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Stir Bar Sorptive Extraction: Recovery of Organic Acids and Amines

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ABSTRACT

The determination of volatile and semivolatile compounds in aqueous solutions using Stir Bar Sorptive Extraction (SBSE) as the extraction step is gaining acceptance in a wide variety of application areas including water, beverages, consumer products and environmental. It has been shown to be simple, sensitive, quantitative, and can often eliminate cumbersome solvent extraction or other sample preparation steps.

Efficiency of partitioning into the polydimethylsiloxane (PDMS) phase on the stir bar can be predicted based on the known analyte partitioning between octanol and water as described by the octanol-water partition coefficient Kow. This prediction usually works well for most compounds, but can overestimate recovery of ionizable compounds like organic acids and amines since only the neutral form can be extracted into the bar.

SBSE of organic acids and amines is evaluated from simple aqueous solutions as well as at pH extremes. Extraction behavior as a function of pH is correlated to published compound dissociation constants. Strategies to enhance extraction efficiency of ionizable compounds are discussed.

INTRODUCTION

Since the commercial introduction of Stir Bar Sorptive Extraction (SBSE) three years ago [1] there have been a growing number of published studies describing the excellent precision and remarkably low detection limits possible with this technique. Sub-ppb or ppt detection limits of PAH's, pesticides, drugs, off-odors, flavors and fragrances in water, urine, fruits, vegetables and beverages attest to the robustness and versatility of SBSE when used to extract and quantify nonpolar target compounds.

Many samples can also contain ionizable compounds- long chain organic acids or amines- that may be important constituents. Examples include fatty acids in beverages (wines, aged distilled spirits, and juices), industrial process monitoring (surfactants, slipaids), and biological fluids. Lipophilic amines can be found as constituents or intermediates in insecticides, biocides, agricultural chemicals, corrosion inhibitors and dyes.

Acids and amines can exist in solution as either neutral or charged species depending on parameters such as solution pH and temperature. The neutral forms of lipophilic acids and amines can be extracted from aqueous solution using the PDMS phase on the Twister stir bar. Since the water solubility of the two forms can differ, the recovery of these type compounds can be influenced by extraction parameters that otherwise would not affect recovery of neutral species. In this study, we explored the effect of solution pH on recovery of acids and amines during SBSE using Gerstel Twister stir bars.

EXPERIMENTAL

Instrumentation. Analyses were performed on a GC (6890, Agilent Technologies) equipped with flame ionization (FID) or mass selective detection (MSD) (5973N, Agilent). Systems were equipped with a PTV inlet (CIS4, Gerstel), Thermal Desorption unit and autosampler (TDS2, TDSA Gerstel).

Analysis Conditions.

TDS 2	splitless
	20°C, 60°C/min, 250°C (5 min)
PTV	0.2 min solvent vent (50 mL/min);
	split ratio 20:1 or splitless
	-120°C, 12°C/s, 280°C (3 min)
Columns	acids (FID):
	30m DB-FFAP (J&W),
	d_i= 0.25mm, d_f= 0.25mm
	amines (MSD):
	30m HP-5 (Agilent),
	d_i= 0.25mm, d_f= 0.25mm
Pneumatics	He, constant flow = 1.2 mL/min
Oven	acids (FID):
	60°C, 15°C/min, 240°C (15 min)
	amines (MSD):
	40°C (2 min), 10°C/min,
	280°C (5 min)

Sample Preparation.

Liquid spikes. 100ug/ml standards of acids and amines were prepared in methanol. 1ul of standard was spiked directly into a thermal desorption tube and the sample was analyzed immediately.

Twister extractions. Solutions were prepared at concentrations of 100ug/l (amines) and 500ug/l (acids) in H2O, unless noted in the figures. When the samples were buffered, 25mM buffer solutions were prepared. Buffers used were as follows: pH 2 (potassium phosphate, monobasic); pH 3 (potassium phosphate, monobasic); pH 5 (sodium acetate); pH 7 (potassium phosphate, dibasic). The pH 10 solution was prepared by pH adjustment with 0.1N KOH.

The samples were extracted with a Twister stir bar at room temperature for 4 hours. Stir bars were removed, rinsed with DI water and placed in a conditioned thermal desorption tube for analysis.

RESULTS AND DISCUSSION

Fatty acid extraction. Initial studies extracting organic acids from water or 10% ethanol solutions using SBSE showed lower than expected recoveries and greater variability compared to similar studies performed with neutral compounds like methyl esters. The acids can exist in either neutral or ionized form in solution, and each form has significantly different water solubility as shown by the estimated [2] octanol:water partition coefficients for several acids in Table 1.

Table 1. Octanol:water partition coefficients for several acids.

Fatty Acid	Neutral acid form log(Kow)	Sodium form log(Kow)
Hexanoic	2.05	-1.76
Octanoic	3.03	-0.78
Decanoic	4.0	0.2
Tetradecanoic (Myristic)	5.98	2.17
Octadecanoic (Stearic)	7.94	4.13

The log(Kow) values in Table 1 suggest the extraction of acids using Twister can be dramatically affected if the solution pH is not controlled or the ionization state

of the acid is altered. We therefore set out to define the differences in behavior between three of these acids and neutral methyl esters in SBSE using thermal desorption equipment (Figure 1).
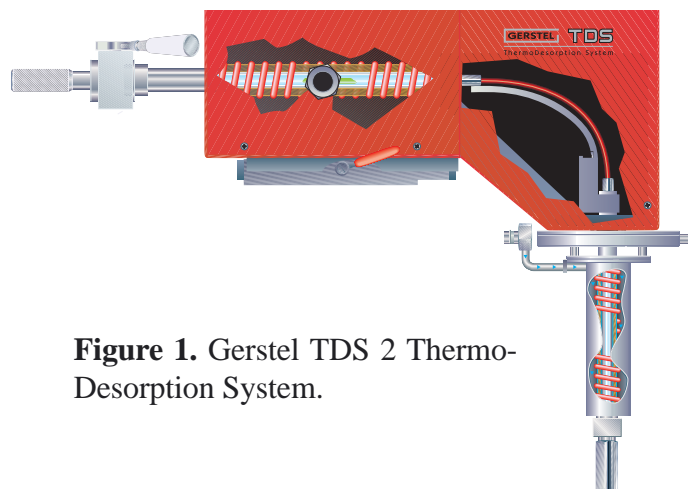

Figure 1. Gerstel TDS 2 Thermo-Desorption System.

Direct thermal desorption of acids and methyl esters. We first verified that acids and methyl esters of fatty acids could be effectively transferred to a GC column by direct thermal desorption (Figure 2). Although the methyl ester peaks are considerably sharper than the acids on the FFAP column, the similarity of peak areas indicate the acids and esters behave similarly in the thermal desorption system.
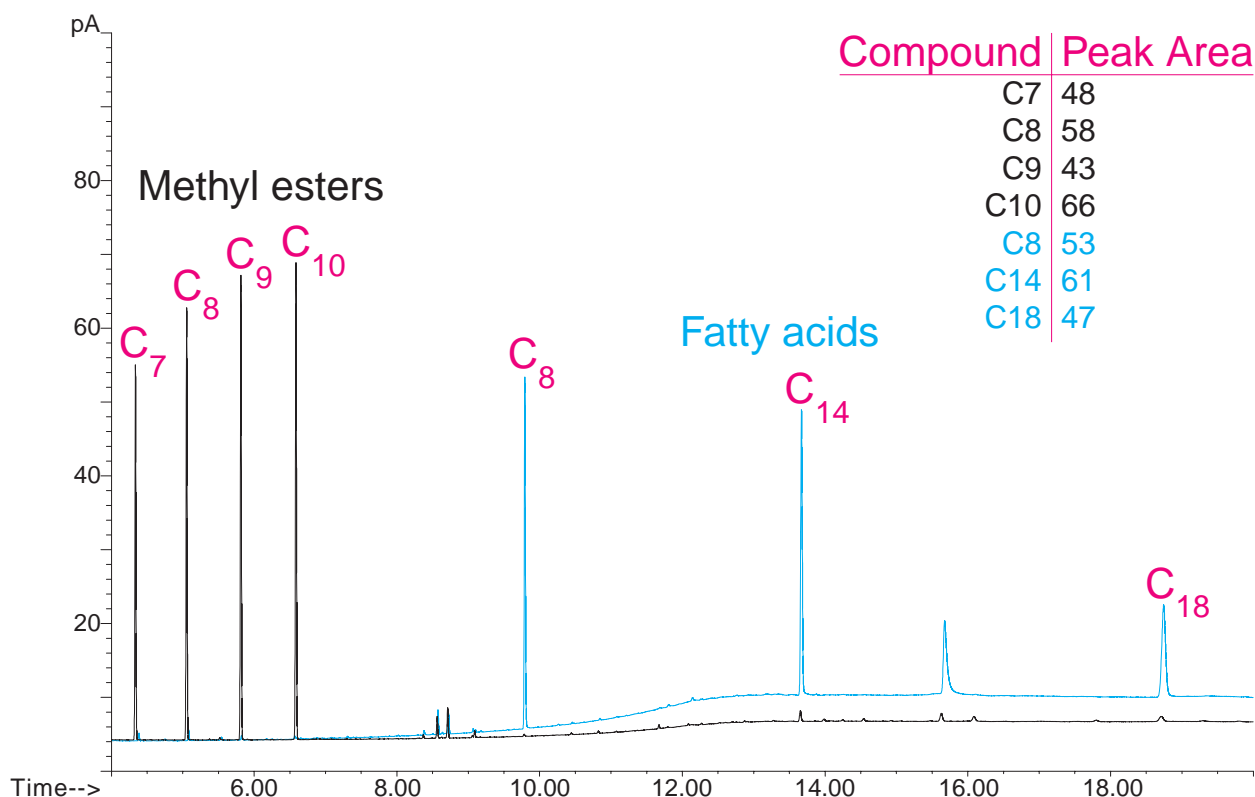

Compound	Peak Area
C7	48
C8	58
C9	43
C10	66
C8	53
C14	61
C18	47

Figure 2. Overlaid chromatograms of long chain methyl esters and acids spiked into empty glass thermal desorption tubes.

Twister extraction of acids and methyl esters. Normal, straight-chain fatty acids like those listed in Table 1 typically exhibit pKa values around 4.7-4.9 in aqueous solution. We therefore chose to extract the acids with Twisters using pH 5 acetate buffer, where a significant fraction of the acid was expected to be ionized. We first determined the extraction time necessary to reach equilibrium, where peak areas of the different acids could be compared. We found for the longer chain acids in particular, that long extraction times (4 hrs) were necessary to reach a steady state (Figure 3). This is in contrast with most results for lower molecular weight compounds suggesting 60-90 minutes is sufficient [3]. We therefore performed all subsequent extractions for 4 hours.

Figure 3. Twister Extraction Time Study for Octanoic, Myristic and Stearic Acids in 25mM Acetate Buffer (pH5).

A mix of acids and methyl esters were then extracted from a pH 5 acetate buffer solution. Ester extraction was found to be unaffected by the acetate buffer, whereas interesting differences in extraction efficiency were seen for the acids. Almost no recovery of the octanoic acid was seen. Myristic acid (C14) was nearly quantitatively recovered, and stearic acid (C18) gave a peak corresponding to significantly reduced recovery (Figure 4). These differences in behavior between different acids therefore warranted further investigation.
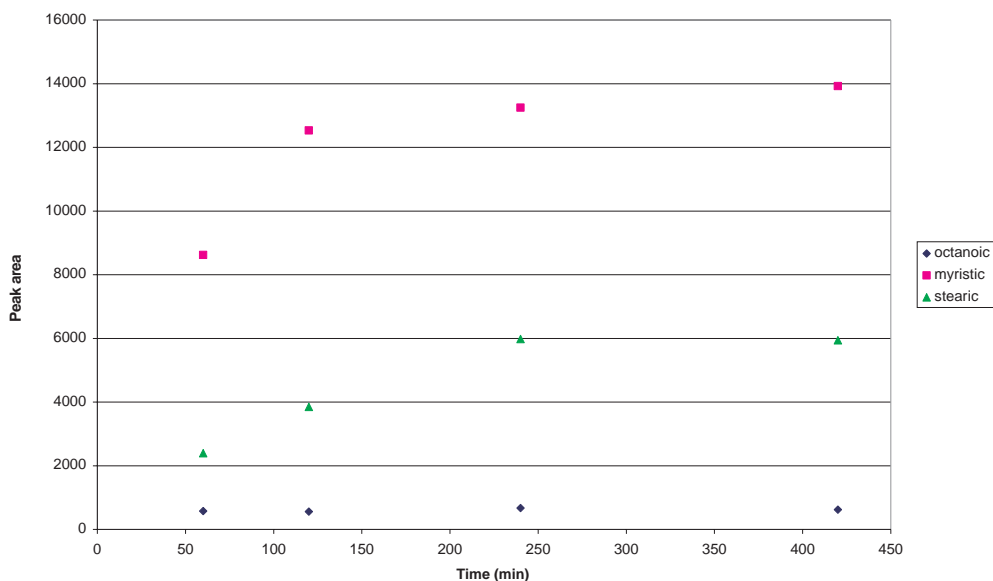

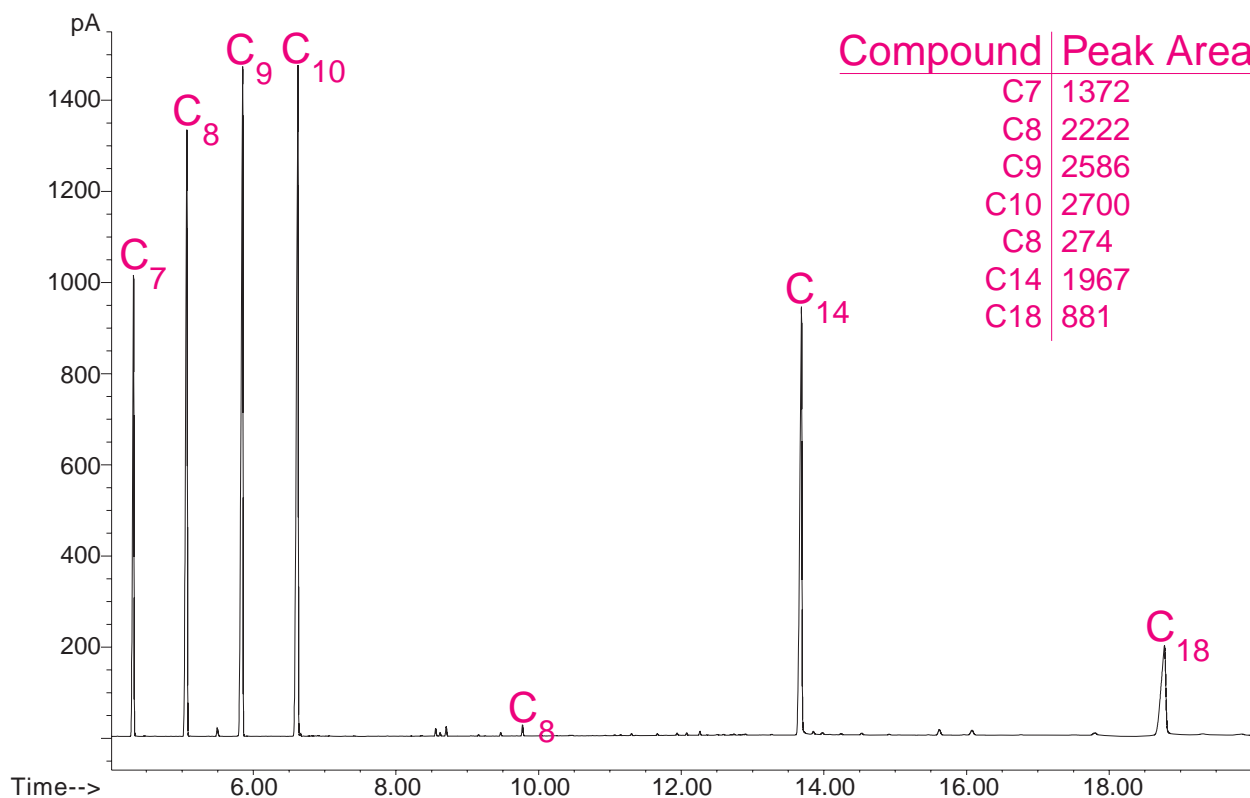| Compound	Peak Area
C7	1372
C8	2222
C9	2586
C10	2700
C8	274
C14	1967
C18	881

Figure 4. Chromatogram of long chain methyl esters and acids extracted from pH 5.0 acetate buffer using Twister.

Twister extraction of acids from different pH solutions. The three fatty acids were prepared in solutions ranging in pH from 2 to 10. Figure 5 shows a plot of the peak areas obtained after Twister extraction for 4 hours at these different pH's.

It is clear from this plot that the solution pH can dramatically affect recovery of the long chain acids. At low pH, carboxylic acids are expected to be fully protonated and exhibit lower water solubility making them most amenable to Twister extraction with the PDMS phase. Both octanoic and myristic acids behave as predicted with maximum recovery at low pH.

As the solution pH is increased toward the pKa (4.8), the equilibrium shifts toward the ionized form of

Figure 5. Twister extraction of fatty acids as a function of pH.

the acid, reducing recovery. Interestingly, the reduction in recovery of octanoic acid occurs at a lower pH than for myristic acid. At pH 10, well above the pKa for the acid the ionized form predominates and little or no recovery is seen.

The behavior of stearic acid is somewhat anomalous compared to the shorter-chain acids. Recovery goes through a maximum around pH 5 and falls off at both pH extremes. It is possible the low recovery at low pH is due to the extreme insolubility of the protonated stearic acid in water. This may promote aggregation of molecules or adsorption on vessel surfaces which effectively reduces the analyte concentration in solution leading to low recovery.
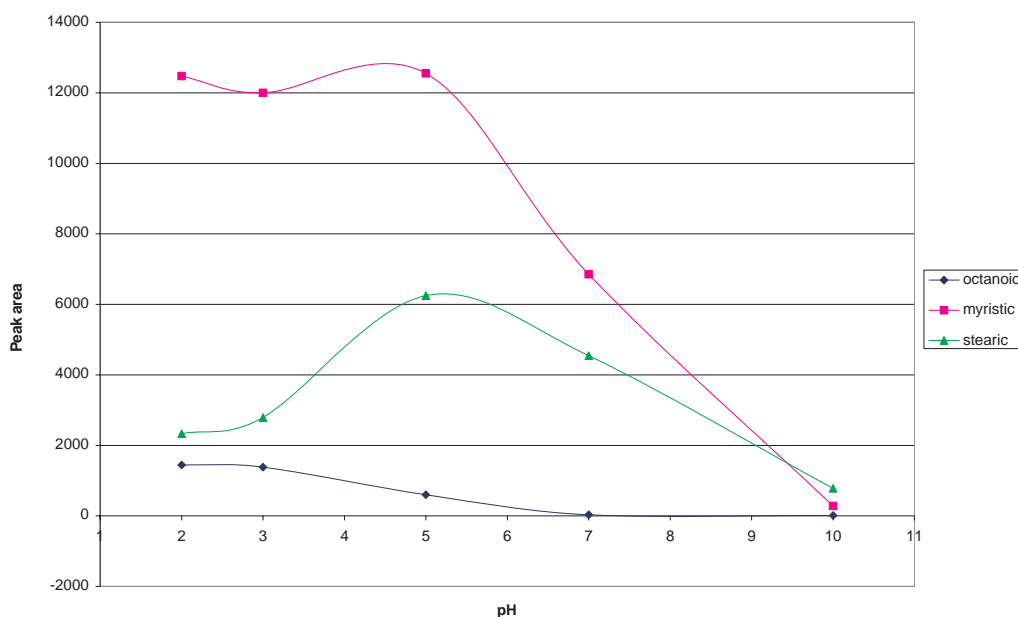
Long chain amine extraction. After investigating the extraction behavior of fatty acids on the Twister stir bar, it was reasonable to assume we would see similar pH effects for amine extractions. Table 2 shows the different water solubility (as indicated by the estimated octanol: water partition coefficients) for the neutral and ionized forms of the amines. We therefore repeated some of the extraction studies with a series of long-chain amines.

Direct thermal desorption of amines. We verified that the target amines could be effectively transferred to a GC column by direct thermal desorption (Figure 6). Trapping conditions and peak shapes (particularly for the primary amines) were not optimized on the nonpolar HP5-MS column, but the longer chain amines gave reasonable peak shapes for this evaluation.

Table 2. Octanol:water partition coefficients for several amines.

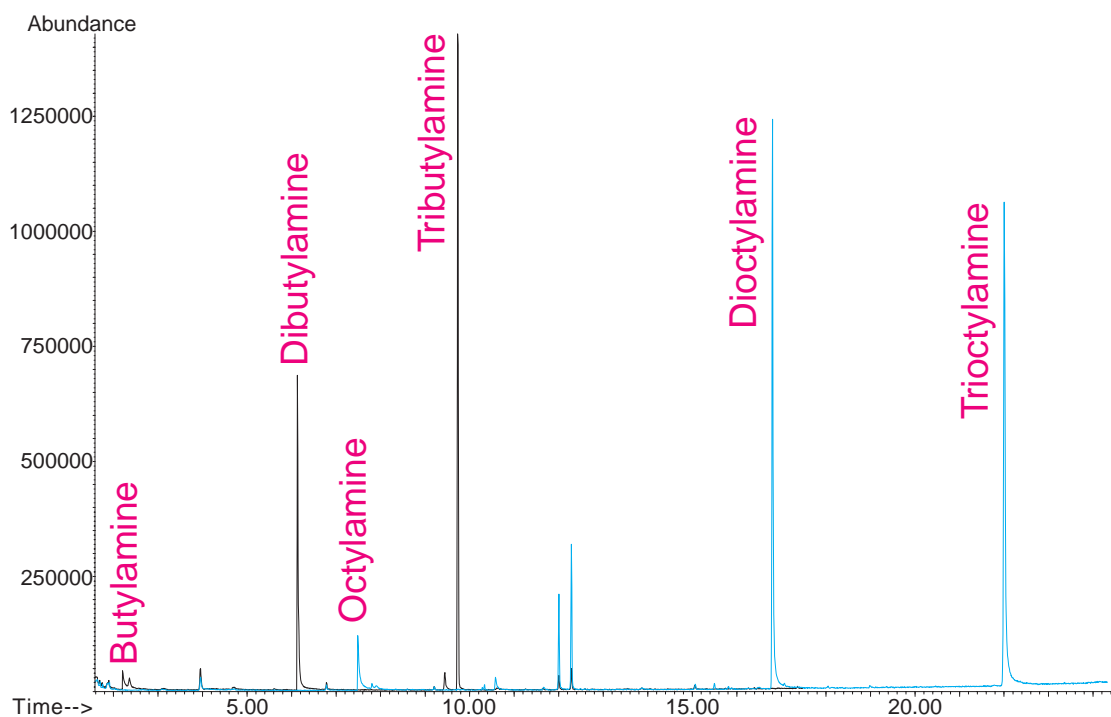| Amine	Neutral base form log(Kow)	Hydrochloride form log(Kow)
Butylamine	0.83	-2.35
Dibutylamine	2.77	-0.33
Tributylamine	4.46	1.69
Octylamine	2.8	-0.39
Dioctylamine	6.7	3.6
Trioctylamine	10.35	7.58

Figure 6. Overlaid chromatograms of butyl and octylamines spiked into empty glass thermal desorption tubes.

Twister extraction of amines. The protonated forms of the amines used in this study exhibit pKa values between 10 and 11 in aqueous solution. We therefore chose to extract the amines with Twisters from aqueous solution adjusted to pH 10, where a significant fraction of the amine was expected to be in the ionized form. We performed all extractions for 4 hours based on the extraction results for the long chain acids.

No recovery of the butylamine was seen (Figure 7), as expected based on the low log(Kow) for both neutral and ionized forms. The remaining five amines were all detectable, with longer-chain (higher log(Kow)) amines generally more efficiently extracted than the shorter chain amines. Trioctylamine showed unusually low recovery compared to the other amines in the study.
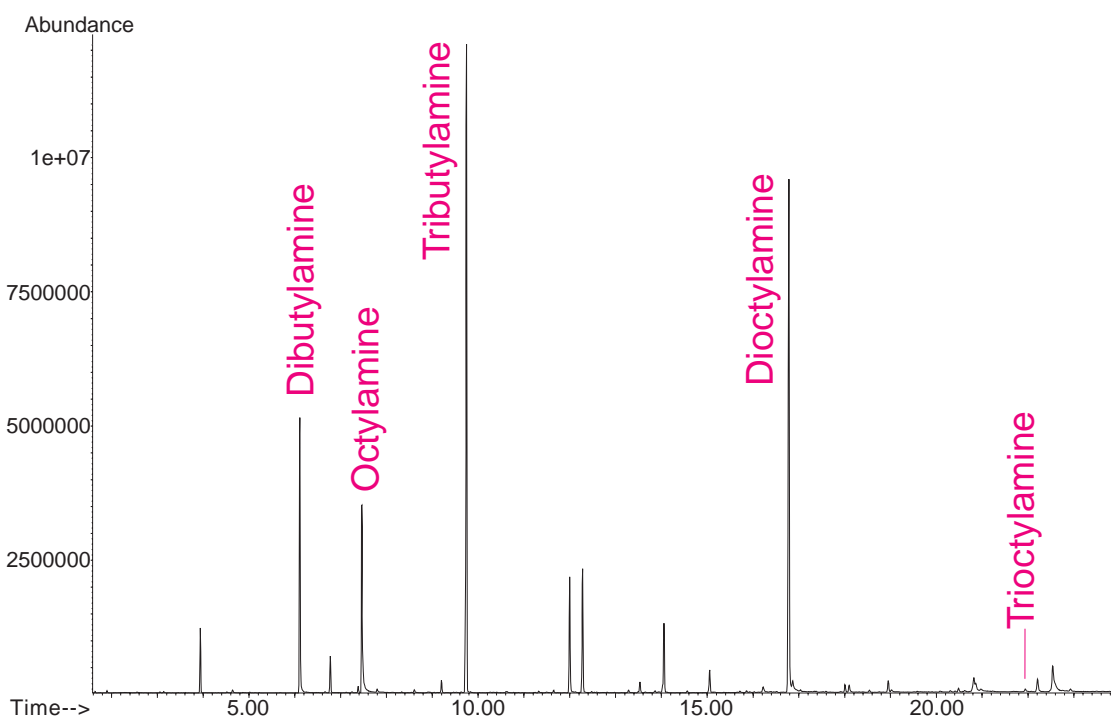

Figure 7. Chromatogram of butyl and octylamines extracted from pH 10 solution using Twister.

Twister extraction of amines from different pH solutions. The six amines were prepared in solutions ranging in pH from 2 to 10. Figure 8 shows a plot of the peak areas obtained after Twister extraction for 4 hours as a function of pH.

It is clear from this plot that the solution pH can dramatically affect recovery of the amines. At high pH, amines are expected to be neutral and exhibit lower water solubility making them most amenable to Twister extraction with the PDMS phase. All of the extractable amines behave as predicted with maximum recovery at higher pH, with the exception of trioctylamine.
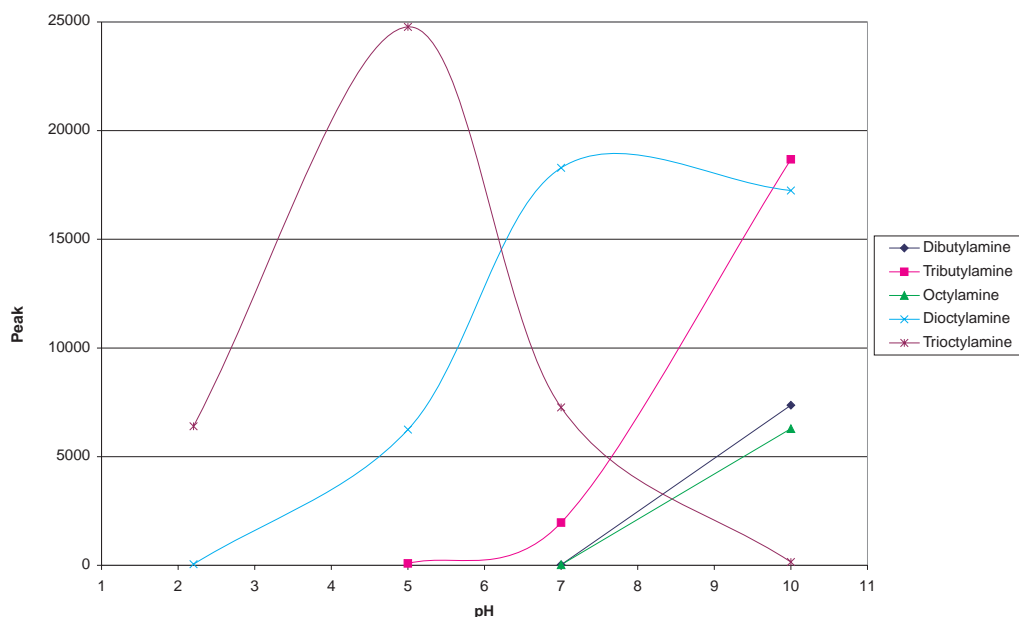
The observed extraction behavior of trioctylamine appears analogous to the behavior observed for stearic acid, since recovery goes through a maximum around pH 5 and falls off at both pH extremes. Like stearic acid, trioctylamine has a very high octanol:water partition coefficient indicating extreme water insolubility, suggesting loss may occur on the vessel walls. Extraction of long chain tertiary amines in the presence of 50% methanol gave improved recovery, consistent with the loss of material on vessel surfaces.

Figure 8. Twister extraction of amines as a function of pH.

These preliminary studies with a limited number of acids and amines illustrate the influence of solution pH on recovery of ionizable compounds in SBSE. Further studies with a broader range of acids and amines may be useful to fully understand the behavior of these compounds in SBSE.

CONCLUSIONS

Stir Bar Sorptive Extraction using the Gerstel Twister can effectively extract ionizable compounds (acids, amines) with sufficient lipophilic character if they are present in aqueous solution in the neutral form.

For good reproducibility, the ionization state of the acid or amine must be controlled. This is most easily done by selecting an appropriate buffer system for the extraction.

Acids and amines with extremely poor water solubility may exhibit anomalously low extraction efficiency from aqueous solution. This is most likely due to loss of analyte on vessel walls or aggregation in solution.

REFERENCES

[1]   Stir Bar Sorptive Extraction (SBSE), a Novel Extraction Technique for Aqueous Samples: Theory and Principles. Baltussen, E., Sandra, P., David, F. and Cramers, C.; J. Microcolumn Separations 11 (10), 737-747 (1999).

[2]   KowWin software, provided as part of EPI Suite™ exposure assessment estimation package (USEPA) http://www.epa.gov/oppt/exposure/docs/episuitedl.htm

[3]   Determination of trace amounts of off-flavor compounds in drinking water by stir-bar sorptive extraction and thermal desorption GCMS. Ochiai, N. et.al., The Analyst, 126, 1652 (2001).

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