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Europe - Issue 25, March 2007, International



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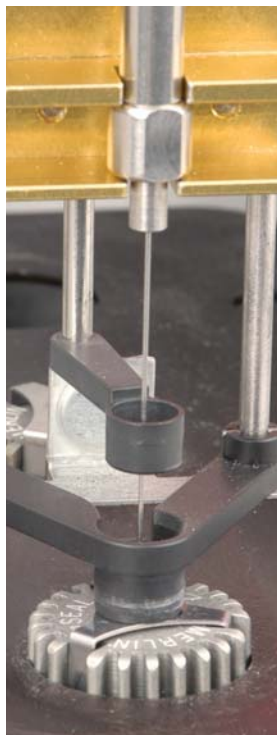
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Expanding SPME Technology through Partnerships



SPME autosampler

Since its discovery by researchers at the University of Waterloo and exclusive license to Supelco over 11 years ago, solid phase microextraction (SPME) has become widely popular with analysts due to its attractive feature as a solvent-less sample preparation technique. The technique has rapidly gained acceptance in areas from foods, flavors and fragrances to environmental, forensics and counter terrorism. SPME is a truly unique sample preparation technology allowing chemists, analysts and researchers to monitor and test samples in ways no other analytical technique allows. With over 2200 publications referencing SPME in its brief existence, its popularity as an evolving, practical and cost-effective analytical sample preparation technique is quite impressive.

Initially commercialized as a manual technique, hundreds of researchers each year use SPME to develop new sample preparation methods. Over time popular methods can evolve to require the need for handling a large number of samples. In these cases automation is necessary to increase sample preparation speed and efficiency. Ever since the early days of SPME, many instrument manufacturers sought to add SPME to their product offerings. Recently Supelco extended access of SPME technology to additional instrument manufacturers. Many analytical instrument manufacturers and equipment suppliers including Varian®, Thermo Electron, CTC Analytics, Gerstel, Alfatech, and Leap Technologies have begun adding SPME to their product portfolio to compliment their current analytical product offering. We continue to work with additional analytical instrument manufacturers anxious to utilize SPME to further innovate and enhance their capabilities and make this unique and valuable tool available to all researchers.

As more and more analytical instrument providers embrace the opportunity to use SPME technology, Supelco will join with these partners to enhance SPME products and integrate it with their automation capabilities to better meet researchers needs. Our overall goal in increasing the access of automated SPME technology to new partners is to encourage other manufacturers to work with Supelco to bundle their technology and know-how with SPME to continue to tackle the challenges of analytical sample preparation.

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Stationary Phase Selectivity in Aqueous Normal-Phase/Hydrophilic Interaction Chromatographic (ANP/HILIC) Separations

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Introduction

The term aqueous normal-phase/hydrophilic interaction chromatography (ANP/HILIC) refers to liquid chromatography performed using highly organic (> 70%) mobile phases and relatively polar stationary phases. The technique offers several potential advantages over traditional reversed-phase chromatography including alternative selectivity, retention of polar analytes and LC-MS compatibility. In reversed-phase chromatography, scientists often use stationary phases of differing chemistry to generate alternative selectivity. In a similar fashion to reversed-phase systems, ANP/HILIC may be accomplished on a number of stationary phases including various polar bonded phases as well as bare silica, which is best known in HILIC applications (1). The purpose of this study was to investigate selectivity differences between a fluorinated stationary phase previously shown to exhibit ANP/HILIC characteristics (2,3) and a modern bare silica phase for the separation of a set of important biogenic amines.

Dopamine, tyramine, and epinephrine (see Figure 1) are regarded as important biogenic amines in neurotransmitter research. Small, highly polar compounds such as these are difficult to retain using traditional reversed-phase chromatography on alkyl (C18, C8) stationary phases. Ion-pair reagents are often used in such systems to impart retention, however, ion-pair methods can suffer from robustness and reproducibility problems and are often not compatible with mass spectrometric detection. An alternative approach for the retention and separation of small, polar compounds is ANP/HILIC. Polar analytes may interact through a partitioning mechanism from an organic-

Figure 1. Structures of Biogenic Amines

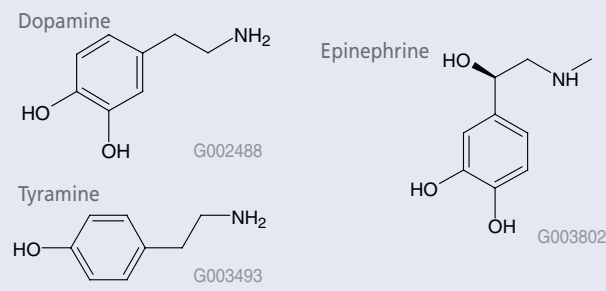
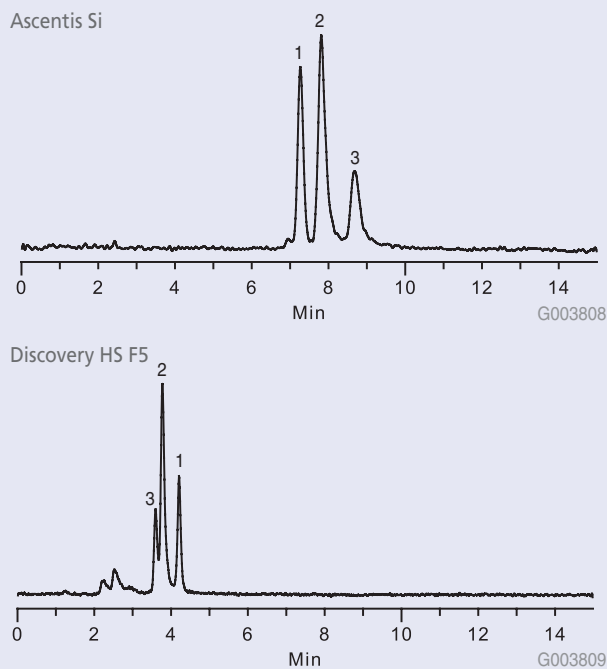


Figure 2. ANP/HILIC Separation of Biogenic Amines on Bare Silica and Polar Bonded Stationary Phases

columns: Ascentis Si, 15 cm x 4.6 mm I.D., 5 μ m particles (581512-U)
Discovery HS F5, 15 cm x 4.6 mm I.D., 5 μ m particles (567516-U)
instrument: Waters 2690 interfaced with a Micromass ZQ quadrupole mass spectrometer via an electrospray ionization source
mobile phase: 15:23.5:61.5, water:acetonitrile:0.1% ammonium acetate in acetonitrile
flow rate: 1 mL/min., split to the MS
temp.: 35 °C
det.: MS, ESI(+) in Single Ion Recording (SIR) Mode
injection: 10 μ L
sample: dopamine, tyramine, and epinephrine in 0.1% ammonium acetate in 10:90 water:acetonitrile

1. Tyramine (0.5 μ g/mL) $m/z = 137.08$
2. Dopamine (2 μ g/mL) $m/z = 153.07$
3. Epinephrine (2 μ g/mL) $m/z = 183.08$



rich mobile phase into an aqueous-rich static phase (HILIC) (4), or by polar and ionic interactions with the bare silica surface (5). The utilization of bonded phases such as a pentafluorophenylpropyl complicates the system by providing even more potential mechanisms for retention and selectivity.

Experimental

Tyramine, dopamine, and epinephrine, (by Sigma-Aldrich, see table on p.4), were run in ANP/HILIC mode on both a bare silica column (Ascentis Si) and a pentafluorophenylpropyl bonded silica column (Discovery HS F5) at high organic modifier (see Figure 2).

Results

Figure 2 (page 3) shows the chromatographic differences obtained when polar bonded phases such as the Discovery HS F5 are utilized in place of bare silica in the ANP/HILIC mode. For the biogenic amines studied, retention order was completely switched between the two phases. Although the retention mechanisms responsible for the alternative selectivity have not been elucidated, neither the retention on the bare silica nor on the fluorinated phase is completely explained by either HILIC or ion-exchange retention mechanisms alone.

Conclusions

This study shows one example of how retention and selectivity may be manipulated in ANP/HILIC mode by utilizing bonded polar stationary phases as alternatives to bare silica. In this non-optimized case, bare silica appears to be the phase of choice for further development, however the fluorinated phase may prove suitable depending on the overall objectives for the method. Similar to the common practice of using alternative stationary phases in reversed-phase method development to impart different selectivity and retention, polar bonded phases should prove to be useful as alternatives to bare silica in ANP/HILIC mode.

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Related Information

For more information regarding retention mechanisms in ANP/HILIC mode, request T406084 (JBT). Available in electronic form only - please include your email address on request form.



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ID (mm)	Length (cm)	Particle Size (µm)	Cat. No.
Ascentis Si Columns for ANP/HILIC			
2.1	5	3	581500-U
2.1	5	5	581507-U
2.1	10	3	581501-U
2.1	15	3	581502-U
2.1	15	5	581509-U
3.0	10	3	581503-U
4.6	5	3	581504-U
4.6	10	3	581505-U
4.6	15	3	581506-U
4.6	15	5	581512-U
4.6	25	5	581513-U
Biogenic Amines			Cat. No.
Tyramine			T90344-5G
Dopamine			H8502-1G
Epinephrine			E4250-1G

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Methanol with 0.1% TFA	34974
Water with 0.1% formic acid	34673
Water with 0.1% formic acid/0.01% TFA	34671
Acetonitrile with 0.1% formic acid	34668
Acetonitrile with 0.1% formic acid/0.01% TFA	34676
Water with 0.1% acetic acid	34675
Acetonitrile with 0.1% acetic acid	34678
Methanol with 0.1% acetic acid	34672
Water with 0.1% ammonium acetate	34674
Acetonitrile with 0.1% ammonium acetate	34669
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Description	Phenyl	Silica
3 cm x 2.1 mm, 3 µm	581602-U	581522-U
3 cm x 2 mm, 3 µm	581606-U	581523-U
5 cm x 2.1 mm, 3 µm	581603-U	581500-U
5 cm x 4.6 mm, 3 µm	581608-U	581504-U
10 cm x 1 mm, 3 µm	581600-U	581520-U
10 cm x 2.1 mm, 3 µm	581604-U	581501-U
10 cm x 3 mm, 3 µm	581607-U	581503-U
10 cm x 4.6 mm, 3 µm	581609-U	581505-U
15 cm x 1 mm, 3 µm	581601-U	581521-U
15 cm x 2.1 mm, 3 µm	581605-U	581502-U

Description	Phenyl	Silica
15 cm x 4.6 mm, 3 µm	581610-U	581506-U
5 cm x 2.1 mm, 5 µm	581611-U	581507-U
5 cm x 4.6 mm, 5 µm	581615-U	581511-U
10 cm x 2.1 mm, 5 µm	581612-U	581508-U
15 cm x 2.1 mm, 5 µm	581613-U	581509-U
15 cm x 4.6 mm, 5 µm	581616-U	581512-U
25 cm x 2.1 mm, 5 µm	581614-U	581510-U
25 cm x 4.6 mm, 5 µm	581617-U	581513-U
25 cm x 4.6 mm, 10 µm		581524-U

*One column per lab. Offer valid until end of April 2007.

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Extraction of the Beta-Agonist Clenbuterol from Urine Using Clenbuterol SupelMIP

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Highly Selective SPE for Trace Analysis in Complex Matrices

Abstract

Selective sample preparation is now available based on molecularly imprinted polymers. These highly cross-linked polymeric sorbents give significant benefits for trace analysis in complex matrices. They provide lower detection limits, significant time and cost savings and enhanced MS-compatibility.

Introduction

Trace analysis of compounds from complex biological samples require often very extensive and time consuming sample preparation procedures due to the insufficient selectivity of traditional SPE sorbents.

SupelMIP cartridges are specifically designed to selectively extract analytes or classes of analytes at trace levels from complex matrices. They contain SPE sorbents based on molecularly imprinted polymers (MIPs).

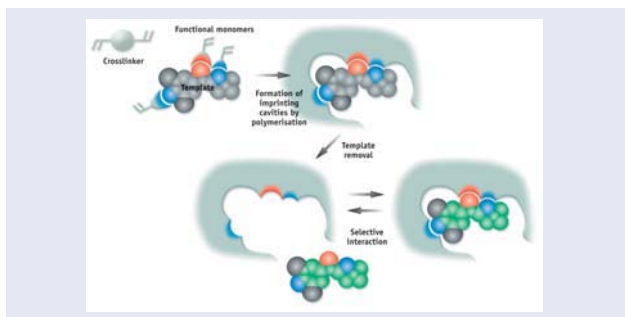


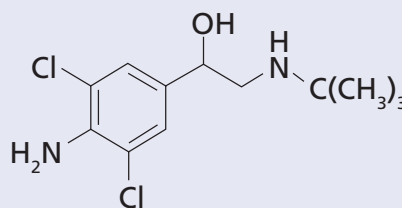
Figure 1. The basic principle of molecular imprinting

Selectivity is introduced during the preparation of the MIP sorbent by choosing one or more functional monomers which in solution will form complexes with a template molecule that mimic the analyte or a sub-fragment of the analyte¹ (Figure 1). Once the polymer is formed the template molecule is removed from the MIP resulting in specific cavities or imprints, sterically and chemically complementary to the analyte or group of analytes of interest. The interactions between the analyte and the functional groups in the cavities are based on hydrogen

bonding, ionic, Van der Waals or hydrophobic interactions. Due to multiple interaction sites binding is stronger compared to random binding to traditional SPE sorbents.

Clenbuterol, a synthetic beta-agonist, is illegally used as a growth promoter both in humans (doping) and in livestock (breeding)² (Figure 2).

Figure 2. Chemical Structure of Clenbuterol



Screening programs are executed all over the world to find the banned drug in food and feed samples, due to the potential health risks associated with beta-agonist residues in meat products. A number of food poisoning cases have been reported with consumption of contaminated meat³, the latest case very recently in Shanghai, where over 300 people were hospitalized⁴.

The determination of beta-agonists in biological samples is at trace levels (typically < 0.5 ng/mL) and in complex biological matrices, such as urine, muscle, liver etc. Conventional SPE materials are normally not selective enough.

The highly selective Clenbuterol SupelMIP is designed for extraction of the most common beta-agonist, clenbuterol, from complex matrices. Available is also a class selective Beta-agonist SupelMIP, which extracts a broad range of beta-agonists from complex biological samples.

Experimental

5 mL bovine urine was extracted on a Clenbuterol SupelMIP column and compared with extraction on three different mixed-phase cartridges (C4, C8 and C18 mixed with a strong cation exchanger)

Protocol for mixed-phase cartridges:

The cartridges were conditioned with 2x1 mL MeOH followed by 2x1 mL 50 mM NH₄Ac buffer pH 6.0. 5 mL urine, pH 6.0 and spiked with 2 ng/mL Clenbuterol was applied to the cartridge. Interferences were eluted with 2x1 mL 50 mM NH₄Ac buffer pH 6.0, 2x1 mL 1 M HAC (cartridges dried for 30 seconds after this wash) and finally 2x1 mL NaOH. Clenbuterol was eluted with 1 mL MeOH/5% ammonia.

Protocol for Clenbuterol SupelMIP cartridges⁵:

The cartridge was conditioned with 1 mL MeOH, 1 mL water and 1 mL 25 mM NH₄Ac pH 6.7. 5 mL urine diluted 1:1 with 25 mM NH₄Ac pH 6.7 was applied to the column. Interferences were eluted by 1 mL of water, followed by 2 minutes of vacuum, 1 mL of acetonitrile/2% acetic acid, 1 mL 0.5 M NH₄Ac pH 5 and 1 mL 70% acetonitrile in water. Finally Clenbuterol was eluted with 2x1 mL MeOH/1% trifluoro acetic acid (TFA).

Each SupelMIP phase is delivered with a data sheet describing a recommended extraction protocol.

Results and Discussion

MIP particles synthesized to be specific for clenbuterol show greater specificity for clenbuterol than conventional SPE particles. Clenbuterol can bind to the polymer with a variety of bonds (ionic, hydrophobic and hydrogen). In the selective cavities on the MIP, these binding possibilities are sterically arranged to fit clenbuterol.

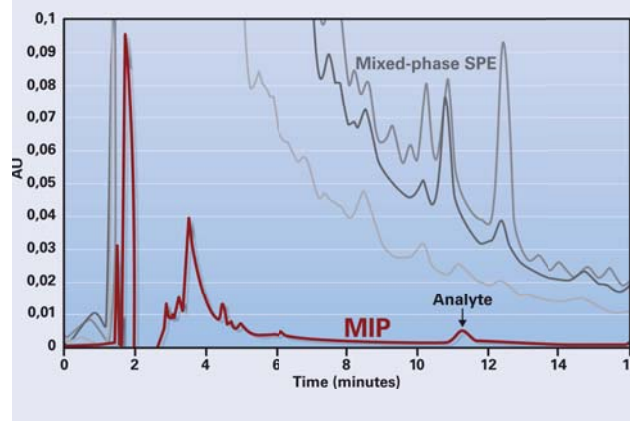
Conditioning consists of wetting the MIP using methanol and water and then adjusting the pH to 6.7 so that the acidic monomer is in a negatively charged state for ionic bonding. During sample loading, clenbuterol is non-selectively retained, together with substances from the urine matrix. In a water environment, interactions between the selective cavities of the MIP and clenbuterol are not well established. Bonding of clenbuterol occurs not only in the selective cavities, but also to the backbone of the polymer. Non-selective binding enables a high total loading capacity of the material. It has been proven that a dilution of urine 1:1 with 25 mM buffer pH 6.7 was optimal for loading⁵. Undiluted urine might decrease the recovery probably due to the high ionic strength of urine that reduces the amount of clenbuterol adsorbed on the polymer during sample loading. To reveal the interactions between clenbuterol and the selective cavities of the MIP the polymer has to be in an acetonitrile environment. By adding 2% acetic acid to acetonitrile it was shown that the MIP phase still retained clenbuterol while interfering compounds were effectively removed. It was observed that some of the clenbuterol was lost if the water content of the polymer was too high and therefore a few minutes of

vacuum was necessary to semi-dry the MIP before the selective wash in order to obtain high recoveries. The interference elution steps described in the protocol for Clenbuterol SupelMIP cartridges in the experimental section must be performed in the described order. Using this protocol, determination of 0.5 ng of clenbuterol/mL urine is possible when using UV detection. Depending on how selective the detector is some of the steps could be left out, but the selective wash (acetonitrile/2% acetic acid) must always be performed.

Elution of clenbuterol was effective by the use of methanol mixed with 1% TFA. Stronger concentrations of TFA can lead to degradation of clenbuterol.

The chromatograms show the SPE extraction of clenbuterol from a 5 mL urine sample on Clenbuterol SupelMIP vs. conventional, mixed-mode SPE particles, which gave high background and misleading responses (Fig 3).

Figure 3. Extraction of Clenbuterol on Mixed-Mode Phases (C4, C8 and C18 in grey) and Clenbuterol SupelMIP (in red). Analysis by HPLC-UV.



The precision and accuracy of the method were determined by analyzing spiked urine samples at 0.6 and 6.0 ng clenbuterol/mL (n=6). The results are presented in Table 1. The results show that the performance of this MIP-based method is well within the limits of that expected of current bioanalytical methods.

Table 1. Precision and Accuracy for the Analysis of Clenbuterol in 5 mL Spiked Urine Samples

	Within-day		Between-days	
	0.6 ng/mL	6.0 ng/mL	0.6 ng/mL	6.0 ng/mL
n	5	6	9	10
Mean + SD	0.58+0.025	5.8+0.12	0.61+0.039	5.9+0.24
SD (%)	4.3	2.1	6.4	4.1
Accuracy (%)	96.7	96.7	101.7	98.3
No. analyses	1	1	3	3

Conclusions

The Clenbuterol SupelMIP exhibits high selectivity for clenbuterol. When the selective binding of clenbuterol is established the MIP can be washed harshly to elute interfering compounds from the matrix without any loss of clenbuterol. This result in much cleaner extracts than those obtained with mixed-phase SPE. Quantitation by HPLC-UV down to 0.5 ng clenbuterol/mL urine is possible with Clenbuterol SupelMIP cartridges. With more selective detectors such as MS even lower detection limits are expected to be reached. Furthermore clenbuterol is extracted with high recoveries and the SPE method is robust with high accuracy and precision. Compared with conventional SPE sorbents such as mixed-phase SPE, the SupelMIP sorbents result in increased cleanliness, improved detection limits, significant time and cost savings and improved MS compatibility.

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SupelMIP For	Bed weight	Column volume	Pk of	Cat. No.
Clenbuterol	25 mg	10 mL	50	53201-U

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SupelMIP For	Bed weight	Column volume	Pk of	Cat. No.
Beta-agonists (class selective)	25 mg	10 mL	50	53202-U
	25 mg	3 mL	50	53225-U
NNAL (4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol)	25 mg	10 mL	50	53206-U
	25 mg	3 mL	50	53203-U
Riboflavin (vitamin B2)	25 mg	10 mL	50	53208-U
Triazines (class selective)	25 mg	10 mL	50	53207-U
Chloramphenicol	25 mg	10 mL	50	53210-U
	25 mg	3 mL	50	53209-U
Beta blockers (class selective)	25 mg	10 mL	50	53218-U
	25 mg	3 mL	50	53213-U
TSNAs (NNK, NNN, NAB, NAT)	25 mg	10 mL	50	53221-U
	25 mg	3 mL	50	53222-U
Full beta receptor (beta agonists and beta blockers)	25 mg	10 mL	50	53223-U
	25 mg	3 mL	50	53224-U

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Improve Sample Prep Selectivity using 96-well SPE Method Development Plates

An Trinh

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In pharmaceutical bioanalysis, researchers are charged with the responsibility of developing and running assays to quantitate drugs, pharmaceutical candidates, and their metabolites in biological fluids such as serum and plasma. With recent advances in combinatorial chemistry, genomics and proteomics, knowledge of drug mechanisms are increasing resulting in drug designs structurally catered to endogenous biomolecules. Such drugs are often more potent allowing for smaller dosages which results in smaller concentrations of the drugs and their metabolites in biological fluids. Although advances in LC-MS technology have reaped overwhelming benefits in terms of increased throughput and sensitivity, good sample preparation has and continues to become more critical.

Bioanalytical scientists are often asked to detect drug levels in the parts-per-trillion to parts-per-quadrillion range. Because solid phase extraction (SPE) technology is based on chromatographic separation, analysts can develop robust methods that offer high analyte recoveries. More importantly, SPE offers the selectivity necessary to specifically target the retention and elution of analytes of interest in the presence of complicated biological matrix components.

Even with SPE technology's advantages, many analysts do have reservations for using the technology. The most

common disadvantage is the wide perception that SPE is overly complex. The wide selection of phase chemistries coupled with the large number of potential reagents/solvents used for each step of the SPE process makes method development and troubleshooting a daunting and time consuming task. In effect, many researchers find it difficult to develop rugged SPE methods that meet their analytical objectives.

96-well SPE MD (Method Development) Plate – BAN, for extracting basic, acidic, and neutral compounds (BAN)

To address this widespread concern, we have developed a 96-well SPE platform to ease the method development process. Our new 96-well SPE MD (Method Development) Plate – BAN contains a selection of 8 different SPE chemistries commonly used in the extraction of basic, acidic, and/or neutral compounds from biological fluids (Figure 1). The mix of phase chemistries contained within this 96-well SPE plate allows researchers to screen for the phase(s) that offer the best analyte recovery, selectivity, and reproducibility when using the generic methods described in Table 1.

The Benefits of Evaluating Multiple Phase Chemistries

Most method developers often focus their method development efforts on popular reversed-phase chemistries such as C18 and hydrophilic polymer phases. Such phases often offer good retention of a broad range

Figure 1. Phase Chemistry Template for 96-well SPE MD Plate-BAN, 25 mg/well (577522-U)

	1	2	3	4	5	6	7	8	9	10	11	12
A	Discovery DSC-PS/DVB (polystyrene divinyl benzene) ¹											
B	Discovery DSC-18 (tC18) ¹											
C	Discovery DSC-8 (C8) ¹											
D	Discovery DSC-CN (cyanopropyl) ¹											
E	Discovery DSC-MCAX (mixed-mode cation exchange) ²											
F	Discovery DSC-WCX (weak cation exchange) ²											
G	Discovery DSC-SAX (strong anion exchange) ³											
H	Discovery DSC-NH ₂ (aminopropyl weak anion exchange) ³											

- ¹ Reversed-phase
- ² Cation-exchange
- ³ Anion-exchange



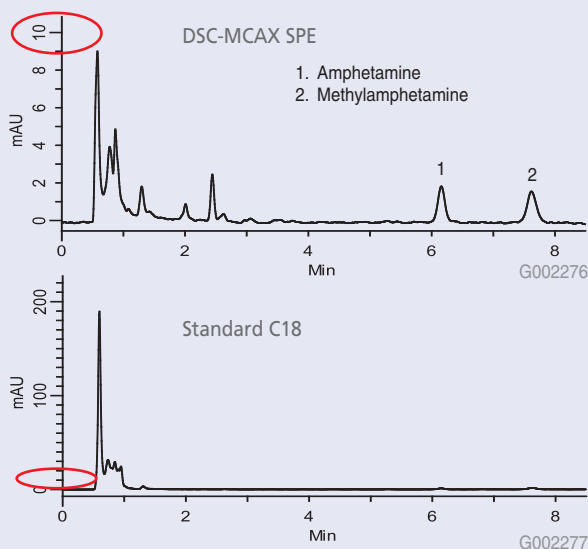
Table 1. Recommended Generic Methods for 96-well SPE MD Plate-BAN

SPE Step	Reversed-phase	Cation-exchange	Anion-exchange
1. Sample Pre-Treatment	Dilute biological sample 1:1 with 10-50 mM buffer (phosphate, ammonium acetate, or ammonium formate) at 2 pH units above analytes' pKa for basic analytes, or 2 pH units below pKa for acidic analytes.	Dilute biological sample 1:1 with 10-50 mM buffer (phosphate, ammonium acetate, or ammonium formate), pH 3 for basic analytes.	Dilute biological sample 1:1 with 10-50 mM buffer (phosphate, ammonium acetate, or ammonium formate), pH 10 for acidic analytes.
2. Condition/Equilibrate	Condition with methanol. Equilibrate with DI water or buffer used in sample pre-treatment.	Condition with methanol. Equilibrate with DI water or buffer used in sample pre-treatment.	Condition with methanol. Equilibrate with DI water or buffer used in sample pre-treatment.
3. Sample Load	Load pre-treated sample from step 1.	Load pre-treated sample from step 1.	Load pre-treated sample from step 1.
4. Wash	Wash off co-retained interferences with 5-20% methanol diluted in DI water or buffer used in sample pre-treatment.	Wash off co-retained interferences with low pH buffer used in sample pre-treatment, followed by 1M acetic acid and 100% methanol.	Wash off co-retained interferences with high pH buffer used in sample pre-treatment, followed by 100% methanol.
5. Elution	Elute with methanol or acetonitrile. pH modification may be necessary to facilitate elution. Use 2% acetic acid in methanol or acetonitrile for basic analytes; or 2% ammonium hydroxide in methanol or acetonitrile for acidic analytes.	Elute basic analytes with 2-5% ammonium hydroxide in methanol or acetonitrile.	Elute acidic analytes with 2-5% acetic acid in methanol or acetonitrile.
6. Evaporate/Reconstitute	Evaporate SPE eluate and reconstitute with analytical mobile phase		

Figure 2. DSC-MCAX SPE vs. C18 SPE for the Extraction of Amphetamine and Methylamphetamine in Urine

SPE tube: Discovery DSC-MCAX, 100 mg/3 mL, standard C18, 100 mg/3 mL
 cat. no.: 52783-U
 HPLC column: Discovery HS F5, 15 cm x 4.6 mm I.D., 5 µm particle size
 cat. no.: 567516-U
 mobile phase: 10 mM ammonium acetate, pH 4.5:MeCN (35:65)
 flow rate: 2 mL/min.
 temp.: 40 °C
 det.: 210 nm, UV
 injection: 10 µL

- Note the Y-axis scale difference between DSC-MCAX and C18 SPE. DSC-MCAX SPE offered a maximum background height of ~9 mAU.
- In contrast, standard C18 background levels were 20 times greater than DSC-MCAX.
- Also, on DSC-MCAX absolute recovery averaged at 100.3 and 101.7%, for amphetamine and methylamphetamine, respectively.
- On standard C18, absolute recovery averaged at 48 and 79% for the two compounds.



of analytes and can typically yield high recoveries under generic methodology. However, because of this broad affinity, matrix interferences can often co-retain and elute with analytes of interest.

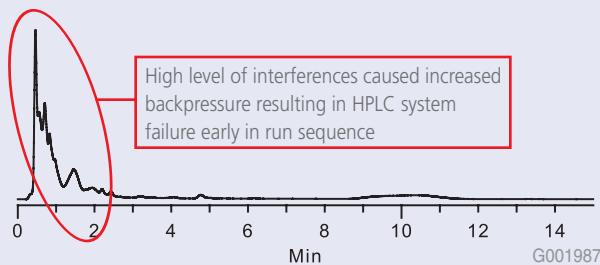
For example, in this application, we compare the extraction of 2 µg/mL amphetamine and methylamphetamine spiked in human urine on both a standard C18 SPE and Discovery DSC-MCAX using the generic protocols described in Table 1 (reversed-phase protocol for C18 and cation-exchange protocol for MCAX) followed by subsequent LC-UV analysis (Figure 2). The MCAX phase offered ~100% absolute recovery, whereas the C18 phase offered 79 and 48% recovery for the compounds tested. Also note that the C18 background was 20 times greater than the MCAX phase.

In this second application, four corticosteroids (0.5 and 1.0 µg/mL) were extracted from urine on both Discovery DSC-CN and standard C18 (100 mg/well) using the reversed-phase procedure described in Table 1 followed by LC-UV analysis. Note that the background level on C18 was so high, HPLC system failure occurred early in the run (Figure 3). DSC-CN also offered excellent recovery for the corticosteroids tested (Figure 4).

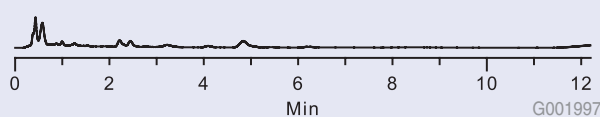
Figure 3. Background Comparison of Blank Urine Sample

column: Discovery HS F5, 5 cm x 4.6 mm I.D., 3 µm particles
mobile phase: 40:60 methanol:deionized water
flow rate: 1.5 mL/min.
temp.: 35 °C
det.: UV at 240 nm
injection: 5 µL

Blank urine extract on conventional C18



Blank urine extract on Discovery DSC-CN



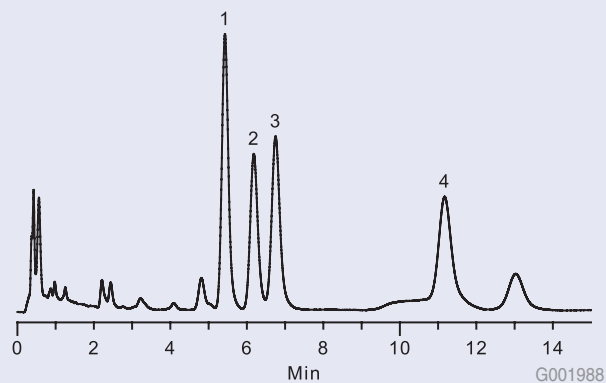
Conclusion

In pharmaceutical bioanalysis, sample prep selectivity and recovery is of vital importance when achieving very low limits of quantitation in difficult sample matrices. Supelco's new 96-well SPE MD Plate-BAN offers a convenient format for researchers to screen an array of SPE phase chemistries during SPE method development. Once one or more phase chemistries are selected, further

Figure 4. Recovery and Example Chromatogram for DSC-CN Extraction of Corticosteroids

Compound	% Recovery ± RSD (n=3)	
	0.5 µg/mL spike level	1.0 µg/mL spike level
1. Hydrocortisone	123.3±1.4%	95.9±1.7%
2. Prednisilone	107.2±1.1%	91.9±1.1%
3. Prednisone	103.2±1.0%	88.4±1.8%
4. Corticosterone	102.0±1.2%	93.1±5.6%

1 µg/mL spiked urine extract on Discovery DSC-CN



method optimization can be conducted to offer maximum assay selectivity, recovery, and accuracy/precision. In this report, we demonstrate the importance of evaluating multiple phase chemistries during method development.

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Description	25 mg/well	50 mg/well	100 mg/well
96-well SPE MD Plates			
96-well SPE MD Plate-BAN (Basic, Acidic, Neutral Compounds)	577522-U	Inquire	Inquire

+ Related Products

Description	25 mg/well	50 mg/well	100 mg/well
Standard 96-well SPE Plates			
Discovery DSC-18	575601-U	575602-U	575603-U
Discovery DSC-18Lt	575604-U	575605-U	575606-U
Discovery DSC-8	575629-U	575628-U	575627-U
Discovery DSC-Ph	575632-U	575631-U	575630-U
Discovery DSC-CN	575626-U	575625-U	575624-U
Discovery DPA-6S (polyamide)	Inquire	Inquire	-
Discovery PS/DVB	575610-U	575611-U	-
Discovery DSC-MCAX (C8/SCX)	575639-U	575640-U	575641-U
Discovery DSC-SCX	575623-U	575622-U	575621-U
Discovery DSC-WCX	575635-U	575634-U	575633-U
Discovery DSC-MANX (C8/SAX)	Inquire	Inquire	Inquire
Discovery DSC-SAX	575620-U	575619-U	575618-U
Discovery DSC-NH ₂	575617-U	575616-U	575615-U
Supelclean PSA	Inquire	Inquire	Inquire
Supelclean LC-4 (wide pore)	Inquire	Inquire	Inquire

TRADEMARKS: Agilent - Agilent Technologies; Ascendis, CHROMASOLV, Discovery, Equity, SLB, Supelclean, Supelco - Sigma-Aldrich Co.; Carbowax - Union Carbide Chemical & Plastics Technology Corp.; CombiPAL - CTC Analytics; FocusLiner - SGE International Pty Ltd.; GERSTEL - Gerstel GmbH; Microseal - Merlin Instrument Company; PerkinElmer - PerkinElmer Corp.; Shimadzu - Shimadzu Corp.; SIR - Airgas; Thermo - Thermo Electron Corp.; Varian - Varian Associates Corp.; Waters - Waters Associates, Inc.

SPME - Technology licensed exclusively to Supelco. US patent #5,691,206; European patent #523,092

A New Polyethylene Glycol (PEG) SPME Fiber Assembly

Robert Shirey

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A new 60 μm polyethylene glycol (PEG) SPME fiber coating has been developed that does not contain an adsorbent polymer. Eliminating the adsorbent produces a more polar, selective fiber. This fiber will replace the existing Carbowax[®]/divinylbenzene (CW-DVB) and Carbowax/templated resin (CW-TPR) coated fibers that are being phased out due to durability and material supply problems.

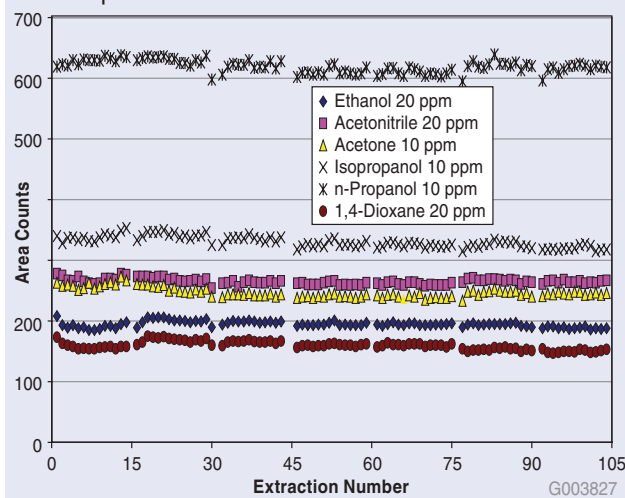
The new PEG phase is coated on an inert metal fiber attached to the standard stainless steel assemblies. The metal fiber enables long lengths of the fiber to be coated at one time to help improve reproducibility between fibers. Also, the metal fiber is unbreakable and the coating bonds to the metal core better than other surfaces.

Durability of Fiber Coating

Since Carbowax is a water-soluble material, the durability of the material often becomes a problem due to swelling and stripping when extracting analytes out of water. A top priority for the PEG fiber coating was to make it more durable. To obtain this parameter, the coating must bond well to the fiber core and not swell significantly when inserted in water. This requires a highly cross-linked polymer.

To test the fibers for durability, they were immersed for 10 minutes in water containing small polar solvents at ppm levels. The Varian 8200 autosampler was used which agitates the fiber similarly to an electric razor. This fast vibration is often detrimental to fiber coatings. With the CW-DVB fibers, the life of the fiber in the system ranged between 1 and 20 extractions before part or all of the

Figure 1. Analyte Response from Repeated Extractions with 60 μm PEG SPME Fiber



coating came off the fiber core. With the new PEG fiber the average life of the fibers was over 100 extractions. After 15 extractions a water blank was extracted to remove salt on the fiber and the fiber was examined. Figure 1 shows the response of the solvents over 100 extractions.

There was a slight reduction in analyte response of about 3% between the first 10 and the last 10 extractions. No visible decay in the appearance of the fiber coating was observed and the film thickness remained constant throughout the study.

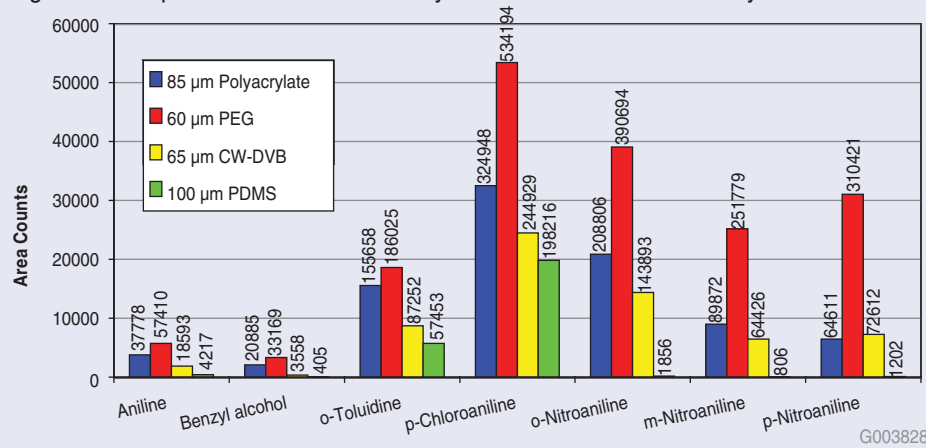
Applications using 60 μm PEG Fiber

Since the 60 μm PEG fiber is being offered as a replacement for the CW-DVB fibers, applications comparing the fibers is important. Several classes of analytes were

evaluated and some examples are shown below. For a more detailed comparison please contact us for additional information.

A mixture containing base-neutral analytes was evaluated with several different SPME fibers. Figure 2 compares the fibers for the extraction of the more polar analytes in the mixture. These analytes were extracted out of buffered salt water (pH 9) by immersion for

Figure 2. Comparison of SPME Fibers by the Extraction of Basic Analytes



30 minutes with agitation using the CombiPAL.

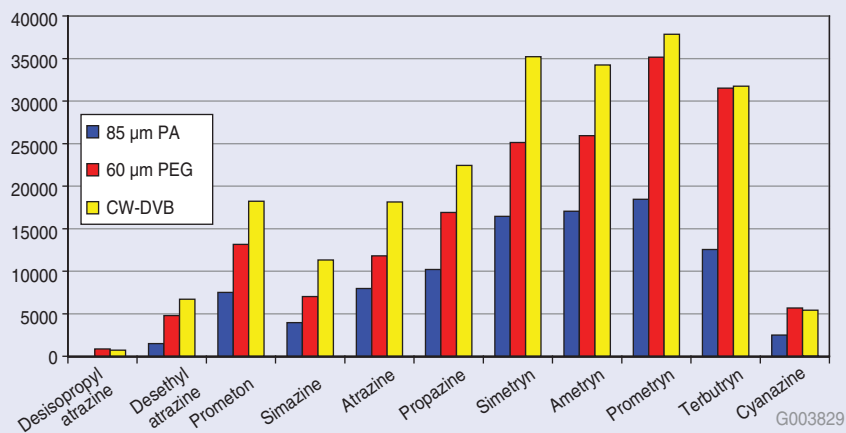
The results show that the 60 μm PEG fiber extracts the more polar analytes better than the other fibers, while it extracts less of the nonpolar analytes compared to the other fibers.

Triazine herbicides have both polar and nonpolar properties and most of the analytes are extracted well with a variety of fibers. However, for the extraction of the metabolites of atrazine, desethyl atrazine and desisopropyl atrazine, more polar fibers are needed. Figure 3 shows a comparison of the fibers for the extraction of the herbicides.

All three polar fibers extract the triazine herbicides well. The most polar analyte, desisopropyl is best extracted by the PEG fiber.

Since the PEG fiber extracts polar analytes well, and reduces the amount of less polar analytes extracted, the fiber is ideal for samples containing both polar and nonpolar analytes. Figure 4 shows a chromatogram of 44 pesticides with the PEG fiber at 10 ppb. The response of the relatively nonpolar chlorinated pesticides is slightly reduced while the most polar analytes are extracted at the trace level.

Figure 3. Comparison of Fiber Coatings for the Extraction of Herbicides



The new 60 μm PEG SPME assembly contains a durable, polar coating that is well bonded to the unbreakable inert metal fiber. Since the coating does not contain an adsorbent, it is more polar and selective than the Carbowax coatings containing DVB. This fiber should be a good compliment with PDMS fibers particularly for the extraction of semi-volatile analytes.

! Related Information

For more information on SPME, request the SPME Application CD (CJQ), or visit sigma-aldrich.com/supelco-spme

Figure 4. Extraction and Analysis of Multiple Pesticides

sample/matrix: 44 pesticides, each at 10 ppb in 0.1 M phosphate buffer, pH 7, with 25 % sodium chloride, in a 10 mL screw cap vial

SPME fiber: 60 μm Carbowax (PEG) on a metal fiber (57354-U)

extraction: immersion for 30 min., with agitation using a CombiPAL™ autosampler

desorption temp.: 250 °C for 3 min.

column: SLB-5ms, 30 m x 0.25 mm I.D., 0.50 μm (28473-U)

oven: 60 °C (1 min.), 25 °C/min. to 150 °C, 5 °C/min. to 230 °C, 18 °C/min. to 310 °C (3 min.)

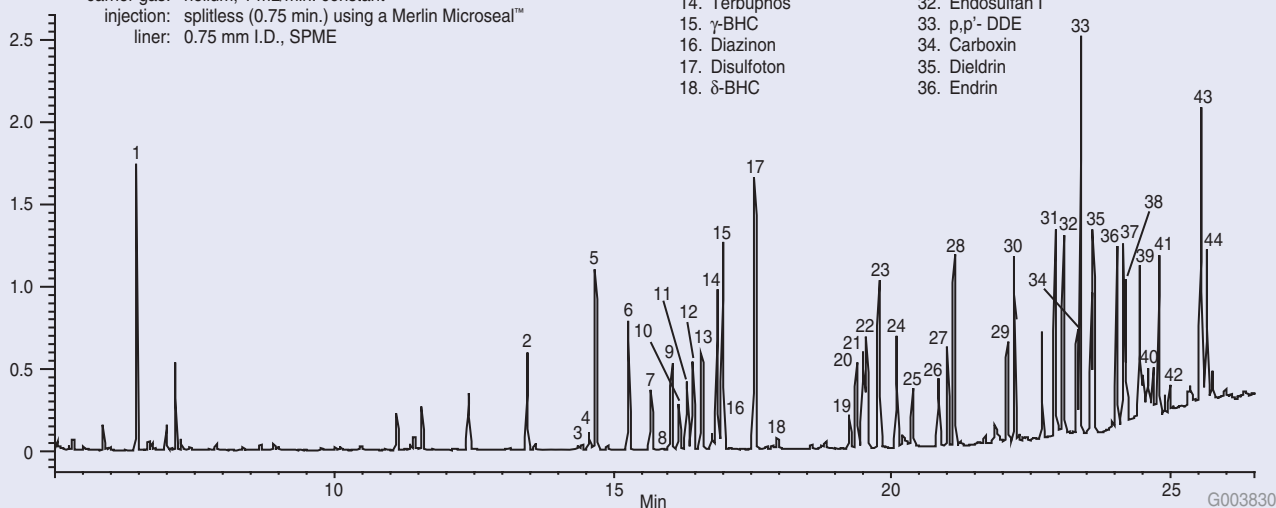
MSD interface: 310 °C

scan range: 60-450 m/z

carrier gas: helium, 1 mL/min. constant

injection: splitless (0.75 min.) using a Merlin Microseal™

liner: 0.75 mm I.D., SPME



Analysis of Adulterated Lemon Essential Oil on the SLB-5ms

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2. Supelco, Bellefonte, Pennsylvania, USA, e-mail: mbuchanan@sial.com

Introduction

Citrus essential oils are of high economical importance in many parts of the world. They are complex substances that are used mainly in the food, beverage, cosmetic and perfume industries. They are extracted by means of cold mechanical pressure applied on the fruit peel. The end products are mixtures of more than 200 components that can be grouped into non-volatile (1-15%) and a volatile (85-99%) fractions. The latter contains different classes of compounds (mainly mono and sesquiterpene hydrocarbons, and their oxygenated derivatives, along with aliphatic aldehydes, alcohols and esters), which are present in a wide range of concentrations. Qualitative and quantitative analysis is fundamental for quality control and, often, for the detection of adulterants. The adulteration of these valuable products through the addition of cheaper compounds is, unfortunately, a common fraud.

Citrus Essential Oil Composition

Citrus essential oils differ from each other mainly in their quantitative compositions, both of the volatile and non-volatile fractions. Hence, citrus oils have different olfactory properties that make some oils, such as bergamot or lemon, more valuable than other oils, such as sweet orange. For example, in 1999 in Italy, 1 kg of winter lemon essential oil cost between 12.9 and 16.5 Euro. During this year, Italy accounted for about 20% of the entire world's production of lemon oil. The same amount of sweet orange oil cost approximately 0.7 Euro in 1999.

Adulteration of Citrus Essential Oils

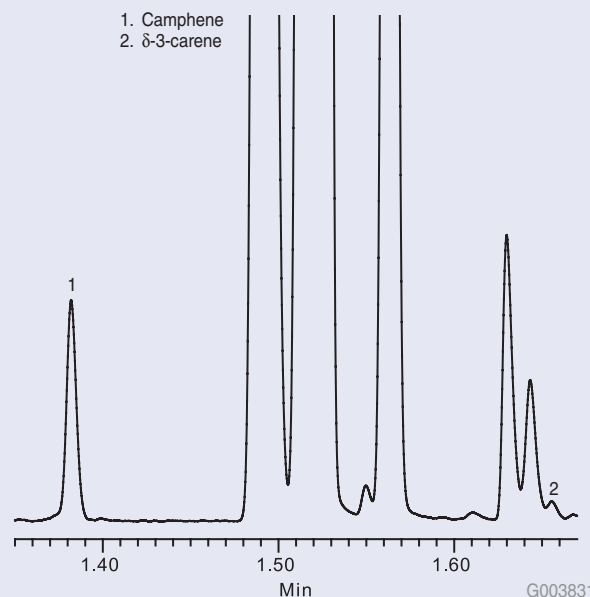
One of the most common adulterations to mandarin, bergamot, bitter orange, and lemon oils (with the latter probably being the most frequent target), is the addition of low-cost lime or orange oil. Sweet orange oils or sweet orange oil terpenes are characterized by the presence of about 0.1% of δ -3-carene. This monoterpene hydrocarbon is either missing or present at trace levels in other citrus oils. The contrary is true for another monoterpene hydrocarbon, camphene, which is practically absent in sweet orange oils or terpenes, but present at a higher level in lemon oil (about 0.06%). The δ -3-carene level and the δ -3-carene/camphene ratio are particularly useful for the detection of the possible addition of these sweet orange oils or orange oil terpenes.

Analysis of Citrus Essential Oils

Figure 1 shows the Fast GC analysis of pure lemon oil on the SLB-5ms column. The SLB-5ms column was chosen for its ability to resolve the analytes of interest, even under Fast GC conditions (fast oven ramp rate, fast carrier gas linear velocity, narrow bore column). The δ -3-

Figure 1. Fast GC Analysis of Pure Lemon Essential Oil

column: SLB-5ms, 10 m x 0.10 mm ID, 0.10 μ m (28465-U)
oven: 40 °C, 30 °C/min. to 85 °C, 80 °C/min. to 320 °C
inj.: 320 °C
det.: FID, 320 °C
carrier gas: hydrogen, 70 cm/sec constant
injection: 0.4 μ L in the split mode (300:1)
sample: lemon essential oil in hexane



carene/camphene ratio cannot exceed a value of 0.140 for the sample to be considered pure lemon oil. Figure 2 shows the Fast GC analysis of pure sweet orange oil on the SLB-5ms column. A very high δ -3-carene/camphene value was obtained. Figure 3 shows the Fast GC analysis of lemon oil adulterated with 5% sweet orange oil. A δ -3-carene/camphene value of 0.275 was obtained, which exceeds the maximum value to be considered pure lemon oil.

Conclusion

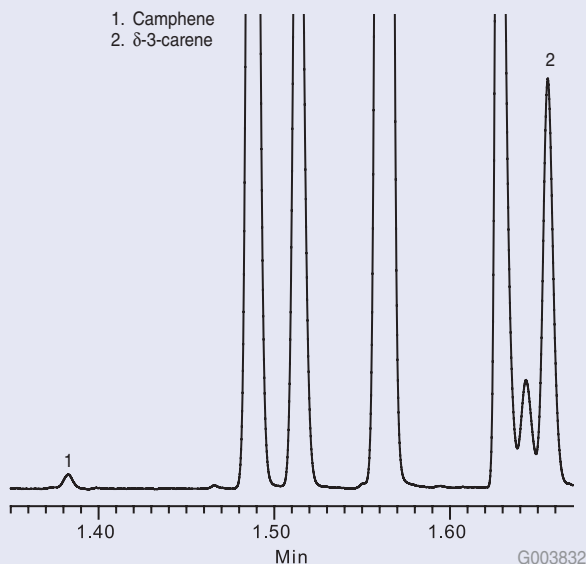
There are two unwanted results when expensive citrus oils are diluted. First, the food, beverage, cosmetic, or perfume manufacturer overpays for a raw material. Second, the end consumer receives an inferior product. Through the use of gas chromatography, chemists in these industries have the ability to verify the purity of the citrus essential oils being used in their products. The SLB-5ms column, available in Fast GC dimensions, proved to be a good choice for this application due to its ability to resolve the analytes of interest.

+ Featured Products

Description	Cat. No.
SLB-5ms, 10 m x 0.10 mm I.D., 0.10 μ m	28465-U

Figure 2. Fast GC Analysis of Pure Sweet Orange Essential Oil

column: SLB-5ms, 10 m x 0.10 mm ID, 0.10 μ m (28465-U)
 oven: 40 °C, 30 °C/min. to 85 °C, 80 °C/min. to 320 °C
 inj.: 320 °C
 det.: FID, 320 °C
 carrier gas: hydrogen, 70 cm/sec constant
 injection: 0.4 μ L in the split mode (300:1)
 sample: sweet orange essential oil in hexane



+ Related Products

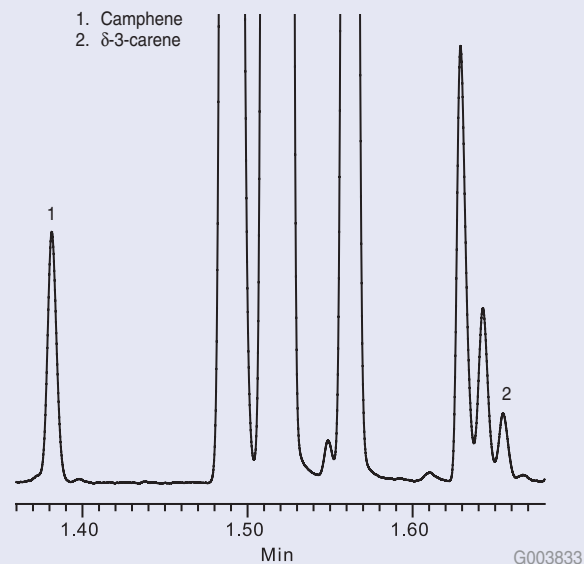
Description	Cat. No.
SLB-5ms Columns	
15 m x 0.10 mm I.D., 0.10 μ m	28466-U
20 m x 0.18 mm I.D., 0.18 μ m	28564-U
12 m x 0.18 mm I.D., 0.30 μ m	28566-U
30 m x 0.18 mm I.D., 0.30 μ m	28575-U
20 m x 0.18 mm I.D., 0.36 μ m	28576-U
30 m x 0.20 mm I.D., 0.20 μ m	28513-U
30 m x 0.25 mm I.D., 0.10 μ m	28467-U
15 m x 0.25 mm I.D., 0.25 μ m	28469-U
30 m x 0.25 mm I.D., 0.25 μ m	28471-U
60 m x 0.25 mm I.D., 0.25 μ m	28472-U
15 m x 0.25 mm I.D., 0.50 μ m	28577-U
30 m x 0.25 mm I.D., 0.50 μ m	28473-U
60 m x 0.25 mm I.D., 0.50 μ m	28474-U
30 m x 0.25 mm I.D., 1.0 μ m	28476-U
15 m x 0.32 mm I.D., 0.25 μ m	28557-U
30 m x 0.32 mm I.D., 0.25 μ m	28482-U
30 m x 0.32 mm I.D., 0.32 μ m	28532-U
15 m x 0.32 mm I.D., 0.50 μ m	28597-U
30 m x 0.32 mm I.D., 0.50 μ m	28484-U
30 m x 0.32 mm I.D., 1.0 μ m	28487-U
15 m x 0.53 mm I.D., 0.50 μ m	28542-U
30 m x 0.53 mm I.D., 0.50 μ m	28541-U
30 m x 0.53 mm I.D., 1.0 μ m	28559-U

! Related Information

For more information on Supelco Low Bleed SLB-5ms capillary columns, visit our website: sigma-aldrich.com/slb

Figure 3. Fast GC Analysis of Adulterated Lemon Essential Oil

column: SLB-5ms, 10 m x 0.10 mm ID, 0.10 μ m (28465-U)
 oven: 40 °C, 30 °C/min. to 85 °C, 80 °C/min. to 320 °C
 inj.: 320 °C
 det.: FID, 320 °C
 carrier gas: hydrogen, 70 cm/sec constant
 injection: 0.4 μ L in the split mode (300:1)
 sample: lemon essential oil + 5% sweet orange essential oil in hexane



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For more information on vials please request your copy of the **vials brochure (IXH)**.



Description	Cat. No.
Screw Top Glass Vials with 9 mm Thread, pk of 100	
Clear w/PTFE/red rubber septa	29056-U
Clear w/PTFE/silicone septa	29057-U
Clear w/PTFE/silicone/PTFE septa	29058-U
Clear w/graduation spot, PTFE/red rubber septa	29064-U
Clear w/graduation spot, PTFE/silicone septa	29065-U
Clear w/graduation spot, PTFE/silicone/PTFE septa	29066-U
Amber w/PTFE/red rubber septa	29061-U
Amber w/PTFE/silicone septa	29062-U
Amber w/PTFE/silicone/PTFE septa	29063-U
Amber w/graduation spot, PTFE/red rubber septa	29067-U
Amber w/graduation spot, PTFE/silicone septa	29068-U
Amber w/graduation spot, PTFE/silicone/PTFE septa	29069-U
Screw Top Glass Vials with Standard Opening (4.6 mm), pk of 100	
Clear w/PTFE/silicone septa	29104-U
Clear w/graduation spot, PTFE/silicone/PTFE septa	29107-U
Amber w/PTFE/silicone septa	29106-U
Amber w/graduation spot, PTFE/silicone/PTFE septa	29108-U
Screw Top Glass Vials with Large Opening (6.0 mm), pk of 100	
Clear w/PTFE/red rubber septa	29116-U
Clear w/PTFE/silicone septa	29118-U
Amber w/PTFE/red rubber septa	29117-U
Amber w/PTFE/silicone septa	29119-U
Crimp Top Glass Vials with Large Opening (6.0 mm), pk of 100	
Clear w/PTFE/red rubber septa	29124-U
Clear w/PTFE/silicone septa	29125-U
Clear w/PTFE/silicone/PTFE septa	29126-U
Amber w/PTFE/red rubber septa	29127-U
Amber w/PTFE/silicone septa	29128-U
Amber w/PTFE/silicone/PTFE septa	29129-U
Snap Seal Glass Vials with Large Opening (6.0 mm), pk of 100	
Clear w/PTFE/red rubber septa	29141-U
Clear w/PTFE/silicone septa	29142-U
Clear w/PTFE/silicone/PTFE septa	29143-U
Amber w/PTFE/red rubber septa	29144-U
Amber w/PTFE/silicone septa	29145-U
Amber w/PTFE/silicone/PTFE septa	29146-U

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GC

A Complete Solution for Forensic Analyses

Patrick Myers
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Forensic science encompasses a very broad range of analytes and analytical techniques. Sigma-Aldrich offers the standards and analytical tools required for quantitative analyses in this expansive field. Our offerings include standards for drugs of abuse testing, therapeutic drug monitoring, explosives determination, fire debris analysis, biogenic amine determination, and even snake venom standards.

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The Sigma-Aldrich website is your best resource for these standards. You can search the website for standards by compound name, CAS#, molecular formula, substructure or synonyms. Price, availability, chemical properties, functions, and safety data are all included for each product.

We also offer tools for sample collection, preparation, and analysis. Solid Phase Microextraction (SPME) is an increasingly popular method of extracting analytes directly from the sample matrix and concentrating them for analysis. Solid Phase Extraction (SPE) offers many advantages over more traditional liquid-liquid extraction. SPME fibers and SPE

tubes are available for the extraction of most analytes from most matrices. Supelco Technical Service can help you determine the correct fiber or tube for your application.

Drug Standards

Sigma-Aldrich offers over 2000 drug compounds available as neat and diluted standards. These include the true illicit drugs like heroine and cocaine, therapeutic drugs that are commonly abused like the benzodiazepines, active components of natural products, and drug metabolites. Most drugs of abuse and many therapeutic drugs are included.

Analyses of drugs of abuse are done by both capillary GC and HPLC. Most screening methods use capillary GC. As shown in Figure 1, Equity™-1701 columns provide a unique selectivity for this common analysis.

HPLC can be used for drug screening and when monitoring for metabolites of drugs. The Ascentis HPLC columns are ideal for these analyses. They are built on the purest silica available, have very high phase coverage and exhaustive endcapping. This all adds up to the ideal columns for the analysis of basic drugs. Figure 2 shows the difficult separation of methadone and its metabolite, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine on an Ascentis C8 column.

The United States Pharmacopeia (USP) oversees the analysis of therapeutic drugs. Most of the HPLC and capillary GC columns described in the USP monographs are available from Supelco. Many of the USP methods

Figure 1. Unique Selectivity of Drugs on Equity-1701

column: Equity-1701, 30 m x 0.25 mm I.D., 0.25 µm (28372-U)
oven: 45 °C (2 min.), 25 °C/min. to 110 °C, 15 °C/min. to 200 °C,
6 °C/min. to 280 °C (3 min.)

inj: 250 °C

MSD interface: 280 °C

scan range: 40-450 m/z

carrier gas: helium, 0.7 mL/min. constant

injection: 0.4 µL, pulsed (40 psi for 0.3 min.), splitless (0.5 min.)

liner: 2 mm I.D., splitless

sample: 15-component drug standard, each at 40 ppm in methanol

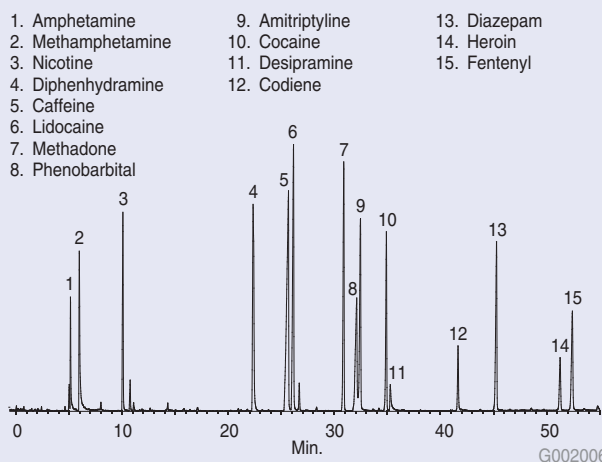


Figure 2. Separation of Methadone on Ascentis C8

column: Ascentis C8, 15 cm x 4.6 mm I.D., 5 µm particles (581424-U)

mobile phase: 50:50, 0.1% ammonium acetate

(pH 6.3 unadjusted), (cat. no. 34674):acetonitrile

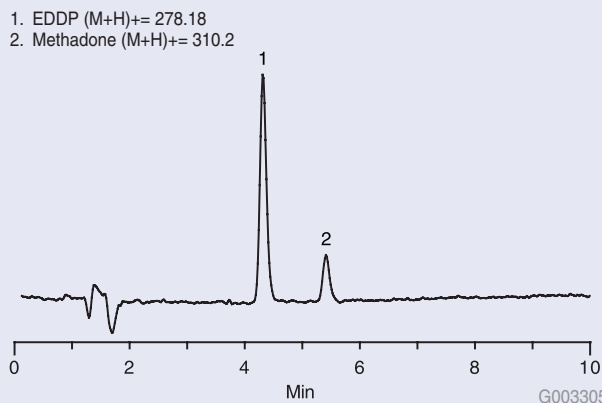
flow rate: 1.0 mL/min.

temp.: 35 °C

det.: UV at 254 nm

injection: 5 µL

sample: 10 µg/mL each in 50:50 water:methanol



require the use of packed GC columns. Supelco is the premier supplier of packed GC columns.

Explosives Standards

Energetic materials monitoring is a growing field. Cleanup of soils contaminated with explosives is a priority for both the US EPA and the US military. There are estimated to be several thousand sites comprising up to 25 million acres of potentially contaminated land. Soil

Figure 3. Analysis of Explosives in Soil Using SPME

sample/matrix: 12 energetic compounds, each at 50 ng/mL in water
 SPME fiber: 65 μ m polydimethylsiloxane/divinylbenzene (57310-U)
 extraction: immersion for 30 min.
 desorption temp.: 180 °C for 5 min.
 column: SPB-1701, 30 m x 0.25 mm I.D., 0.25 μ m (24113)
 oven: 95 °C (3 min.), 8 °C/min. to 182 °C (4 min.), 8 °C/min. to 250 °C (6 min.)
 det: ECD, 250 °C
 carrier gas: nitrogen, 60 mL/min.
 injection: split/splitless

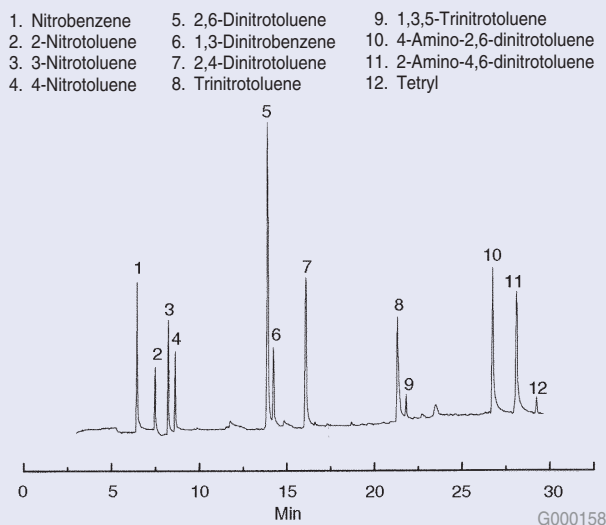


Figure courtesy of José Almirall, Crime Laboratory Bureau, Metro-Dade Police Department, Miami, Florida, USA; and Grace Bi and Kenneth Furton, Department of Chemistry, Florida International University, Miami, Florida, USA.

must be monitored before remediation to determine the extent of contamination and after remediation to clear it for replacement.

The entire range of compounds regulated in US EPA Method 8330 is available as single component standards, as a kit of single component standards, and as two multi-component mixes.

Explosives analysis represents an excellent opportunity for using SPME. This technique allows the solventless extraction of explosives from aqueous matrices whether solid or liquid. Figure 3 shows the GC analysis of 12 explosives extracted from an agitated soil/water slurry using SPME.

Standards for Fire Debris Analysis

Supelco offers multiple standards for fire debris analysis including several gasolines, fuel oils, military fuels, and kerosene. These can be supplemented with any of hundreds of solvent standards as required.

The current methods of extracting accelerant compounds from fire debris include several methods that concentrate the sample before analysis such as, solvent extraction, dynamic headspace concentration and static headspace concentration. These methods are cumbersome, time-consuming and require the use of hazardous solvents. The use of SPME to extract accelerant compounds from fire debris, as shown in Figure 4, is faster, simpler, more economical and more sensitive.

Figure 4. Accelerant Compounds Extracted from Fire Debris Using SPME

sample/matrix: 0.1 μ L gasoline in a headspace vial
 SPME fiber: 100 μ m polydimethylsiloxane (57300-U)
 extraction: headspace for 20 min.
 desorption temp.: 220 °C for 10 sec
 column: poly(dimethylsiloxane), 30 m x 0.25 mm I.D., 0.25 μ m
 oven: 35 °C (2 min.), 10 °C/min. to 220 °C (2 min.), 30 °C/min. to 300 °C (5 min.)
 det: FID, 300 °C
 carrier gas: helium, 1 mL/min.
 injection: splitless (3 min.), 50:1 split
 liner: 2 mm I.D.

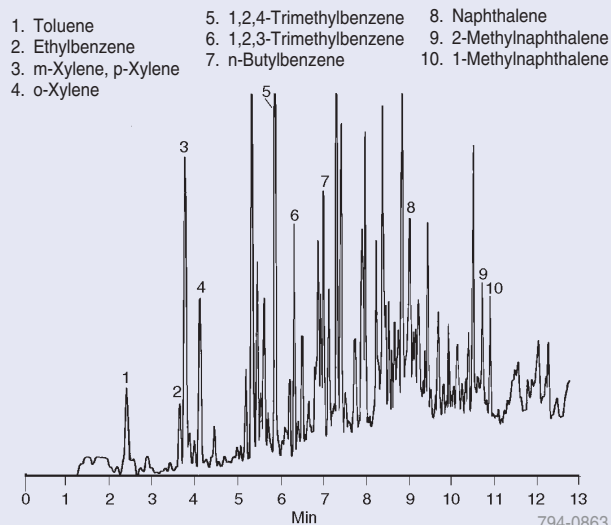


Figure courtesy of José Almirall, Crime Laboratory Bureau, Metro-Dade Police Department, Miami, Florida, USA; and Kenneth Furton and Juan Bruna, Department of Chemistry, Florida International University, Miami, Florida, USA.

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