SL} with Strata XC at pH 3.5 are not presented in any table. These K_{SL} values obtained are the following: o-aminoacetophenone (2981), methyl anthranilate (4079), indole (27,684), 2-isobutyl-3-methoxypyrazine (6697), 2,3,5-trimethylpyrazine (2626) and 2-acetylpyrazine (626).

This suggests that, leaving aside indole, basic compounds are effectively retained in a mixed mode. This was confirmed in part by the elution with dichloromethane containing an alkali. This solvent was able to elute the compounds in cationic form, although only in the cases of methyl anthranilate and isobutyl 2-methoxypyrazine recoveries were high. In any case, this result suggests that highly selective isolation procedures can be obtained for basic analytes, although the elution of the resins with GC-compatible solvents may turn difficult. In the case of acid compounds, results in Table 7 show that dichloromethane cannot elute hexanoic acid and 3-mercaptohexyl acetate, and that only 4-ethylguaiacol is easily eluted by this solvent. The second solvent, containing now formic acid, was able to elute significant amounts of hexanoic acid and of m-cresol. 3-Mercaptohexyl acetate could not be detected, perhaps because it was degraded by formic acid. Results in this case, confirm that also for some acid analytes is possible to design highly selective isolation schemes using mixed-mode anion exchanger, but that again, difficulties linked to the elution of the compounds with GC-compatible systems may be found.

4. Conclusions

This work has shown that among all the tested sorbents it is possible to find one at a given pH condition able to provide a high retention ($K_{SL} > 1000$) for all the tested analytes, except for the small alcohols such as isoamyl alcohol and cis-2-hexenol. The highest retention is observed in nearly all cases with non-functionalized high surface polymeric sorbents. It has also been shown that retention of small ionogenic volatile compounds can be just slightly improved by using mixed-mode sorbents. In particular, it has been shown that although these sorbents are in general more selective towards ionogenic molecules, they do not provide more retention than neutral polymeric sorbents of high surface. The study has also shown that selectivity depends on the pH, the exact kind of mixed-mode sorbent and on the polarity of the analyte. In some surprising cases, the highest selectivity has been achieved with mixed-mode sorbents with the same charge of the analyte, which suggests that secondary interactions can be of paramount importance. On the contrary, the highest selectivity can be obtained by combining retention in mixed mode, a rinsing with a non-polar solvent and further elution with a solvent containing a neutralizing agent. However, not all the ionogenic molecules seem to be retained in ionic mode in the conditions tested and the complete elution of some analytes can be difficult, which suggests that analyte-specific isolation procedures should be analyzed case by case.

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