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Application of mixed-mode, solid-phase extraction in environmental and clinical chemistry

Combining hydrogen-bonding, cation-exchange and Van der Waals interactions

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ABSTRACT

Silica- and styrene–divinylbenzene-based mixed-mode resins that contain C_8, C_{18} and sulphonated cation-exchange groups were compared for their efficiency in isolation of neutral triazine compounds from water and of the basic drug, benzoylecgonine, from urine. The triazine compounds were isolated by a combination of Van der Waals and hydrogen-bonding interactions, and benzoylecgonine was isolated by Van de Waals interactions and cation exchange. All analytes were eluted with a polar organic solvent containing 2% ammonium hydroxide. Larger recoveries (95%) were achieved on copolymerized mixed-mode resins where C_{18} and sulfonic acid are in closer proximity than on "blended" mixed-mode resins (60–70% recovery).

INTRODUCTION

Solid-phase extraction (SPE) is a sample-preparation tool used for the isolation, concentration, and purification of analytes from complex matrices such as serum, urine, saliva, tissue, food, and contaminated water. Reversed-phase (C_2, C_4, C_8 and C_{18}), cation- and anion-exchange, and various polar phases (silica, alumina, and cyano phases) are used in SPE. The technique of SPE has wide-range application in drug-screening laboratories, environmental-monitoring programs, and the food and cosmetic industry. An expansive literature on the subject of SPE has developed during the last 10 years [1,2].

Recently, various phases of SPE have been blended or copolymerized in order to use multiple interactions for isolation and purification of analytes, called mixed-mode resins [3–6]. These resins have the potential to recover analytes covering a wider-range of polarity by utilizing specific, simultaneous interactions. Furthermore, elution steps can be selective by sequentially cancelling specific mechanisms of interaction in operation on one resin. Prior to the advent of mixed-mode resins, there had been many reports on mixed-mode interactions in high-performance liquid chromatography (HPLC) [7–10] and in SPE resins [11–14]. For example, silica-based HPLC columns and SPE resins show mixed-mode interactions between active silanol sites and

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polar functional groups of the organic analyte, especially for basic analytes containing nitrogen. However, the utilization of such interactions for enhanced isolation of more polar compounds has been realized only recently [13,14].

To capitalize on the duality of isolation mechanisms, either multiple functionalities are bonded onto a common frame (copolymerized), or different resins are blended into one cartridge. Although some papers have dealt with the tandem use of resins [15,16], few papers have dealt with the merits and drawbacks of mixed-mode resins [3,4] or have discussed the diversity of mixed-mode resins presently available. Therefore, the objectives of this paper are to: (1) present current research on the mechanisms of mixed-mode interactions for two suites of analytes, triazine herbicides and drugs of abuse, and (2) compare and contrast the efficiency of isolation of "bonded" and "blended" mixed-mode resins using both environmental and clinical compounds as examples.

EXPERIMENTAL

Reagents

Methanol (Burdick & Jackson, Muskegon, MI, USA), ethyl acetate, and isooctane (Fisher Scientific, Springfield, NJ, USA) were the pesticide-grade solvents used. Deionized water was charcoal filtered and glass distilled prior to use. The triazine herbicides ametryn, atrazine, prometon, prometryn, propazine, simazine and terbutryn were obtained from Supelco (Bellefonte, PA, USA), and the triazine metabolites, 2-amino-4-chloro-6-ethylamino-*s*-triazine and 2-amino-4-chloro-6-isopropylamino-*s*-triazine, were obtained from Ciba Geigy (Greensboro, NC, USA). The benzoylecgonine and [$^2H_3$]benzoylecgonine were obtained from Sigma (St. Louis, MO, USA) and pentafluoropropanol and pentafluoropropionic anhydride were obtained from Alltech (Deerfield, IL, USA). The MP-3 mixed-mode resin (Interaction Chromatography, Mountain View, CA, USA), which included C_{18} chains and sulfonic acid groups (0.8 mequiv./g), contained 100 mg of 45-μm particles of styrene–divinylbenzene. The Bond-Elut Certify[a] mixed-mode resin (Varian Sample Preparation, Harbor City, CA, USA) contained 300 mg of 40-μm particles of silica which included either C_{18} chains or sulfonic acid groups (1 mequiv./g). All sulfonic acid groups were in hydrogen-saturated form. Standard solutions were prepared in methanol. [$^2H_{10}$]Phenanthrene (US Environmental Protection Agency, Cincinnati, OH, USA) was used as an internal standard for gas chromatography–mass spectrometry (GC–MS) quantification of the triazine herbicides.

Solid-phase extraction

SPE cartridges for the triazine herbicides were preconditioned sequentially with 2 ml methanol and 2 ml distilled water on a Millilab workstation by the robotic probe (Waters, Milford, MA, USA). Samples were passed by positive pressure through the cartridge at a flow-rate of less than 2.0 ml/min as greater flow-rates were not physically possible due to back pressure. The herbicides were eluted by the workstation with 4 ml ethyl acetate, which contained 2% ammonium hydroxide, and the eluate was robotically spiked with 500 μl of internal standard [$^2H_{10}$]phenanthrene (0.2 ng/μl). Approximately 100 μl of water trapped in the cartridge were co-eluted with the ethyl acetate. The ethyl acetate and water layers were mixed after addition of the internal standard, and after settling, 3.5 ml of the homogenized ethyl acetate layer were drawn off the denser water layer by the robotic probe into a new centrifuge tube. Eluates were evaporated down to 100 μl by a Turbovap (Zymark, Hopkinton, MA, USA) at 45°C under a nitrogen stream and transferred to a glass-lined vial for GC–MS analysis.

SPE cartridges for benzoylecgonine were preconditioned with 2 ml of methanol followed by 2 ml of 0.1 *M* phosphate buffer at pH 6.0 on a vacuum manifold (Supelco, Bellefonte, PA, USA). A 1-ml volume of sample was spiked with 1000 ng of [$^2H_3$]benzoylecgonine in 100 μl of deionized water. Then, 2.0 ml of 0.1 *M* phosphate buffer at pH 6.0 were added, and the sample was passed through the cartridge under medium vacuum. The sample was washed with 3.0 ml of deionized water, 3.0 ml of 0.1 *M* HCl, and 9.0 ml of methanol. The cartridge was eluted with 2.0 ml of methylene chloride–isopropanol (80:20, v/v) with 2% ammonium hydroxide. The sample was then evaporated to dryness and derivatized by adding 50 μl of pentafluoropropionic

[a] The use of trade names in this paper is for identification purposes only and does not constitute endorsement by the US Geological Survey.

anhydride and 25 μl of pentafluoropropanol to a screw-cap test tube and heating at 75°C for 15 min. The sample was evaporated to dryness at 50°C under nitrogen and taken up in 100 μl of ethyl acetate for GC–MS analysis.

GC–MS analysis

Automated GC–MS analysis of the eluates were performed on a Hewlett-Packard Model 5890 GC (Palo Alto, CA, USA) and a 5970A mass selective detector. Operating conditions for the triazine herbicides and benzoylecgonine were as follows: ionization voltage, 70 eV; an ion source temperature of 250°C, electron multiplier, 2200 V; direct capillary interface at 280°C, tuned daily with perfluorotributylamine; and a 50-ms dwell period. Separation of the herbicides and benzoylecgonine was accomplished with a fused-silica capillary column of methyl silicone (HP-1) with a film thickness of 0.33 μm, 12 m × 0.2 mm I.D. (Hewlett-Packard). Helium was used as the carrier gas at a flow-rate of 1 ml/min and a head pressure of 35 kPa. The column temperature was held at 50°C for 1 min, then ramped at 6°C/min to 250°C where it was held for 10 min. Injector temperature was 280°C.

The filament and multiplier were not turned on until 5 min into the analysis. Quantification of the base peak of each triazine was based on the response of the 188 (amu) ion of the internal standard, [$^2H_{10}$]phenanthrene. Confirmation of the compound was based on the presence of the molecular ion and two confirming ions with a retention-time match of ± 0.2% relative to [$^2H_{10}$]phenanthrene and correct area ratios for confirming ions. All triazine herbicides were selectively monitored. Benzoylecgonine was monitored with selected ions of 300, 316, and 421 u; and the [$^2H_3$]benzoylecgonine was monitored with 303, 319, and 424 u ions. Quantification of benzoylecgonine was by internal standard using the [$^2H_3$]benzoylecgonine.

Adsorption isotherm experiments

Adsorption isotherms for the triazine herbicides and metabolites were performed in triplicate on MP-3 and MP-1 resins from distilled water. SPE cartridges (containing 100 mg of packing) were preconditioned sequentially with 3 ml methanol and 3 ml distilled water. The cartridge was cut open, and the resin emptied into a PTFE-lined glass vial containing 40 ml distilled water. Analytes were spiked into the water from individual 1-mg/ml stock solutions in methanol. The samples were allowed to equilibrate for 24 h on a mechanical shaker, and the resin was allowed to settle. Approximately 35 ml of clear water sample were pipetted off into a clean, weighed test tube to determine the exact volume of water present. The sample then was passed through a disposable 0.45-μm filter (Millipore Filtrex) and preconditioned C_{18} cartridge (Waters Millipore) (conditioned sequentially with 3 ml methanol, 3 ml ethyl acetate, 3 ml methanol and 3 ml distilled water) at 20 ml/min [17]. The cartridge was eluted with 3 ml ethyl acetate, and the eluate was spiked with 500 μl of internal standard, [$^2H_{10}$]phenanthrene (0.2 ng/μl). The ethyl acetate layer was drawn off the co-eluted, denser water layer and evaporated to 100 μl for transfer to a glass-lined vial for analysis by GC–MS with selective ion monitoring.

RESULTS AND DISCUSSION

Mixed-mode isolation of s-triazine compounds

To understand the mechanism of isolation of triazine herbicides on a mixed-mode resin, it is first necessary to discuss the structure of the suite of analytes. Fig. 1 shows the structure of the two dealkylated metabolites and the parent *s*-triazine suite. Common to each member of the suite is a triazine ring, with variations in the length of the alkyl side chains at the 4′ and 6′ positions and the moiety at the 2′ position. The 2′ position is occupied either by a chlorine atom (deethylatrazine, deisopropylatrazine, atrazine, simazine and propazine), a methylthio group (ametryn, prometryn and terbutryn), or a methoxy linkage (prometon). Primary amine moieties are present on the dealkylated metabolites, and secondary amines are present on all parent triazine herbicides. Retention of chlorine-containing triazine herbicides is reported to follow structural changes on the reversed-phase C_{18} resins, with capacity of the herbicides increasing in a linear logarithmic fashion with the addition of methylene groups to the alkyl side chain [18], which indicates Van der Waals interactions. Deisopropylatrazine, which contains only two carbon atoms in alkyl side chains, is retained the least on C_{18}, and propazine, which contains six carbon atoms, is retained the most.

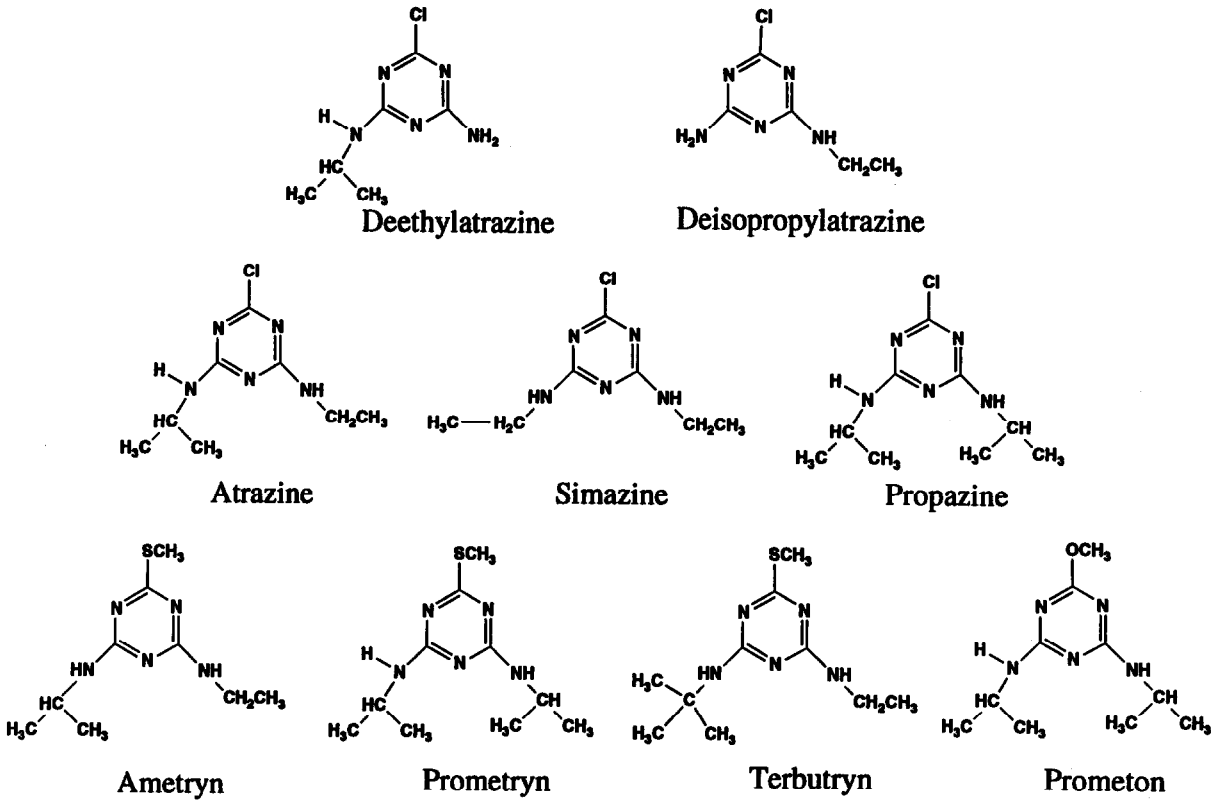


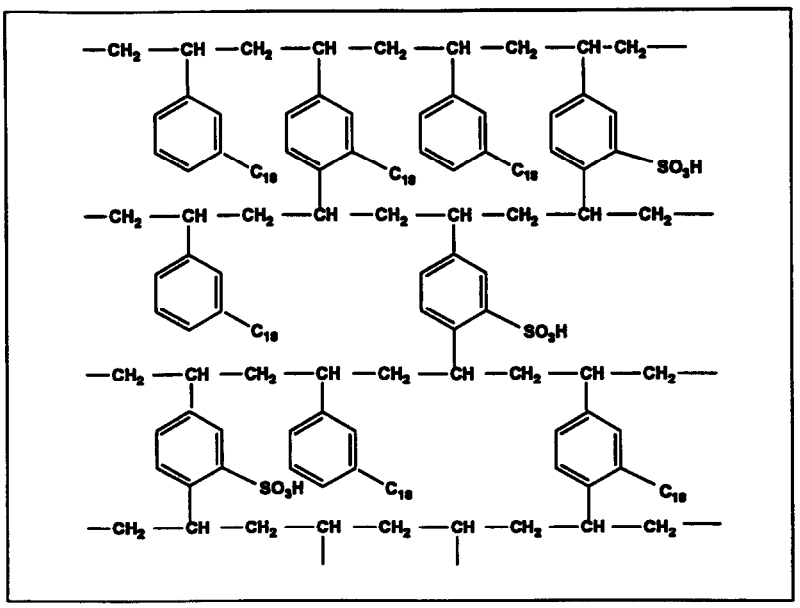
Fig. 1. Structure of the *s*-triazine suite of herbicides.

The capacity of silica-based C_{18} resin for isolation of deisopropylatrazine from pure water was compared to the capacity of two other resins —a mixed-mode resin (MP-3) and a polymeric reversed-phase resin (MP-1). Fig. 2 shows the structure of the two polymeric resins. MP-3 comprises a styrene–divinylbenzene framework onto which both C_{18} and sulfonic acid groups are bonded. MP-1 comprises a styrene–divinylbenzene framework with bonded C_{18} groups only. The C_{18} reversed-phase covering is comparable for both MP-1 and MP-3.

Fig. 3A shows the breakthrough curves for deisopropylatrazine in pure water on the three resins. Deisopropylatrazine was retained the least on MP-1, with over 10% breakthrough occurring after 10 ml of water had passed through the resin, and 100% breakthrough between 30 and 40 ml. On the silica-based C_{18} resin, 10% breakthrough of deisopropylatrazine occurred after 30 ml, and 100% breakthrough was reached after between 60 and 70 ml of water had been passed through the resin. The MP-3 resin had the largest retention for deisopropylatrazine, with 10% breakthrough still not reached after 1.8 l of water had passed through the resin. The large retention on the MP-3 resin suggests that a polar or hydrogen-bonding interaction is occurring between analyte and sulfonic acid groups, as well as Van der Waals interactions. The pK_b of the triazine compounds is small (< 2.0), [19] and at pH 7.0, the triazine compounds remain neutral analytes, which rules out the possibility of any cation-exchange interactions. It is hypothesized, therefore, that polar interactions in the form of hydrogen bonding occur between the primary amine moiety of the metabolite and the hydrogen of the sulfonic acid moiety.

The breakthrough of deisopropylatrazine on C_{18} and MP-3 was repeated out of tap water, which had

MP-3



MP-1

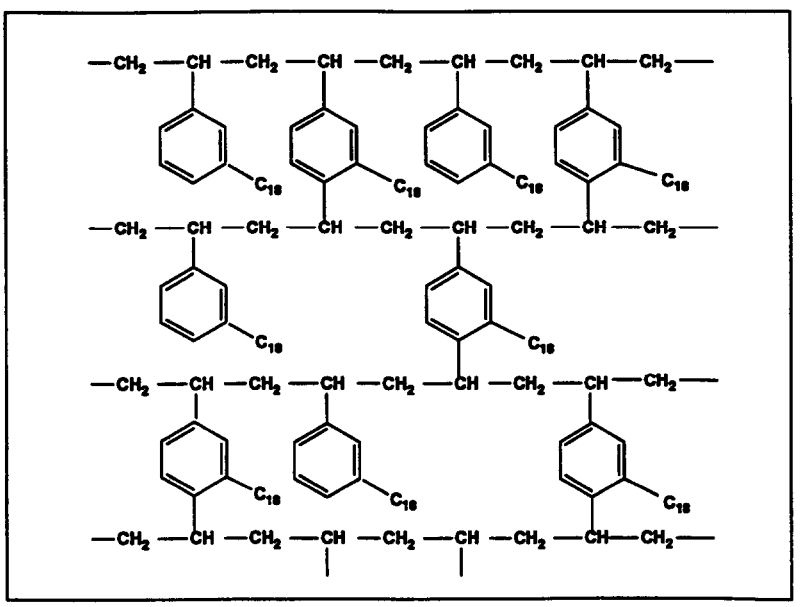


Fig. 2. Structure of copolymerized resin, MP-3, which comprises a styrene–divinylbenzene framework onto which both C_{18} and sulfonic acid groups (0.8 mequiv./g) are bonded, and MP-1, which comprises a styrene–divinylbenzene framework onto which only C_{18} groups are bonded.

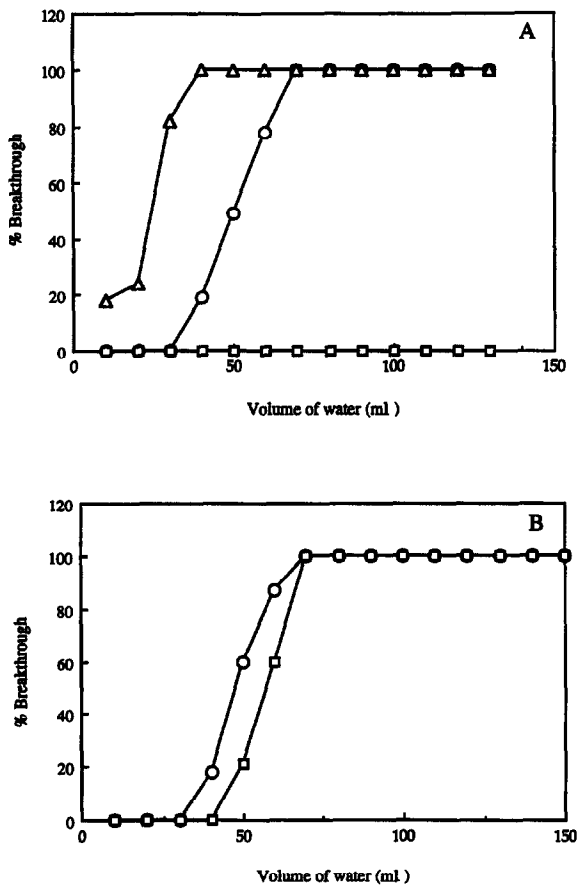


Fig. 3. (A) Breakthrough curves for deisopropylatrazine in pure water on (○) silica-based C_{18} resin, (△) polymeric reversed-phase resin (MP-1) and (□) copolymerized mixed-mode resin (MP-3). (B) Breakthrough curves for deisopropylatrazine in tap water (conductivity 200 μs) on (○) silica-based C_{18} resin and (□) copolymerized mixed-mode resin (MP-3).

a conductivity of 200 μs (Fig. 3B). The breakthrough of deisopropylatrazine on the C_{18} resin was comparable to the breakthrough out of pure water and was unaffected by the inorganic ions in solution. On the MP-3 resin, however, over 10% breakthrough occurred after only 50 ml of tap water had passed through the resin, and 100% breakthrough occurred after 70 ml of water. This indicates that hydrogen-bonding interactions were cancelled by displacement of the sulfonic acid hydrogen by the positive cations in solution. This is strong evidence that hydrogen bonding is the mechanism enhancing retention of the triazines on MP-3, because as soon as the resin is not in hydrogen-saturated form, breakthrough of deisopropylatrazine occurs rapidly.

Further evidence for a hydrogen-bonding mechanism of retention for the triazine suite was obtained from the isolation characteristics of the herbicides and metabolites on the MP-1 and MP-3 resins from organic solvents of different polarities. The retention of all compounds from methanol, ethyl acetate, acetonitrile and hexane onto the MP-1 resin was zero (all analytes remained in the organic solvent), which indicates weak Van der Waals interaction of the analytes with the non-polar styrene–divinylbenzene matrix and the C_{18} chains in comparison with that between analytes and the organic solvent. Fig. 4 shows recoveries of the triazine suite isolated from the same solvents on the MP-3 resin. From the polar solvents methanol, ethyl acetate and acetonitrile, there was smaller retention of polar triazine metabolites deisopropylatrazine and deethylatrazine, and larger retention of the remaining parent herbicides (Fig. 4). From the non-polar solvent hexane retention of the polar metabolites was 65–75%, and retention of parent triazine was 100%. Due to the presumed greater solubility of triazines in more polar solvents, such as methanol, ethanol, acetonitrile, and ethyl acetate, the retention of analytes by MP-3 resin through hydrogen-bonding interactions is decreased. As the polarity of the solvent decreases (hexane), triazine interactions with the MP-3 resin are enhanced. It appears that the solubilities of all triazines become more comparable in the solvent as the solvent polarity decreases, and the difference in retention of members of the triazine suite diminishes.

Sorption isotherms were conducted for four members of the triazine suite, deisopropylatrazine, deethylatrazine, atrazine and ametryn, from distilled water on MP-3 and MP-1 resins, at small concentrations relative to the solubility of the analytes. Linear sorption isotherms for all analytes on the MP-3 and MP-1 resins were obtained. Chiou [20] has emphasized that when the concentration of the solute is small relative to the solutes solubility, a linear sorption isotherm will result, as stoichiometric interactions are incomplete. At larger solute concentrations, such interactions are complete, and the isotherm will become non-linear. The isotherm for

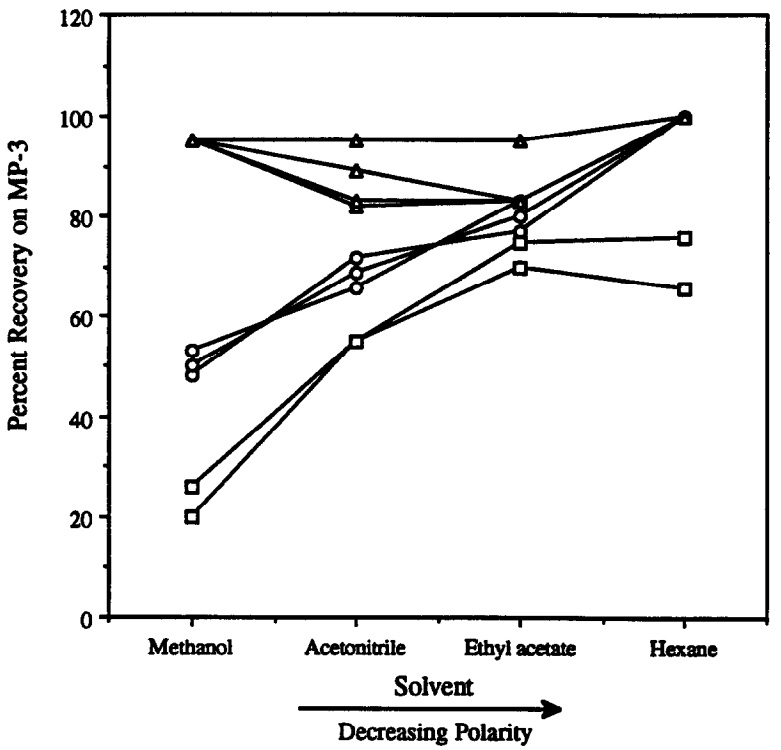


Fig. 4. Recovery of triazine suite on MP-3 mixed-mode resin isolated from polar and non-polar organic solvents. △ = Prometon, ametryn, prometrym terbutryn; ○ = simazine, atrazine, propazine; □ = deisopropylatrazine, deethylatrazine.

the MP-3 resin may become non-linear at larger solute concentrations, and this would support a secondary interaction taking place to explain the enhanced isolation of the triazine compounds.

The slope of the plot of analyte sorbed onto the solid phase (ng/g) *versus* the concentration of analyte in the water phase (ng/ml) is defined as the partition coefficient (K_d). Table I lists the K_d values for each analyte on MP-1 and MP-3 resins. Because the MP-1 and MP-3 resins differ only in structure by the presence of sulfonic acid groups, the difference in the log K_d for MP-1 and MP-3 resins ($\Delta\log K_d$) for the same analyte can be attributed to the enhanced interactions (hydrogen bonding) with the sulfonic acid groups. The $\Delta\log K_d$ value was approximately 1.9 for both triazine metabolites, each of which contain a primary amine moiety. From the expression [$\Delta G = 2.303RT(\Delta\log K_d)$], the free energy of adsorption (ΔG) associated with the enhanced interactions is 2.59 kcal/mol. This is in the energy range for hydrogen bonding. The $\Delta\log K_d$ value for the parent herbicides, atrazine and ametryn, which both contain secondary amine moieties, was approximately 1.3, which equates to an energy of adsorption of 1.77 kcal/mol. These $\Delta\log K_d$ values and small energies of adsorption indicate that the enhanced interactions could be due to hydrogen-bonding interactions on the MP-3 resin, and that

TABLE I

PARTITION COEFFICIENTS (K_d VALUES) FOR DEISOPROPYLATRAZINE, DEETHYLATRAZINE, ATRAZINE AND AMETRYN ON MP-1 AND MP-3 POLYMERIC RESINS

Compound	K_d (ml/g)	
	MP-1	MP-3
Deisopropylatrazine	400	58 600
Deethylatrazine	1600	112 500
Atrazine	40 000	625 000
Ametryn	184 600	4 000 000

TABLE II

PERCENT RECOVERY OF THE TRIAZINE SUITE (± 5%) FROM PURE WATER FOLLOWED BY A METHANOL WASH ON COPOLYMERIZED AND BLENDED MIXED-MODE RESINS

Compound	Recovery (%)	
	Co-polymerized mixed-mode	Blended mixed-mode
Deisopropylatrazine	95	61
Deethylatrazine	95	65
Ametryn	95	95
Atrazine	95	65
Prometon	95	95
Prometryn	95	95
Propazine	95	65
Simazine	95	65
Terbutryn	95	95

these interactions are stronger between primary amine groups and the sulfonic acid groups than between the secondary amines.

A comparison was made of the recovery of the triazine compounds on two types of commercially available mixed-mode resins —the co-polymerized mixed-mode resin (MP-3) and a blended mixed-mode resin, where a mixture of reversed-phase resin and cation-exchange resin are combined in one SPE cartridge. Table II shows recovery of the triazine suite from pure water followed by a 6-ml methanol-wash step prior to elution with ethyl acetate containing 2% ammonium hydroxide. Recovery of the triazine compounds from pure water was comparable for the two types of resin, with recoveries of 95% for all triazine herbicides and metabolites. When a methanol-wash step was added following isolation, recoveries of polar metabolites and the parent herbicides, atrazine, propazine, and simazine decreased to 65% on the blended mixed-mode resin but remained unchanged on the copolymerized mixed-mode resin. The recovery of the more nonpolar parent triazine herbicides, ametryn, prometon, prometryn and terbutryn remained at 95%. This result indicates that the proximity of the cation-exchange moiety to the C_{18} phase is important in ensuring full recovery of more polar analytes when using organic solvents as part of the procedure. Because cation-exchange and C_{18} reversed-phase moieties are separated by the particle size of the silica in blended mixed-mode resins, the analyte is retained by either reversed-phase or secondary interactions, and is not dually held by both mechanism. During a methanol-wash step, a polar triazine retained by Van der Waals interactions only is easily eluted. Only the analyte retained by hydrogen-bonding interactions will remain. The less-polar triazine compounds also will be eluted with methanol from a relatively non-polar organic phase, as evidenced by zero retention of any triazine herbicides or metabolites out of organic solvent on the MP-1 resin. However, the more non-polar compounds can partition more readily out of the polar solvent and back onto a hydrogen-bonding site than polar compounds, as seen by their consistently large recoveries out of organic solvents onto MP-3. The close proximity of the two moieties on the copolymerized mixed-mode resin is important, therefore, to hold the more polar compounds by a dual mechanism at all times. Van der Waals interactions can then be disrupted with a methanol wash, but the triazine is retained by the secondary hydrogen-bonding interactions with the sulfonic acid group.

Mixed-mode isolation of benzoylecgonine

To design an efficient method for solid-phase extraction of benzoylecgonine, first it is necessary to examine the structure of the drug and its basicity. Fig. 5 shows the structure of benzoylecgonine. It is an amphoteric compound with both a carboxyl group and a tertiary amine. Thus, in a SPE procedure, the drug may be sorbed most efficiently at a neutral to slightly acidic pH by using a non-specific partition interaction, such as with C_8 resin. At an acid pH, benzoylecgonine will be a cation with protonation of the tertiary amine. This side of the drug's structure can be exploited by cation exchange.

First, the SPE procedure isolates the benzoylecgonine with passage of the urine sample through the

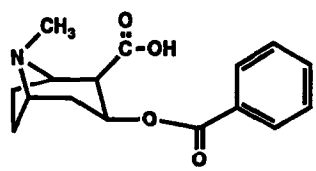


Fig. 5. Structure of benzoylecgonine.

TABLE III

RECOVERY OF BENZOYLECGONINE FROM VARIOUS MIXED-MODE RESINS

Resin	Recovery of benzoylecgonine (%)
Blended SPE 1 resin	60
Blended SPE 2 resin	70
Mixed-mode MP-3 resin (copolymerized)	95
C_{18} resin followed by cation-exchange resin	90

cartridge at pH 6.0. At this pH, the drug is ionized at the carboxyl group but should adsorb by Van der Waals interactions to the C_8 resin. Many urine interferants also will sorb at this pH. To wash the resin of these interferants, first water is used to remove excess salts and extremely soluble organic compounds. Next, acid is used to regenerate the cation-exchange resin, which has lost some of its hydrogen saturation by introduction of the urine sample. Next, an organic solvent, such as methanol, is required. However, if methanol is used, it will elute the benzoylecgonine at the same time. To remove the interferants without loss of the drug, the basic side of the molecule can be exploited by use of acid. By passing acid through the column, not only will the cation-exchange capacity be regenerated but also the carboxyl group will be protonated, and the basic amine will become cationic. Thus, a SPE cartridge that contains both a hydrophobic group and a strong cation-exchange group should work well on this compound and indeed on any basic drug.

This procedure was tested on four different mixed-mode resins (Table III). Two blended mixed-mode resins from different manufacturers, consisting of C_8 and strong cation-exchange resins in their hydrogen-saturated form, were compared with a copolymerized mixed-mode resin (MP-3) and a combination of C_{18} resin followed by a strong cation-exchange resin. The blended resins were commerically available. The recovery of benzoylecgonine varied from 60 to 70% of the applied amount for the blended resins. This decreased recovery probably is caused by the fact that the benzoylecgonine can desorb from the C_8 resin and re-adsorb by cation exchange on an adjacent resin bead.

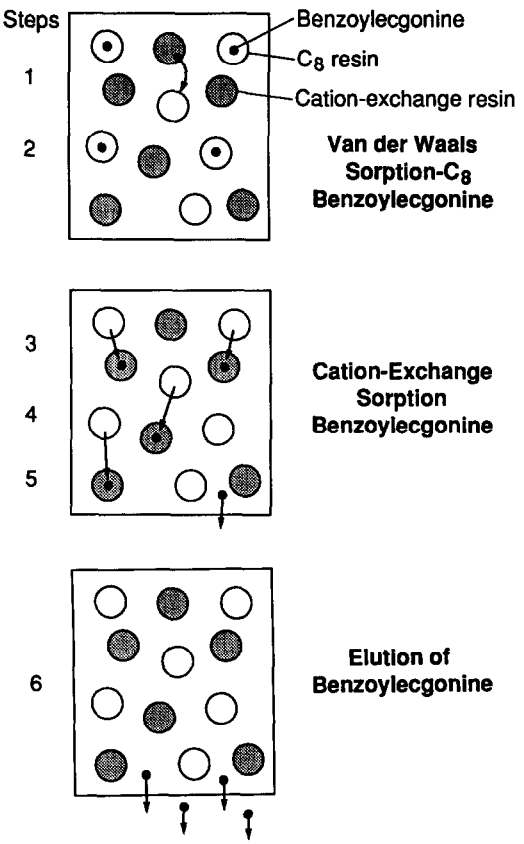


Fig. 6. Procedure and mechanism of isolation of benzoylecgonine on blended mixed-mode resin. White circles: C_8; grey circles: AR-SO_3H. Steps: 1 = condition column; 2 = apply sample with benzoylecgonine; 3 = wash with water; 4 = acid wash (proton lock); 5 = methanol wash; 6 = elute with methylene chloride–isopropanol (80:18) with 2% ammonium hydroxide.

For example, the method depicted in Fig. 6 shows that the blended resin contains both C_8 and cation-exchange beads. During the initial phase of sorption, the C_8 beads sorb the benzoylecgonine via Van der Waals interactions. During the water wash, the urine interferants are washed out, but the benzoylecgonine remains sorbed by the C_8 resin. The methanol-wash step, however, elutes the benzoylecgonine from the C_8 resin. The data in Table II indicate this result by the 90% recovery of benzoylecgonine when the methanol eluate of the C_{18} resin is passed through the cation-exchange resin. However, because an acid-wash step precedes the methanol rinse, the carboxyl group of the benzoylecgo-

nine is protonated, and the tertiary amine takes on a positive charge. This cation then is retained on the cation-exchange resin that is blended in the SPE cartridge during the methanol wash step. Thus, the molecules must desorb from the reversed-phase bead and move to a cation-exchange bead for retention. Consequently, in the blended resin some of the molecules are washed from the cartridge before the cation exchange occurs. Because cation exchange often is a slow process the flow-rate may easily affect recovery of the benzoylecgonine (Table III, recovery 60–70%).

When the resin contains both the reversed-phase and ion-exchange groups in close proximity, as on the co-polymerized MP-3, then the recovery is nearly total (95%). This may be explained easily by the desorption of the benzoylecgonine from the hydrophobic matrix and the immediate sorption by cation exchange without transport to the next bead. Thus, recovery is high in spite of the large wash volume of 9 ml of methanol.This mechanism of sorption, which is activated by an acid wash with subsequent sorption by cation exchange, is called a "proton-locking" step. This procedure is quite important for all of the classes of basic drugs that contain nitrogen that are easily protonated by acid.

The methanol-wash step on the mixed-mode resins was quite effective at removing nonvolatile, colored urinary metabolites. The eluates from C_8 resins were more colored than eluates from the mixed-mode resins because they were not washed with methanol. Injection of non-volatile urinary acids onto the gas chromatograph will shorten the life of the column due to activation, reduce sensitivity, and cause peak broadening. Furthermore, cleaner chromatograms were obtained with the mixed-mode eluates compared to the C_8 eluates. Thus, the application of mixed-mode resins to basic drugs in urine provides an efficient clean-up procedure.

CONCLUSIONS

The retention of both polar *s*-triazine metabolites and non-polar parent herbicides on copolymerized mixed-mode resin MP-3 is achieved by a combination of Van der Waals and hydrogen-bonding interactions. The dual-retention mechanism is demonstrated by retention of all triazine compounds from both polar and nonpolar organic solvents. Hydrogen-bonding interactions were completely disrupted only by adding 2% ammonium hydroxide to the eluting solvent; therefore, organic solvents can be used to selectively elute any interferents from the resin held by Van der Waals interactions only. Adsorption isotherms for the triazine compounds were linear on the MP-1 and MP-3 resins, with a difference in free energy of sorption between the two resins of 1.77 kcal/mol for parent triazine herbicides and 2.59 kcal/mol for the polar triazine metabolites. This is in the range of hydrogen-bonding interactions.

The basic drug, benzoylecgonine, was retained from urine on mixed-mode resin by a combination of Van der Waals and cation-exchange interactions. The basic drug was applied to the resin in neutral form and protonated "on resin" by an acid-wash step, which also re-protonated the cation-exchange groups. During a methanol-wash step, Van der Waals interactions were disrupted, but the drug was held by cation exchange while neutral interferences were washed off. The drug was eluted with organic solvent containing 2% ammonium hydroxide.

A comparison of retention efficiency of copolymerized and blended mixed-mode resins showed smaller recoveries for polar triazine compounds and benzoylecgonine on blended resin and larger recoveries on the copolymerized resin. This result indicates that the closeness of reversed-phase and sulfonic acid moieties is important. In blended resins, these groups are separated physically by the particle size of the silica. Mixed-mode resins are an innovative contribution to solid-phase sorbents for the enhanced isolation of polar analytes with non-polar analytes from complex matrices.

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