Comparison of mixed-mode anion-exchange performance of N-vinylimidazole-divinylbenzene sorbent

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1. Abstract

A newly synthesized copolymer based on N-vinylimidazole-divinylbenzene (NVIm-DVB) was evaluated as a mixed-mode anion-exchange sorbent for solid-phase extraction (SPE) since the NVIm monomer apart from its hydrophilic properties can be protonated at certain pH, and then, perform as anion-exchanger. To investigate the behavior of the NVIm-based sorbent, the SPE performance was evaluated under reversed-phase, weak anion-exchange and strong anion-exchange conditions. The results for NVIm-DVB sorbent were also compared to commercial reference sorbents from each group: Oasis HLB, Oasis WAX and Oasis MAX, respectively. From this evaluation, NVIm-DVB showed suitable SPE results as reversed-phase material, compared to Oasis HLB, as well as potential as strong anion-exchange sorbent, compared to Oasis MAX, since under the proper conditions it was able to fraction and quantitatively recover a group of selected solutes.

2. Introduction

At present, solid-phase extraction (SPE) is undoubtedly the most extensive pretreatment technique used to isolate and concentrate analytes from complex samples [1-4]. To further enhance the results in SPE, in the last few years, there has been a growing interest in developing commercial or laboratory-made sorbents with improved extraction efficiency. There are several available sorbents that improve the sensitivity (i.e. polymeric sorbents with hypercrosslinked structure or/and hydrophilic character) [5,6] and those that improve selectivity (i.e. immunosorbents –ISs- or molecularly imprinted polymers –MIPs-) [7-9].

In multiresidue analysis, however, a compromise between sensitivity and selectivity must be reached. Restricted-access materials (RAM), which are silica-based materials intended for the analysis of small molecules in a complex matrix, could be a suitable option to clean-up the sample, but not always to achieve sensitivity [10,11].

Mixed-mode SPE sorbents, which combine an effective reversed-phase chemistry with ion-exchange sites, can be used to extract the analytes of interest from the rest of matrix interferences, ideally, by properly controlling the SPE conditions (mainly the pH as well as the percentage of organic solvent) [12]. Therefore, these types of sorbents are promising materials for the net extraction of pharmaceuticals, pollutants or many other types of compounds from complex matrices [11,13-15]. Polymeric ion-exchange resins have a higher exchange capacity and a wider pH operating range than the classical silica-based ion-exchangers [1]. Waters Corporation pioneered the introduction of mixed-mode polymeric sorbents with Oasis MCX (with sulfonic groups) and

Oasis MAX (with primary amine groups). More recently, the same company commercialized weakly dissociated ion-exchange sorbents -Oasis WCX (with carboxylic acid groups) and Oasis WAX (with piperazine groups)- which may allow for milder elution conditions.

In a previous work [16] we evaluated a laboratory-made N-vinylimidazole-divinylbenzene (NVIm-DVB) polymeric sorbent [17] as a new hydrophilic sorbent to extract polar compounds of environmental interest from aqueous samples, with the aim of determining if polar interactions between the imidazole group and polar analytes increased retention. However, apart from hydrophilicity, N-vinylimidazole monomer adds another feature. This group can be protonated depending on the pH. Then, the NVIm-based resin can perform as mixed-mode anion-exchange sorbent. The goal of the present paper is to explore both hydrophilic and anion-exchanger mechanisms on NVIm-DVB. With this proposal, we have tested in detail the behavior of NVIm-DVB sorbent under reversed-phase, weak anion-exchange and strong anion-exchange conditions for the SPE of a group of small pharmaceutical solutes. We have also compared the NVIm-DVB sorbent to the commercial Oasis HLB, Oasis WAX and Oasis MAX sorbents, which are based on N-vinylpyrrolidone-divinylbenzene and make them structurally comparable to NVIm-DVB one.

3. Material and methods

- Reagents and standards

The analytes selected (whose concentrations in μg ml^{-1} are enclosed in brackets) to check the sorbent under reversed-phase conditions were: amitriptyline (7.5), propranolol (40.0), N-acetyl procainamide (7.5), caffeine (7.5), acetaminophen (5.0), betamethasone (2.5), practolol (5.0), phenylacetic acid (150.0) and naproxen (5.0), and ibuprofen (500.0) as internal standard. The analytes selected to check the sorbent under mixed-mode conditions were: 2-naphthalenesulfonic acid (2.5), 4-propylbenzoic acid (30.0), secobarbital (12.5), ketoprofen (15.0), salicylic acid (7.5) and amitriptyline (5.0), and propylparaben (100.0) as internal standard. All the analytes were supplied from Aldrich (St. Louis, MO, USA). The structure and pK_a values of all the analytes are in Figure 1.

Standard solutions at the given concentration of each compound were prepared in methanol/water mixtures. The mixture of all the compounds was prepared by diluting the standard solutions with isotonic saline, pH = 7. The isotonic saline was prepared by dissolving: 0.2 g KCl, 8.0 g NaCl, 0.2 g KH_2PO_4 and 1.2 g Na_2HPO_4 in 1 liter of Milli-Q water adjusted to pH = 7 with concentrated H_3PO_4. The salts used to prepare the isotonic saline were supplied from Aldrich.

Acetonitrile, methanol, isopropanol, phosphoric acid (all supplied from JT Baker (Paris, KY, USA)), formic acid from EM Science (Gibbstown, NJ, USA) and ammonium hydroxide from Aldrich were all HPLC grade. Sodium dihydrogen phosphate was purchased from Aldrich.

- Chromatographic equipment

The HPLC system consisted of 600E pumps on-line connected to 717plus Autosampler, a column heater unit and 2487 UV detector (all from Water, Milford, MA, USA). The analytical column depended on the evaluation. A 75 x 4.6 mm I.D. Waters SymmetryShield RP18, 5 μm was used to analyse the SPE fractions from the reversed-phase evaluation. A 50 x 4.6 mm I.D. Waters SymmetryShield RP18, 5 μm for the weak anion-exchange evaluation and, a 150 x 4.6 mm I.D. Waters Atlantis dC18, 5 μm for the strong anion-exchange evaluation.

A vacuum manifold was used for the solid-phase extraction process.

- Polymerization

The polymer beads were obtained by an optimized suspension polymerization method described in previous work [17].

The sieved copolymer with fraction in the range 32-50 μm was selected for the SPE evaluation.

The resin was characterized by measuring its surface area (627 $m^2\ g^{-1}$) by N_2 sorption and applying the BET model, and the nitrogen content (6.26 wt.% N) with elemental analysis. The anion-exchange capacity is 1.130 ± 0.005 meq g^{-1} measured by performing chloride exchange followed by tritation with silver nitrate.

- Chromatographic conditions

In the reversed-phase conditions, the mobile phase consisted of Milli-Q water acidified with 0.01% formic acid (A) and acetonitrile (B). The gradient profile and flow-rate are: 100% A initially, 35% A after 7.33 min at 2 ml min^{-1}, 100% B at 8.60 min increasing the flow to 3 ml min^{-1}, then held at 100% B for 0.24 min and

increasing the flow rate to 4 ml min^{-1}, finally, the gradient and flow-rate were returned to initial conditions at 9 min. The sample was analyzed at 30ºC and the injection volume was 10 μl for all the samples.

For the weak anion-exchange evaluation, the chromatographic separation was performed under isocratic conditions. The mobile phase was 42% methanol and 58% aqueous phase, which contained 20 mM of sodium dihydrogen phosphate adjusted to pH = 2.7 with concentrated H_3PO_4. The column oven was set at 30 ºC and the flow-rate was 1.8 ml min^{-1}. The injection volume was 15 μl for all the samples.

As for the strong anion-exchange conditions, the mobile phase was the same as the weak anion-exchange conditions (i.e. solvent A= 20 mM NaH_2PO_4, pH = 2.7 and solvent B= methanol), but the chromatographic separation was performed using the following gradient: 75% A initially, 5% A at 10 min held for 2 min and then returned at initial conditions, which are maintained till 20 min. The column oven was set at 30 ºC and the flow-rate was 1.5 ml min^{-1}. The injection volume was also 15 μl for all the samples.

The wavelength used to detect the analytes under reversed-phase conditions was 254 nm. The wavelength to detect the analytes under weak anion-exchange conditions was 214 nm and under strong anion-exchange was 220 nm.

- Solid-phase extraction conditions

The materials tested in solid-phase extraction procedure were: the in-house synthesized NVIm-DVB sorbent (in the fraction whose particle size was between 32–50 μm) and the commercial Oasis HLB, Oasis WAX and Oasis MAX (see structures in Figure 2). All these sorbents were used as 30 mg bed

format, 1 ml cartridges. The SPE experiments were performed with a vacuum manifold set to 5 mmHg (except in the drying step) procured from Waters.

The SPE procedure differed depending on whether the sorbent was being evaluated as a reversed-phase, weak anion-exchange or strong anion-exchange sorbent. The procedures for each condition are detailed in Table 1. To control the different steps of each procedure an internal standard was added. This addition is as follows:

- Reversed-phase. 0.5 ml of ibuprofen at 0.50 mg ml^{-1} in 50/50 (v/v) methanol/water were added as the internal standard to the collected fractions from steps 3-5. The collected fractions from steps 4 and 5 were further diluted with 0.333 ml of 40/60 (v/v) acetonitrile/ isopropanol and from step 6 with 0.750 ml of isotonic saline.

- Weak anion-exchange. 0.5 ml of 0.100 mg ml^{-1} propylparaben in 70/30 (v/v) acetonitrile/water solution was added as the internal standard to the collected fractions from steps 4-7. The collected fractions from steps 4 and 5 were further diluted with 1 ml of 2% NH_4OH acid in 20/80 (v/v) methanol/acetonitrile. The collected fractions from step 6 were further diluted with 1 ml of isotonic saline. The collected fractions from step 7 were further diluted with 0.250 ml of isotonic saline.

- Strong anion-exchange. 0.5 ml of 0.100 mg/mL propylparaben in 70/30 (v/v) acetonitrile/water solution was added to the collected fractions from steps 4-7. The collected fractions from steps 4 and 5 were further diluted with 1 ml of 2% formic acid in 20/80 (v/v) methanol/acetonitrile. The collected fractions from steps 6 and 7 were further diluted with 1 ml of isotonic saline.

4. Results and discussion

In previous work [16,18] the N-vinylimidazole-divinylbenzene (NVIm-DVB) (the structural details of this copolymer are in Figure 2) sorbent was evaluated for the solid-phase extraction (SPE) of polar compounds such as oxamyl, methomyl, phenol or 4-nitrophenol from aqueous samples. We worked at a loading pH ~ 3 in which the sorbent was protonated but the analytes studied, whose pK_a-values are higher than 4, were predominantly in the neutral form. Under these conditions we were attempting to exploit the hydrophilicity of the imidazole group. In these experiments, we did not evaluate ion-exchange interactions. This effect is equally important because the imidazole group can act as an anion-exchanger at pH values less than seven (the nitrogen atom in this group has a pK_a ~ 7), and therefore, this atom can transfer proton donating effects. In the present study, we studied both hydrophilic and anion-exchanger mechanisms on NVIm-DVB.

- Reversed-phase conditions

The NVIm-DVB sorbent was evaluated under reversed-phase loading conditions for a group of small molecules (Figure 1(a)). The experiments were carried out under wet and dry conditions, which means with or without drying (step 2) in the conditioning stage. The NVIm-DVB sorbent showed no difference in recoveries for the small molecule solutes studied under both fully wet and dry conditions. The elution recovery values were 90-100% for all of the solutes except for practolol (51.9%) and N-acetyl procainamide (63.5%), which are the earliest eluting compounds in the HPLC method with retention times of 1.30 min. and 1.62 min., respectively. Both hydrophilic bases have low elution

recovery. We believe that under the extraction conditions, the positively charged, hydrophilic practolol and N-acetyl procainamide are not hydrophobic enough (due to electrostatic repulsion) to be retained in the positively charged NVIm-DVB bed.

In addition to the breakthrough of the hydrophilic bases, phenylacetic acid behaves unexpectedly on the NVIm-DVB sorbent. Phenylacetic acid did not break through during the load and equilibrate step. The enhanced retention of this solute is presumably due to the anion-exchange interaction between the positively charged sorbent and the deprotonated phenylacetic acid; however, this interaction is not strong enough to prevent elution of the solute upon exposure to a purely organic solvent (40/60 (v/v) acetonitrile/isopropanol).

These results were compared to Oasis HLB (Waters) in order to further demonstrate any differences in the retention characteristics of the sorbents. Oasis HLB is an N-vinylpyrrolidone-divinybenzene (NVP-DVB)-based sorbent (see Figure 2). The NVP monomer does not have any nitrogen that is similar to the nitrogen in NVIm (i.e. can become positively charged at the pH conditions in the SPE procedure (i. e. pH ~ 7)). Unlike NVIm-DVB, Oasis HLB can only interact with the solutes through hydrophilic and hydrophobic interactions. This can be clearly seen by comparing the SPE results; thus, all the analytes presented recoveries near to 100% when extracted with Oasis HLB, except for phenylacetic acid. Unlike NVIm-DVB, the phenylacetic acid completely breaks through on Oasis HLB during the water equilibrate step. Since the NVIm-DVB did not behave like the Oasis HLB for the negatively charged phenylacetic acid, the next step was to investigate the anion-exchange capabilities of the sorbent.

- Weak anion-exchange conditions

The anion-exchange characteristics of NVIm-DVB sorbent were evaluated with a modified set of solutes (see Figure 1 (b)) using the experimental conditions described earlier (Table 1).

The sorbent performed similarly under fully wet and dry conditions (Table 2 shows the results under wet conditions); thus, further demonstrating resistance to dewetting. It is clear from the data (Table 2) that quantitative recovery of the acidic solutes from NVIm-DVB was not achieved under weak anion-exchange elution conditions. NVIm-DVB did not show breakthrough of the solutes during the load and equilibration steps under both fully wet and dry conditions, but the sorbent failed to completely retain the weakest acids (ketoprofen and 4-propylbenzoic acid) by anion-exchange during the basic solute elution step (to elute the basic analytes that are in a neutral form) using 100% methanol. The stronger acids (salicylic acid and 2-naphthalenesulfonic acid) did not breakthrough when exposed to methanol; however, elution with 2% NH_4OH in 20/80 (v/v) methanol/acetonitrile (to deprotonated the sorbent, and, hence, elute acidic analytes, due to the effect of the organic solvent) fails to quantitatively recover the salicylic acid or the 2-naphthalenesulfonic acid.

In order to address the breakthrough of the weak acids during basic solute elution, the pH of the equilibration buffer was increased to 5.0 (slightly above the pK_a values for ketoprofen –4.8– and 4-propylbenzoic acid –4.4–) to increase anion-exchange interactions with the sorbent. By increasing the pH of the equilibration step, we ensure the degree of deprotonation of those solutes which in turn enhances anion-exchange interactions with the positively charged NVIm-DVB sorbent. As shown in Table 3, increasing the pH of the equilibration step

improves fractionation selectivity, especially in the case of ketoprofen, presumably by increasing the anion-exchange interactions between the solutes and the sorbent. However, the increase in anion-exchange interactions was sufficient to substantially decrease the recovery of salicylic acid during the subsequent acidic solute elution step (with 2% NH_4OH in 20/80 (v/v) methanol/acetonitrile).

Based upon the pK_a values of the weak acids and an estimation of the pK_a of the imidazole groups on the sorbent ($6 < pK_a < 8$), it was reasonable to believe that using water would insure the deprotonation of the weak acids while maintaining sufficient, but not excessive anion-exchange capacity, because at the water pH some of the imidazole functionalities would not become protonated (i.e. neutral). Using water (pH = 5.8) for the equilibration step (Table 3) decreased the anion-exchange retention enough to improve salicylic acid recovery during the acidic solute elution step; however, this led to complete breakthrough of the weak acids prior to the acidic solute elution step. Despite the apparent decrease in anion-exchange capacity, the 2-naphthalenesulfonic acid still failed to elute from the sorbent under these conditions.

A third experiment was conducted to determine if the combination of using pH = 5 equilibration buffer and a more alkaline elution solvent for the acidic solutes (5% NH_4OH in 20/80 (v/v) methanol/acetonitrile) would maximize anion-exchange retention yet still allow for quantitative recovery of all of the acidic solutes. As shown in Table 3, this combination only worked well for ketoprofen. Salicylic acid recovery remained low and 2-naphthalenesulfonic acid was still not being released from the sorbent despite the use of 5% NH_4OH in 20/80 (v/v)

methanol/acetonitrile. Therefore, none of the conditions used could elute all of the selected analytes.

Based on previous experience with NVIm-DVB in off-line SPE [16], one could think that the volume (1 ml) of either the basic or acid elution was insufficient to elute the highly retained 2-naphthalenesulfonic acid. This possibility was ruled out by additional elution steps that failed to improve recovery.

Another alternative to improve the elution of acidic compounds without increasing the elution volume would be to use stronger elution conditions, such as an increase in the percentage of NH_4OH; however, again, this option was discarded due to the perception that injecting highly alkaline solutions results in HPLC column damage and potential problems from solvent mismatch between the sample and the mobile phase.

Next, we compared NVIm-DVB results under the first experimental conditions (see Table 1) to those of a “well-established” weak anion-exchange sorbent: Oasis WAX (Figure 2) (Waters), which has the same underlying polymeric structure as Oasis HLB, with additional piperazine functionalities that make the sorbent positively charged at pH lower than 6.

As we can see from Table 4, Oasis WAX provided retention, fractionation selectivity, and quantitative elution recovery under these test conditions as expected for a weak anion-exchange sorbent. From this last comparison and bearing in mind the results for NVIm-DVB, we can conclude that this newly synthesized sorbent does not act as a weak anion-exchange sorbent. To further probe the SPE potential of the NVIm-DVB sorbent, we tested this sorbent in strong anion-exchange conditions to determine if it behaved as strong-anion-exchange sorbent.

- Strong anion-exchange conditions

In this set of experiments, we used the same solutes as in the previous section, but we modified the SPE conditions, as detailed in Table 1. Basically, we changed the equilibration conditions from pH 4 or 5 (weak) to pH 7 (strong) and we eluted with a strong acid additive (2% formic acid) to protonate the solutes in the elution step rather than a basic solution (NH_4OH) that deprotonates the sorbent under the weak anion-exchange conditions. The experiments were only performed under wet conditions because it was demonstrated in previous experiments that NVIm-DVB performs similarly under both wet and dry conditions. The SPE results are summarized in Table 5. It is very clear from the results that using formic acid in the elution solvent rather than the NH_4OH used to elute acids from a weak anion-exchanger dramatically increased the recovery of salicylic acid and improved the recovery of ketoprofen. However, 4-propylbenzoic acid was partially eluted between basic and acidic elution. This retention behavior might be explained by conjugation between the carboxylic acid group and the benzene ring in 4-propylbenzoic acid. The conjugation leads to charge delocalization in 4-propylbenzoic acid (i.e. lower charge density) and reduces the strength of its anion-exchange interactions with NVIm-DVB, thus making it more susceptible to breakthrough. Alternatively, ketoprofen shows very little breathrough during the basic solute elution step (100% methanol). The carboxylic acid group in ketoprofen is directly bonded to an aliphatic group, thus making the charge much less delocalized. The higher charge density of ketoprofen enhances its anion-exchange interactions with the NVIm-DVB sorbent. It should also be noted that

the sorbent failed to release the 2-naphthalenesulfonic acid during the acidic solute elution step.

This type of elution behavior, where it is necessary to change the charge of the solutes to elute them rather than to change the charge of the sorbent, indicates that the imidazole groups on the NVIm-DVB sorbent are behaving as *strong* anion-exchange sites because their protonation state is hard to modify. NVIm-DVB provides adequate fractionation selectivity for most of the solutes and nearly quantitative recovery using these SPE conditions. NVIm-DVB works reasonably well as a mixed-mode strong anion-exchanger.

As discussed in previous sections, we did in parallel the same experiments using Oasis MAX (Figure 2) (Waters), which is a strong anion-exchange sorbent (quaternary ammonium functionality). Table 5 shows the results for Oasis MAX. These recovery results are similar to the ones for the NVIm-DVB with the exception of 4-propylbenzoic acid. This solute was partially eluted between the basic and acid elution with NVIm-DVB sorbent, while it is totally eluted during acidic elution (as expected) with Oasis MAX. The differences in the retention of this solute on both sorbents (NVIm-DVB and Oasis MAX) can be also explained by charge delocalization on the NVIm-DVB. The amine functionality of Oasis MAX is a quaternary ammonium group bonded to the polymer network through an aliphatic carbon and this charge is not delocalizated because the charge center is not in conjugation with the polymer. Alternatively, the positive charge in imidazole group of NVIm-DVB is delocalizated across the imidazole ring.

Thus, although NVIm-DVB acts as a strong anion-exchange sorbent, its charge delocalilization along the aromatic ring may explain why NVIm-DVB is less retentive than Oasis MAX. This charge delocalization and, therefore, the

lower charge density, might allow us to use the NVIm-DVB sorbent under milder anion-exchange SPE conditions, which might be an advantage in the extraction of certain families of analytes or applications than other “well-established” strong anion-exchange sorbents such as Oasis MAX.

6. Concluding remarks

NVIm-DVB polymeric sorbent provides good results as both a unique reversed-phase and also as a mixed-mode anion-exchange sorbent. The imidazole functionality contributes hydrophilicity to the sorbent, thus enhancing polar interactions with the solutes. This group can be protonated depending on pH, thus allowing for anion-exchange interactions with almost all the studied compounds.

The data clearly indicate that NVIm-DVB behaves as a strong anion-exchanger. The sorbent provides adequate fractionation selectivity for most of the solutes tested and quantitative recovery. The results also illustrate that judicious choice of SPE conditions is critical to achieving the desired selectivity and solute recovery.

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Table 1. SPE conditions for reversed-phase, weak anion-exchange and strong anion-exchange evaluation.

	SPE CONDITIONS		
	Reversed-phase	**Weak anion-exchange**	**Strong anion-exchange**
1. Condition	1 ml MeOH	1 ml MeOH	1 ml MeOH
2. Dry (optional)	10 mmHg for 10 min.	10 mmHg for 10 min.	-
3. Condition	1 ml H_2O	1 ml H_2O	1 ml H_2O
4. Load	1 ml solute-spiked isotonic saline, pH 7	1 ml solute-spiked isotonic saline, pH 7	1 ml solute-spiked isotonic saline, pH 7
5. Equilibrate	1 ml H_2O	1 ml 25mM sodium acetate buffer, pH 4 or pH 5	1 ml 95/5 (v/v) 25mM sodium acetate buffer, pH 7 / MeOH
6. Basic & neutral elution	4 x 0.25 ml 40/60 (v/v) ACN/Isoprop.	1 ml MeOH	1 ml MeOH
7. Acidic elution		4 x 0.25 ml of 2% or 5% NH_4OH in 20/80 (v/v) MeOH/ACN	2 x 1 ml of 2% formic acid in 20/80 (v/v) MeOH/ACN

Table 2. Weak anion-exchange SPE recovery (%) of NVIm-DVB sorbent under wet established conditions.

Solute	Recovery	
	Basic elution	*Acidic elution*
2-Naphthalene-sulfonic acid	0.0	0.0
Salicylic acid	0.0	68.8
Ketoprofen	59.1	36.0
4-Propylbenzoic acid	99.0	0.0
Amitriptyline	100.1	0.0
Secobarbital	101.5	0.0

Relative standard deviation % (R.S.D. %) (n=3) lower than 8%.

Table 3. Weak anion-exchange SPE recovery (%) of NVIm-DVB sorbent under modified conditions.

Solute	Equilibrate at pH 5[a]			Equilibrate with H_2O	
	Basic elution	*Acidic elution*[b]	*Acidic elution*[c]	*Basic elution*	*Acidic elution*[b]
2-Naphthalene-sulfonic acid	0.0	0.0	0.0	0.0	0.0
Salicylic acid	0.0	23.0	67.9	0.0	85.7
Ketoprofen	0.0	88.1	92.9	96.0	0.0
4-Propylbenzoic acid	52.5	34.0	36.3	96.4	0.0
Amitriptyline	100.9	0.0	0.0	96.7	0.0
Secobarbital	102.3	0.0	0.0	101.9	0.0

R.S.D. %) (n=3) lower than 8%. a) Two set of experiments just different in the acidic elution conditions used. Acidic elution with: b) 2% NH_4OH or c) 5% NH_4OH in 20/80 (v/v) methanol/acetonitrile.

Table 4. Weak anion-exchange SPE recovery (%) of Oasis WAX sorbent under wet established conditions.

Solute	Recovery	
	Basic elution	*Acidic elution*
2-Naphthalene-sulfonic acid	0.0	97.4
Salicylic acid	0.0	96.4
Ketoprofen	0.0	95.7
4-Propylbenzoic acid	0.0	95.0
Amitriptyline	94.5	0.0
Secobarbital	94.5	0.0

R.S.D. % (n=3) lower than 6%.

Table 5. Strong anion-exchange SPE recovery (%) of NVIm-DVB and Oasis MAX sorbent under wet established conditions.

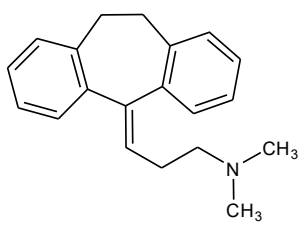
	Recovery					
	NVIm-DVB			Oasis MAX		
Solute	*Load + Equilib.*	*Basic elution*	*Acidic elution*	*Load + Equilib*	*Basic elution*	*Acidic elution*
2-Naphthalene-sulfonic acid	5.3	0.0	0.0	0.0	0.0	0.0
Salicylic acid	0.0	0.0	95.6	0.0	0.0	98.7
Ketoprofen	0.0	1.4	94.3	0.0	0.0	99.5
4-Propylbenzoic acid	0.0	44.4	54.2	0.0	0.0	99.6
Amitriptyline	0.0	98.4	0.0	0.0	94.4	2.9
Secobarbital	0.0	98.2	0.0	0.0	96.6	2.0

R.S.D. % (n=3) lower than 9%, except for the recovery values that are lower than 10%.

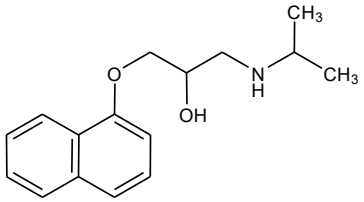
Figure captions

Figure 1. Structures and pK_a values of the selected analytes evaluated under: (a) reversed-phase conditions and (b) anion-exchange conditions.

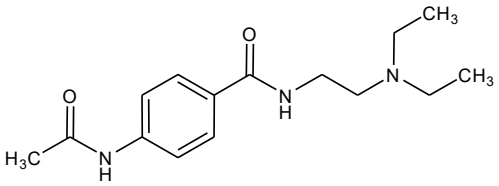
Figure 2. Structures of the sorbents tested: NVIm-DVB, Oasis HLB, Oasis WAX and Oasis MAX.



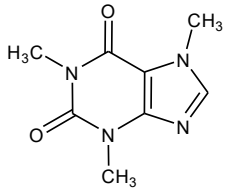
Amitriptyline, pK_a 9.4



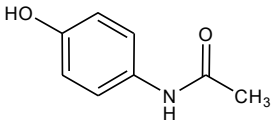
Propranolol, pK_a 9.5



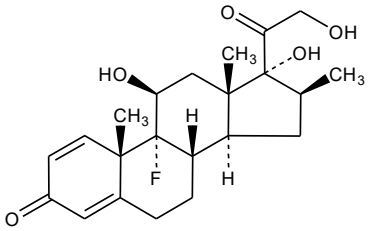
N-acetyl procainamide, pK_a 9.2



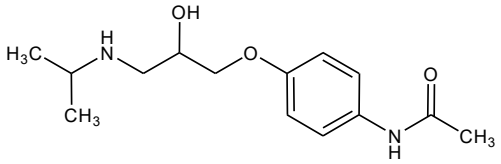
Caffeine, pK_a >14, 0.6



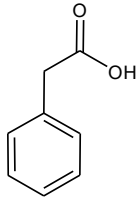
Acetaminophen, pK_a 9.7



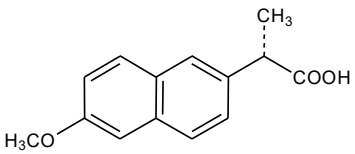
Betamethasone, pK_a >> 14



Practolol, pK_a 9.5

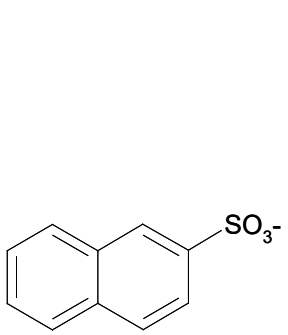


Phenylacetic acid, pK_a 4.3

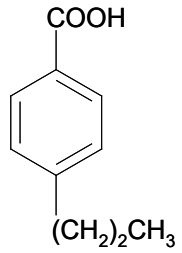


Naproxen, pK_a 4.2

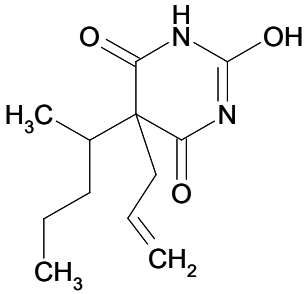
Figure 1 (a)



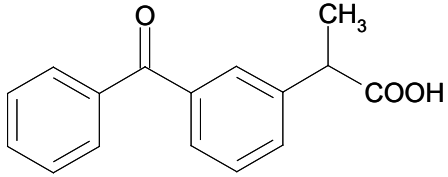
2-Naphthalenesulfonic acid, pK_a << 1



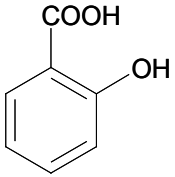
4-Propylbenzoic acid, pK_a 4.4



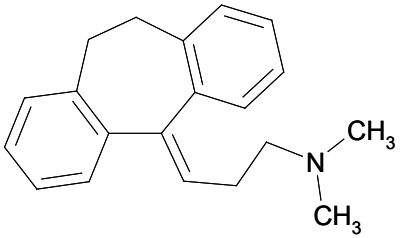
Secobarbital, pK_a 7.9



Ketoprofen, pK_a 4.8

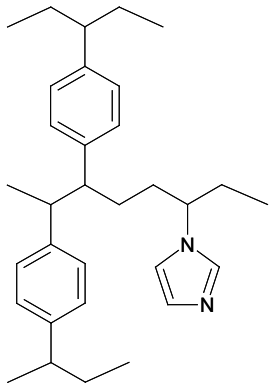


Salicylic acid, pK_a 3.0, 13.4



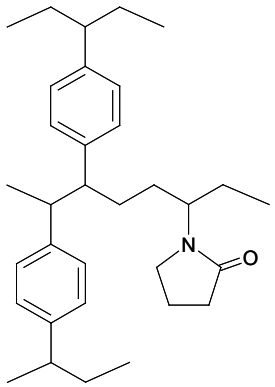
Amitriptyline, pK_a 9.4

Figure 1 (b)

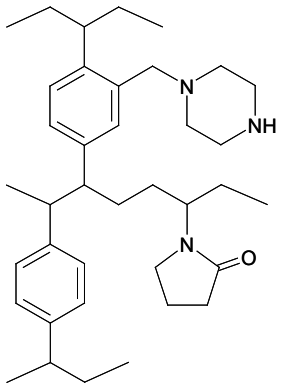


NVIm-DVB

pK_a ~7

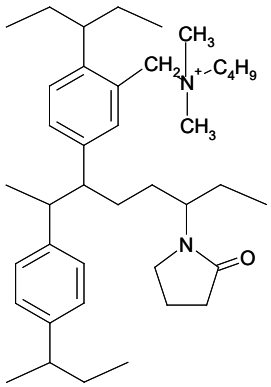


Oasis® HLB



Oasis® WAX

pK_a ~6



Oasis® MAX

pK_a ~18

Figure 2