

Reporter

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Assessing the Benefits of a “Hike in the Mountains” Using SPME

HPLC/LC

- Ascentis Express®:
Improved USP Method
for Lansoprazole 3
- Preparative HPLC:
Contract Services 5

Sample Handling

- Assessing the Benefits of
a “Hike in the Mountains”
Using SPME 8
- Extraction of Amphetamine
and Related Drugs using
SupelMIP™ SPE 11

GC

- Fast GC Analyses of
Volatiles 15

Standards and Reagents

- Trace analysis of
triazines, beta-agonists,
chloramphenicol and
clenbuterol 21

Breathing the mountain air of
the Paneveggio Natural Park in
the Dolomite region of Italy

Reporter

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Frank Michel, PhD
Sales Development Specialist
Analytical
Sigma-Aldrich Germany

Dear Colleague,

In research and routine analysis, SPME has for many years been and is still the established microextraction method. This was clearly demonstrated by the acceptance of the first SPME user meeting organised by Supelco/Sigma-Aldrich in Munich, Germany. More than eighty SPME users from Austria, Switzerland and Germany participated in the two-day meeting.

The meeting was intended to be a platform for SPME users to exchange tips and tricks. Due to this the majority of the presentations were presented by well-known guest speakers with a lot of practical experience in SPME. The main topics covered in the seminar were:

- Screening
- Quantification
- Troubleshooting
- Derivatisation
- Automation
- Recent developments

Besides a lot of valuable general information such as how to quantify correctly or how to apply SPME to polar compounds, many interesting and exciting application solutions were shown, e.g. automated SPME fibre exchange and horizontal sampling for permeation studies.

Additionally, several experts from Supelco delivered lectures on general SPME topics such as SPME troubleshooting, derivatisation in SPME and recent developments in SPME. The meeting was completed by an exhibition and the lectures from different instrument manufacturers offering devices for the automation of SPME.

The evening event, a relaxed dinner with a lot of conversation, was especially valued as an opportunity to discuss current applications, and to exchange ideas and know-how in this microextraction technique.

The first SPME user meeting was highly appreciated by the participants and was rated by many as one of the best seminars held by Supelco. It will be repeated in two years' time in 2009; we look forward to inviting you to an even better, second SPME user meeting.

In the meantime, stay informed about the seminars Supelco is offering in Europe this year: please visit our web page at: www.sigma-aldrich.com/events

Kind regards,

A handwritten signature in black ink that reads "F. Michel". The signature is written in a cursive, slightly slanted style.

Frank Michel, PhD
Sales Development Specialist Analytical
Sigma-Aldrich Germany

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Improving USP Monograph Methods using new Analytical Technologies

The Advantage of Ascentis Express® for the Current USP Method for Lansoprazole

Kai Li, Jiajie He, and Xiaoya Ding, PPD - 8551 Research Way, Suite 90, Middleton, WI 53562

Introduction

Compendial methods from the USP (United States Pharmacopeia) are widely used in pharmaceutical drug product and raw materials testing. However, not all methods in the USP use modern technologies. In chromatographic methods, it is not uncommon that older brands of columns are specified. Therefore, the USP methods are under continuous revision to improve existing procedures or to allow the user to obtain better results.

Due to the improved resolving power of Fused-Core™ particles, the method was optimised with a shorter runtime without sacrificing resolution.

In an effort to improve the compliance of drug product, drug substance, and excipient monographs with current scientific/regulatory standards, USP is seeking the submission of proposals for improved methods. The intention is to replace the current procedures that may be deficient, flawed, or unsafe (e.g. <http://www.usp.org/USPNF/submitMonograph/improveMon.html>). Requests for revision of an existing monograph are encouraged by USP in light of advances in analytical technologies. Furthermore, ease of operation, suitability for automation, and potential for high-throughput analysis can be considered in a revision. To develop the best possible analytical test method for its intended use, a fully integrated method development process such as the selection of column, mobile phase, detection technology, and LC hardware by utilising the most advanced technologies viable should be considered to ensure the methods are robust, consistent, and easy to use.

In this study, the USP method for lansoprazole was considered for improvement. Several drawbacks in the current USP monograph for lansoprazole prompted the investigation. These drawbacks include sample solution instability, use of different columns and samples preparations for the evaluation of assay and impurity, the requirement of using internal standard for assay and a long HPLC run-time (60 min). A new HPLC column, Ascentis Express C18, based on Fused-Core particle technology was investigated for this study. The Ascentis Express HPLC column claims high efficiencies as a result of a 0.5 µm layer of porous silica on a 1.7 µm solid silica core. An additional advantage to the column is that standard HPLC instrumentation can be used as opposed to UHPLC that is required for sub 2-µm columns.

Results and Discussion

Initially, a traditional 5 µm C18 column as specified in the USP monograph was compared to the 2.7 µm Ascentis Express C18, using the standard USP conditions for chromatographic purity for lansoprazole (1, 2). Improved resolution and sensitivity were obtained using the Fused-Core column that allowed us to make several significant improvements to the method. Due to the improved resolving power of Fused-Core particles, the method was optimised with a shorter run-time without sacrificing resolution. The total run time was reduced from 60 min to 40 min (Table 1). Moreover, the

improved sensitivity allowed for the reduction in concentration of the test sample for chromatographic purity from 250 µg/mL to 100 µg/mL, the level required for assay in the USP monograph. Therefore, simultaneous evaluation of assay and chromatographic purity is achieved. Finally, a change of diluent pH was implemented to improve sample solution stability, removing the requirement of injecting the sample within 10 minutes after preparation.

Table 1 Method Parameters for Improved Lansoprazole Method

Column:	Ascentis® Express C18, 15 cm x 4.6 mm, 2.7 µm (53829-U)		
Mobile phase A:	Water		
Mobile phase B:	Acetonitrile: 0.5% Triethylamine in Water, pH=7.0 [80:20]		
Flow rate:	0.8 mL/minute		
Column temp.:	Ambient		
Autosampler temp.:	5°C		
Injector volume:	15 µL		
Detector wavelength:	285 nm		
Run time:	40 min		
Gradient:	Time (Min)	%A	%B
	0.0	90	10
	30.0	20	80
	35.0	20	80
	35.1	90	10
	40.0	90	10

Figure 1 Linearity Curve from 50 to 150% Nominal Concentration

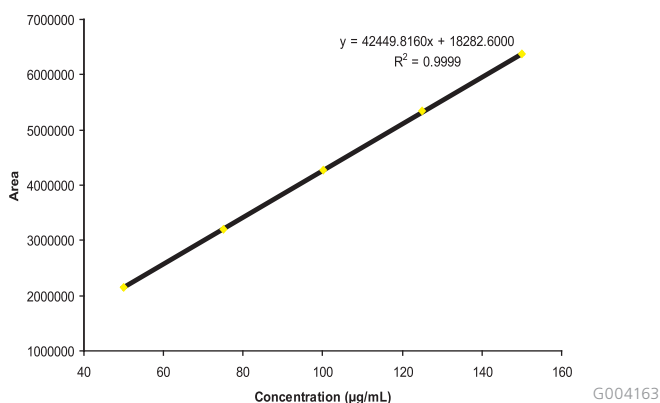


Figure 2 Linearity Curve from 0.05 to 2.0% Nominal Concentration

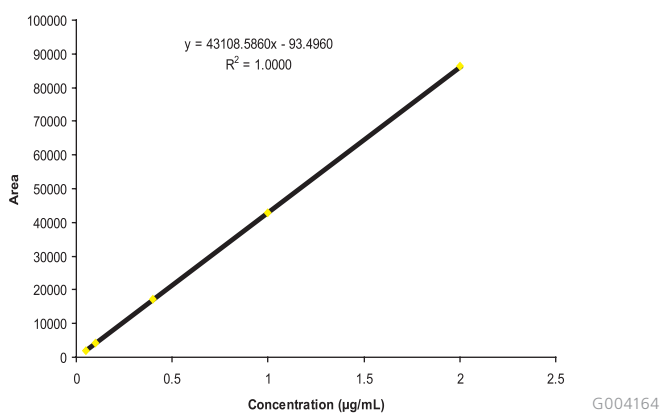
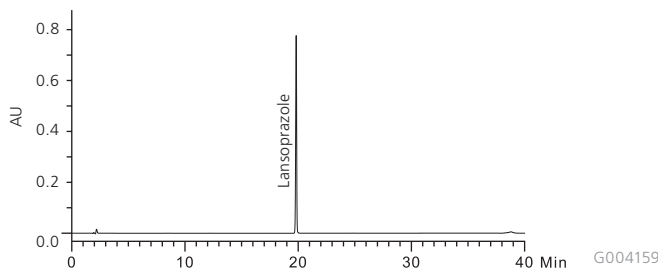
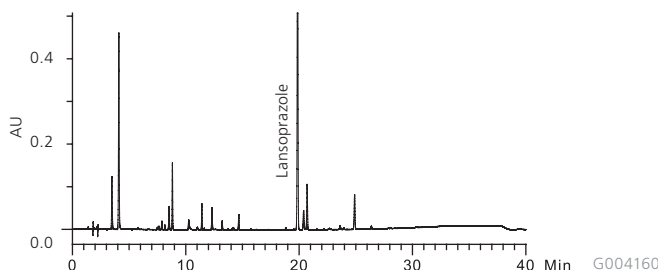


Figure 3 Analysis of Lansoprazole Using Improved Method with Ascentis Express C18 HPLC Column

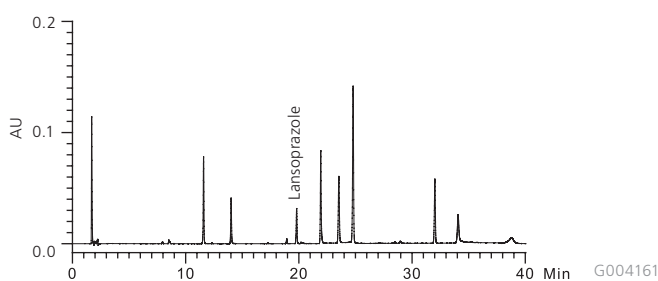
Unstressed Lansoprazole



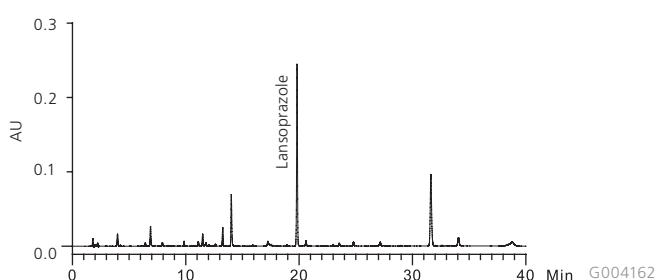
Aged Resolution Solution



Degraded Lansoprazole Exposed to 0.1 N HCl



Degraded Lansoprazole Exposed to 0.05% H2O2



The new method was shown to be linear from 0.05% to 150% of nominal concentration of 100 µg/mL, with quantitation limit less than 0.05%. The broad range of linearity allows for simultaneous impurity and assay analysis. The linearity data are shown in Figures 1 and 2. The RSD of five replicate injections of standard solution was 0.11%. In additional experiments, the method was evaluated by analysis of degraded lansoprazole drug substance. Lansoprazole was stressed under four separate conditions by exposure to acid, base, heat and hydrogen peroxide. The chromatograms of the acid and peroxide exposed drug substance along with the unstressed

drug substance are shown for reference. The resolving power of the Ascentis Express HPLC column makes it very suitable for these types of studies.

Conclusion

The method developed using Ascentis Express C18 column provided significant improvements in comparison with the original USP method in terms of resolution, run time and sensitivity. As a result, the consolidated single method can be used for both assay and impurity quantitation. The advantages of Ascentis Express columns as an alternative for sub-2 µm columns without using new UHPLC instruments could be appealing for pharmaceutical testing. Furthermore, this paper has presented one of the ways (a road map) that could be utilised by analytical scientists in the pharmaceutical field to improve USP monographs for their intended purposes using modern analytical technologies.

Featured Products

ID (mm)	Length (cm)	Ascentis Express C18	Ascentis Express C8
2.1	3	53802-U	53839-U
2.1	5	53822-U	53831-U
2.1	7.5	53804-U	53843-U
2.1	10	53823-U	53832-U
2.1	15	53825-U	53834-U
3.0	3	53805-U	53844-U
3.0	5	53811-U	53848-U
3.0	7.5	53812-U	53849-U
3.0	10	53814-U	53852-U
3.0	15	53816-U	53853-U
4.6	3	53818-U	53857-U
4.6	5	53826-U	53836-U
4.6	7.5	53819-U	53858-U
4.6	10	53827-U	53837-U
4.6	15	53829-U	53838-U

Related Information

For more information on Ascentis Express columns, request T407044 (JHD) or visit sigma-aldrich.com/express

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PPD is a leading global contract research organisation (CRO) providing discovery, development and post-approval services as well as compound partnering programmes for the pharmaceutical industry. The cGMP laboratory, located in Middleton, WI, provides analytical services for all phases of the drug development process and for all dosage forms.

References

- USP Monograph: Lansoprazole, USP 30-NF 25 through second supplement.
- Pharmacopeial Forum, In-Process Revision, Lansoprazole, Vol. 32(6), 1710–12 (2006).

Preparative HPLC – The Complete Service from Stationary Phase Selection to Contract Purification

Denise Wallworth denise.wallworth@sial.com

Introduction

Innovative design of stationary phases and an expanded service lab is now leading Supelco into new strengths in preparative HPLC. Supelco uses in-house technology and expertise to provide a complete service for prep HPLC – optimised stationary phases and columns, plus comprehensive contract services that include method development and purification. The Ascentis® range of phases were developed with robustness, selectivity choice and high capacity in mind, and the new addition from Astec of the Chirobiotic and Cyclobond ranges of chiral stationary phases extends capabilities further to enantiomeric separations. All of these are used in proven screening protocols to provide purification methodologies for those who need efficient and versatile outsourcing facilities.

Prep HPLC services

The service lab develops prep methods based on the best possible separation and compound solubility combined with the maximum possible loading and good recovery through method optimisation and loading studies. Predictable scale-up is one of the first requirements for successful scale-up. The Ascentis range of phases is based on very stable and diverse chemistries, identically manufactured in 3, 5 and 10 µm particle sizes to provide the natural progression from fast, highly efficient analytical separations through to high-capacity prep HPLC. Figure 1 shows an unchanging selectivity as the particle size increases, while figure 2 shows a typical predictable scale-up. Surface area variation is one of the main causes of batch-to-batch irreproducibility in bonding chemistry, and the Ascentis range of columns has the narrowest surface area specification available with, a surface area range of +/- 15m²/g.

Both chiral and achiral purification studies form part of the service facilities. Project quantities can be as little as a few mg, through to multi-gram. With simulated moving bed (SMB) and pilot and process scale elution prep LC within the Sigma-Aldrich Fine Chemicals (SAFC) group, large projects can be readily accommodated. All of these benefits can also be realised in your own lab through the Ascentis and Chirobiotic™ range of prep columns and media.

Ascentis Bonded Phases

Advanced end-capping techniques and a proprietary single-step bonding process mean that there are no unpredictable additional mechanisms that might interfere. In addition, this gives a high resistance to hydrolysis such that the columns in the Ascentis range can be used successfully between pH 2–8 routinely and pH 1.5–10 under certain conditions. This highly stable bonding chemistry makes the Ascentis perfect for mass-directed prep.

For all types of polar compounds, a solution is available. Ascentis RP Amide and Phenyl exhibit different selectivity than C18 or C8 for a wide range of polar molecules (especially polar aromatics for Ascentis Phenyl and phenols and acids for Ascentis RP-Amide); instead of using additives in the mobile phase, the selectivity component is built into the stationary phase. The RP-Amide shows improved peak

shape for bases and is more retentive than C18 for acids, while Ascentis Phenyl is often more retentive for nitro-substituted aromatics and many heterocyclic compounds. Both are highly stable in 100% aqueous mobile phases.

Table 1 Ascentis range of HPLC phases

Phase	%C	Surface Area (m ² /g)	End-capped?	Particle Sizes (µm)
Ascentis C18	25.5	450	Yes	3, 5 and 10
Ascentis C8	16	450	Yes	3, 5 and 10
Ascentis RP-Amide	19.5	450	Yes	3, 5 and 10
Ascentis Phenyl	19.5	450	Yes	3 and 5
Ascentis Silica	0	450		3, 5 and 10
Discovery F5	12.4	300	Yes	3, 5 and 10

Chiral prep HPLC

Traditionally run in normal phase solvents, chiral prep HPLC ideally needs the ability to be run in different solvent types in order to maximise solubility – often far more important than any other prep HPLC variable. The Supelco Chirobiotic range of columns and media for prep LC provide chiral selectivity in polar, normal or reversed phase mobile phases – and all can be run on the same column. Where solubility in hexane/heptane is poor, the Chirobiotic phases can offer an alternative often providing high selectivities in polar solvents such as methanol and acetonitrile. Such mobile phase systems, known as the polar ionic and organic modes, also typically provide short retention times, enabling high throughput for prep. These columns can be used in all prep HPLC techniques, including elution and recycle chromatography, mass-directed prep and simulated moving bed (SMB). In terms of loading capacity, a 250 x 21 mm column has medium to high loadings, from a few mg to over 300 mg per injection.

In a study requiring the purification of Thalidomide for the production of standards, an α value of 3.35 in 100% methanol and a retention in under 10 minutes was obtained on a Chirobiotic T column. Since this molecule is fairly insoluble in pure methanol, it was possible to change the purification method to a methanol/dioxane mixture enabling a 3.5-fold increase in solubility with only a slight reduction in selectivity (figure 3).

Table 2 Range of Chirobiotic chiral phases

Phase	Chiral ligand	Particle Sizes (µm)
CHIROBIOTIC V, V2	Vancomycin	5, 10 and 16
CHIROBIOTIC T, T2	Teicoplanin	5, 10 and 16
CHIROBIOTIC TAG	Teicoplanin Aglycone	5, 10 and 16
CHIROBIOTIC R	Ristocetin A	5, 10 and 16



Ascentis Express®

Scale-up from an analytical method that uses Ascentis Express (the new Fused-Core particle) can be readily accomplished on Ascentis materials: a high correlation between the capacity factors for a set of 180 compounds for the two phases confirms that selectivities and retention mechanisms are similar for these two phases. Conversion of an analytical method that takes advantage of the exceptional high speed and efficiency of the Ascentis Express can be made to take advantage of the high capacity and robustness of Ascentis prep media.

Conclusion

Comprehensive resources for prep HPLC are available to all from Supelco. The Ascentis and Chirobiotic ranges are the ideal choice for prep HPLC and are used extensively and successfully in Supelco's service labs. With ultra-stable bonding chemistry, a variety of different chemistries available for optimum selectivity and a range of different particle sizes that are manufactured by the same reliable and reproducible process, Ascentis can fulfil the demanding needs of prep HPLC.

Table 3 Key products for prep HPLC: Ascentis

ID (mm)	Length (cm)	Ascentis C18	Ascentis RP-Amide	Ascentis Phenyl	Ascentis Silica
5 µm	21.2	581344-U	565345-U	On request	On request
	25	581347-U	565348-U	581619-U	581515-U
10 µm	5	581356-U	565358-U	On request	On request
	10	581357-U	565359-U	On request	On request
	15	581358-U	565360-U	On request	On request
	25	581359-U	565361-U	On request	581517-U

Other column sizes and the Ascentis C8 range are available on request.

Table 4 Key products for prep HPLC: CHIROBIOTIC Chiral phases

ID (mm)	Length (cm)	CHIROBIOTIC V	CHIROBIOTIC V2	CHIROBIOTIC T	CHIROBIOTIC T2	CHIROBIOTIC TAG	CHIROBIOTIC R
5 µm	21.2	11044AST	15044AST	12044AST	16044AST	14054AST	13044AST
	30.0	11054AST	15054AST	12054AST	16054AST	14054AST	13054AST
10 µm	50.0	11150AST	15150AST	12150AST	16150AST	14150AST	13150AST

Details of other column sizes and bulk media in 10, 16 µm are available on request.

Figure 1 Effect of changing particle size on efficiency and selectivity for Ascentis

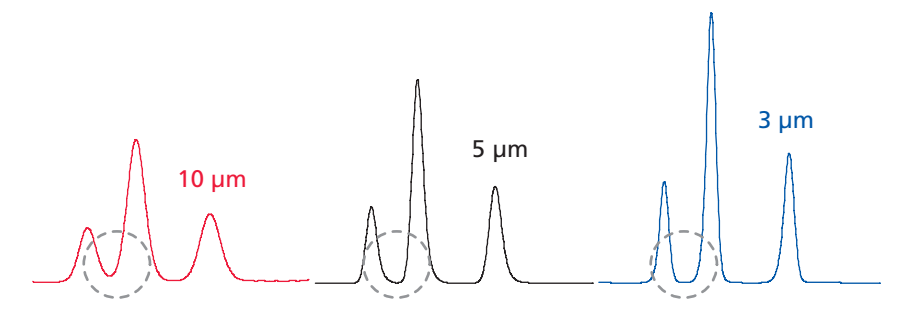
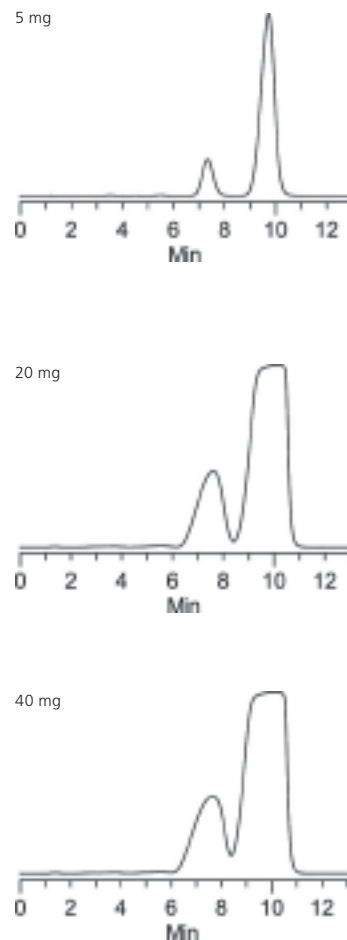
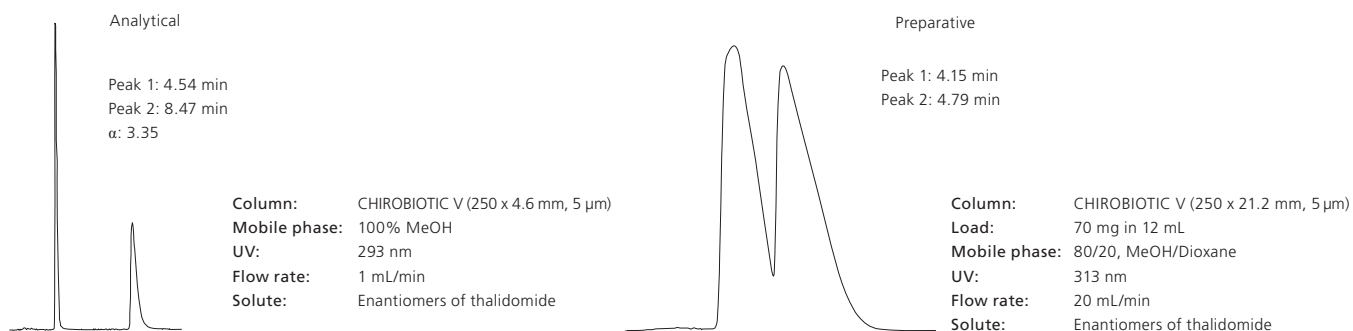


Figure 2 Typical loading study using Ascentis C18



Column: Ascentis C18, 10 micron, 15 x 21.2 mm
 Flow rate: 25 mL/min
 Solutes: Quinidine and Dihydroquinidine

Figure 3 Chiral prep example: purification of thalidomide standards



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Assessing the Benefits of a “Hike in the Mountains” Using SPME

Loris Tonidandel¹, Ettore Sartori² and Pietro Traldi¹ pietro.traldi@adr.pd.cnr.it

¹ CNR-ISTM Corso Stati Uniti, 4 35100 Padova (Italy)

² Parco Naturale Paneveggio Pale di San Martino, 38054 Tonadico (TN) Italy

Mountain hikers often claim to feel refreshed and renewed when climbing in mountains surrounded by coniferous trees. This may seem surprising, since from 200 to 1500 metres the density of air decreases from 1.22 to 1.08 Kg/m³, which corresponds to a decrease of oxygen of about 12%. In principle this would make it more difficult to breathe; however some people feel that hiking in a mountain forest leads to easier and more beneficial respiration. This phenomenon is further supported by past approaches in the cure of pulmonary disease, which recommended spending time in the mountains. This suggests the presence, in a forested mountain environment, of substances that aid in respiration.

In this study, most of the attention was focused on the detection of terpenoids and other secondary metabolites released from plants, indicated as biologically active compounds. The project was based on the determination of a map related to the plant metabolites present in the atmosphere of the Paneveggio Natural Park in the Dolomite region (north-east region of Italy) in order to obtain an evaluation of the quality of the air present in the park (Figure 1).

The study was based on the sampling of biological volatile organic compounds (BVOCs) by portable SPME field samplers placed in different points of the park. Table 1 shows the atmospheric conditions and the predominant plants in the different sampling regions. An SPME fibre with a Carboxen™/Polydimethylsiloxane (75 µm) stationary phase was used to capture both non-polar and medium polarity volatile metabolites released from these plants (Figure 2). Considering the low concentration of BVOCs (at ppb level), fibre exposure was maintained for five hours. To prevent sample degradation and loss of the trapped compounds, each SPME fibre was stored at 0°C and kept at the same temperature until sample analysis (about 24 hours later).

Figure 3 shows a series of peaks due to the volatile terpenic species, while aldehydes and phenols are present in lower quantity. In zone 1, Norwegian Spruce (*Picea Abies*) prevails, together with silver firs (*Abies Alba*) and beech trees (*Fagus sylvatica*). The results

Table 1 Environmental Parameters of the Sampling Zones

ZONE	CONDITIONS	PREVALENT PLANTS
1	Temperature: 21°C Altitude: 1050 m Wind: absent	Norwegian Spruce, Silver Fir, European Beech
2	Temperature: 22°C Altitude: 1250 m Wind: absent	Broadleaf Plant, European Beech, Norwegian Spruce
3	Temperature: 22°C Altitude: 1600 m Wind: gentle breeze	Mugo Pine
4	Temperature: 22°C Altitude: 1520 m Wind: absent	Larch
5	Temperature: 22°C Altitude: 1200 m Wind: absent	Grass surrounded of European Beech and Norwegian Spruce

obtained are in agreement with volatile metabolites typical of these kinds of plants. Zone 2 has a larger distribution of broad-leaved plants, which leads to slightly different results: some monoterpenic species (β -pinene and limonene) are present at lower concentrations, while there is a clear increase of C₁₂-C₁₃ hydrocarbons. The results obtained in the five different zones are compared in Table 2.

The experimental data obtained allow us to make the following observations:

1. Atmospheric sampling by SPME followed by GC-MS is highly effective for the qualitative determination of the BVOCs released from plants and present in a forest environment.
2. The semi-qualitative approach adopted in these preliminary studies allows us to establish the relative concentration of different BVOCs for each zone.

Figure 1 Location of the Paneveggio Natural Park and Sampling Zones

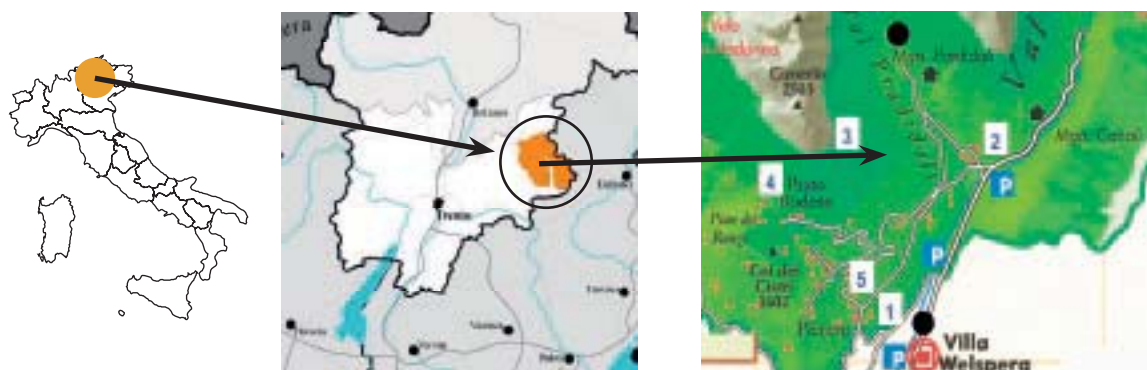


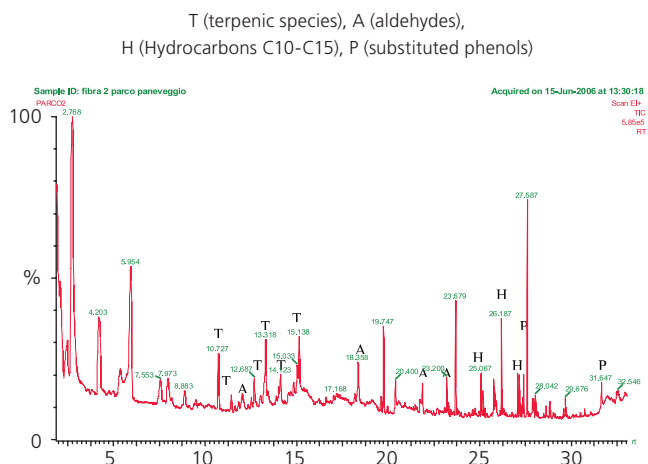
Figure 2 An SPME Fibre with Carboxen/Polydimethylsiloxane (75 µm) Stationary Phase Captures Both Non-Polar and Medium Polarity Volatile Metabolites Released from Plants



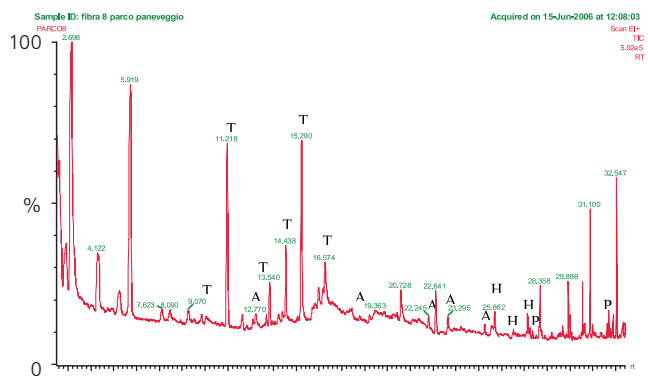
The results summarized in Table 2 (page 10) show that the BVOC content in air exhibits clear differences, depending on altitude and surrounding vegetation.

From a therapeutic point of view, terpenoids represent an interesting class of BVOCs, due to their anti-inflammatory and antimicrobial activity for man and in general for animals. For more than a century, essential oils (e.g. trementine, pine extract) have been employed in the treatment of respiratory ailments. Trementine of larch, pine essences, and trementine medicinal essences are the most common and are described in international pharmacopoeia. The therapeutic effect is mainly due to the most volatile constituents of oil and is related to their antioxidant activity. Considering that pulmonary and bronchial diseases and some skin infections are associated with the formation of the reactive oxygenated species (ROS), the antioxidant action of terpenes can reduce as well as prevent inflammation. Recent studies have demonstrated that terpenes, through their antioxidant activity, are also active in the prevention of arteriosclerosis, stroke and heart attack. They inhibit low-density lipoprotein (LDL) oxidation, limiting the formation of products, which represent one of the causes of the above-cited pathologies. These volatile constituents of essential oil are present, in a forested mountain environment, and the concentration in a forest can reach in excess of 10 ppb.

Figure 3 A) Chromatogram of zone 1



B) Chromatogram of zone 4



IT is therefore possible to recognise how healthy and therapeutic the atmosphere in a forest mainly covered by conifers is (like the Paneveggio Natural Park). It is necessary to emphasise that the quality of the air we breathe is not only related to the absence of toxic pollutants in the atmosphere, but also to the presence of volatile plant metabolism products, whose biological properties have a beneficial effect.

(continued on page 10)

Table 2 The Most Abundant VOCs Identified and Their Relative Abundance

No.	R.T. (min)	Compounds	Relative Abundance				
			Zone 1	Zone 2	Zone 3	Zone 4	Zone 5
1	2.488	Benzene	+	+	-	-	-
2	4.203	Toluene	++	+	+	+	+
3	7.553	o-Xylene	+	+	+	+	+
4	7.973	m,p-Xylene	+	+	+	+	+
5	8.883	Benzene substituted	+	+	+	+	+
6	10.725	α -Pinene	++	++	++	+++	++
7	11.427	Camphene	+	+	+	+	+
8	12.034	Benzaldehydes	+	+	+	+	+
9	12.687	β -Pinene	++	+	+	++	+
10	13.434	Cumene	+	+	+	+	+
11	14.123	3-Carene	+	+	+++	+++	+
12	14.834	Compound not identified	+	+	++	+	+
13	15.033	Limonene	++	+	++	+	+
14	15.138	1,8-Cineole	++	++	+	+	+
15	18.358	Nonanal	++	+	+	+	++
16	20.400	Bicycle 3,10 hexan-2one	+	+	+	+	+
17	21.870	Aldehydes C ₁₀ -C ₁₂	+	+	+	+	+++
18	23.200	Aldehydes ramified C ₁₀ -C ₁₃	+	+	+	+	+
19	25.067	Hydrocarbons C ₁₃ -C ₁₂	+	++	++	-	+
20	25.184	Tetradecanal	+	-	+	+	+
21	26.187	Pentadecane	++	++	++	+	+
22	27.074	Pentane-1,3-dioldiisobutirrate	+	+	+	-	++
23	27.156	Hexadecane substituted	+	++	+	+	+
24	27.401	Phenol substituted A	+	+	+	+	++
25	27.587	Compound not identified	+++	+++	+++	+	++
26	29.676	Esters > C6	+	+++	-	+	+
27	31.647	Phenol substituted B	+	+	+	-	+

Featured Products

Film Thickness (μm)	Phase	Qty.	Cat. No.
SPME Portable Field Sampler			
100	PDMS	2 pk	504823
75	Carboxen/PDMS	2 pk	504831
65	PDMS/DVB	2 pk	57359-U

Related Information

For more information on this study, please email the author at: pietro.traldi@adr.pd.cnr.it or download the complete article at sigma-aldrich.com/spme

TRADEMARKS: Ascentis, Carboxen, Equity, HYDRANAL, Omegawax, radiello, SLB, SP, SPB, Supelco, SUPELCOWAX, SupelMIP™, VOCARB, VOCOL – **Sigma-Aldrich Biotechnology LP;** Fused-Core – **Advanced Materials Technology;** SPME – Technology licensed exclusively to Supelco. US patent #5,691,206, European patent #523,092.

The Extraction of Amphetamine and Related Drugs using SupelMIP™ Molecularly Imprinted Polymer SPE

Christine Widstrand, Staffan Bergström, Anna-Karin Wihlborg¹ and An Trinh² an.trinh@sial.com

¹ MIP Technologies AB, Scheelevägen 22, 220 07, Lund, Sweden

² Supelco, 595 North Harrison Road, Bellefonte, PA, 16823, USA

Introduction

Amphetamine and amphetamine-related drugs consist of a class of stimulants and hallucinogens used by some students, athletes, and recreation drug users. Over the last 15 years, abuse of these drugs has become a global problem. In 2004, there were over 17,000 methamphetamine lab seizures in the US; as a result, The Combat Methamphetamine Act of 2005 became law in 2006 to regulate over-the-counter sales of ephedrine, pseudoephedrine, and phenylpropanolamine products, which are precursors used for the illicit manufacture of amphetamine and methamphetamine (1). In a 2005 report conducted by the European Monitoring Centre for Drugs and Drug Addiction, use of amphetamine and Ecstasy has been growing throughout Europe (2). In recent years, a variety of screening/detection techniques have been commercially available such as ELISA and other immuno-based colorimetric and point of collection (POC) kits/devices. Although such assays are fast and efficient, many are typically not class-selective and will only detect some amphetamines and not others (3). Some immunoassays are not selective enough, and recognise such a wide range of sympathomimetic amines that the positive predictive value (PPV) can range from 0–90% (4). In either case, an alternative and highly selective class-specific confirmation assay is required to verify immuno-based screening results.

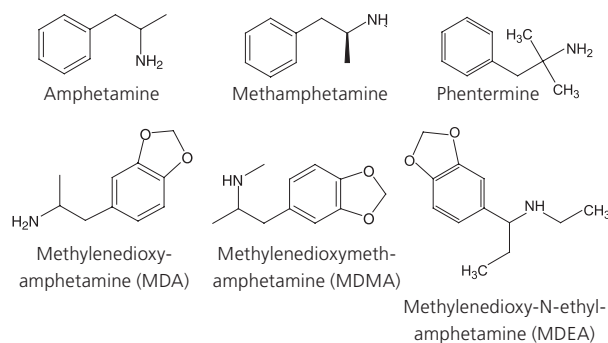
In this report we describe a simple, fast and class-selective method for trace extraction of amphetamine-related drugs from urine samples, using a molecularly imprinted polymer SPE (SupelMIP™) specifically designed for selective extraction of amphetamine-related drugs (Figure 1). The SupelMIP technique is also compared with a recently published extraction technique using a conventional hydrophilic polymer SPE phase (5).

Molecularly Imprinted Polymers

Molecularly imprinted polymers (MIPs) are a class of highly cross-linked polymer-based molecular recognition elements engineered to bind one target compound or a class of structurally related compounds with high selectivity. Selectivity is introduced during MIP synthesis in which a template molecule, designed to mimic the analyte, guides the formation of specific cavities or imprints that are sterically and chemically complementary to the target analyte(s). As a result, multiple interactions (e.g. hydrogen bonding, ionic, Van der Waals, hydrophobic) can take place between the MIP cavity and analyte functional groups. The strong retention offered between an MIP phase and its target analyte(s) allows for the use of exhaustive wash procedures during solid phase extraction that results in superior sample cleanup prior to analysis.

This leads to cleaner extracts, lower detection limits and a more efficient sample cleanup process. An illustration of the selective cavity is shown in Figure 2.

Figure 1 Chemical Structures of Amphetamines and Related Drugs Investigated



G003864, G003870, G004176, G003873, G003874, G004177

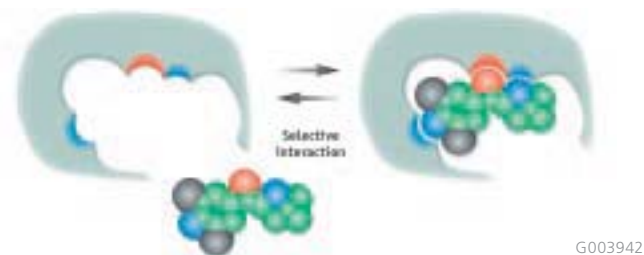
Extraction and Analysis of Amphetamine and Amphetamine Related Drugs

Amphetamine and the related drugs methamphetamine, phentermine, MDA, MDMA and MDEA were extracted from urine using both the SupelMIP SPE and a conventional hydrophilic polymer SPE via the procedures described in Table 1. Extracts were further analysed via LC-MS-MS (Table 2).

Lower Limits of Quantitation Achieved using SupelMIP SPE

The amphetamine drugs listed in Figure 1 were spiked into urine at the levels of 15 and 50 ng/mL, and extracted/analysed using the procedures described in Tables 1 and 2. The lower limit of quantitation (LLOQ) for each analyte was estimated for both the SupelMIP SPE and conventional hydrophilic SPE methods (signal to noise ratio of 10:1) and summarised in Table 3. Using the SupelMIP method, LLOQs in the range of 2.5 – 43.0 pg/mL (an order of magnitude lower than the conventional hydrophilic polymer SPE method) were achieved. To further illustrate the high selectivity achieved using the SupelMIP SPE

Figure 2 Illustration of a Selective MIP Cavity



G003942

(continued on page 12)



Table 1 The Most Abundant VOCs Identified and Their Relative Abundances**SupelMIP SPE – Amphetamines Method****Sample Pre-Treatment**

Human urine samples were spiked with internal standard (methamphetamine-d8 and MDMA-d5) and diluted 1:1 (v/v) with 10 mM ammonium acetate buffer, pH 8. Adjust to pH 7.5-8.5 using NH₃ or HAc

SPE Procedure

SupelMIP SPE – Amphetamines, 25 mg/3 mL (53228-U)

1. Condition and equilibrate MIP phase with 1 mL methanol, and 1 mL 10 mM ammonium acetate buffer, pH 8.
2. Load 1 mL pre-treated sample on to the cartridge.
3. Wash (elute interferences) using the following wash scheme:
 - 2 x 1 mL DI water (Do not let column dry!)
 - 1 mL 60/40 MeCN/DI water followed by 5–10 minute vacuum (-1 bar, -20 inHg, or -70 kPa) to dry the column
 - 1 mL 1% HAc in MeCN
4. Elute the amphetamine drugs with 2 x 1 mL 1% formic acid in methanol. Apply -0.4 bar (-12 in Hg) between each fraction.
5. Evaporate under nitrogen to dryness and reconstitute with 150 µL LC mobile phase (90% A and 10% B) prior to LC-MS-MS analysis.

Published Amphetamine Method Using Conventional Hydrophilic Polymer SPE Phase (5)**Sample Pre-Treatment**

Human urine samples were spiked with internal standard. Spiked and blank urine acidified with 100 µL 5 M HCl per 10 mL urine.

SPE Procedure

Conventional Hydrophilic Polymer SPE Phase, 30 mg/1 mL

1. Condition and equilibrate SPE phase with 1 mL methanol and 1 mL DI water.
2. Load 1 mL pre-treated sample on to the cartridge.
3. Wash (elute interferences) with 1 mL 5% methanol containing 2% ammonium hydroxide and with 1 mL 20% methanol containing 2% ammonium hydroxide.
4. Elute the amphetamine drugs with 0.5 mL 20% methanol with 2% acetic acid.
5. Evaporate under nitrogen and reconstitute with 150 µL LC mobile phase prior to LC-MS-MS analysis.

extraction method, urine was spiked with 15 µg/mL amphetamine and extracted/analysed as above. An amphetamine peak was easily detected using the SupelMIP SPE extraction method. In contrast, no amphetamine response was observed using the conventional hydrophilic polymer SPE method (Figure 3).

Improved Recovery & Reduced Ion-Suppression Achieved using SupelMIP SPE

Urine samples were spiked with the amphetamine drugs listed in Figure 1 at the levels of 0.010, 0.015, 0.50, 1.0, and 5.0 ng/mL and extracted/analysed using the methods described in Tables 1 and Table 2. Relative recovery for each spike concentration was determined against deuterated internal standards. Greater than 80% relative recovery was achieved for all the spike levels tested (with the exception of MDA) using the SupelMIP SPE method. In contrast, 0% recovery was observed for all the amphetamine drugs at spike levels 0.010 and 0.015 ng/mL, and at spike levels 0.5, 1.0 and 5.0 ng/mL; recoveries above 80% were only observed for methamphetamine and MDMA using the conventional hydrophilic polymer method (data not shown).

Blank urine samples were cleaned up according to both SPE procedures. The SPE extracts were spiked with a mixture of the amphetamine drugs post extraction. Standards for the calibration curve were prepared in the reconstitution solvent.

Table 2 LC-MS-MS Conditions

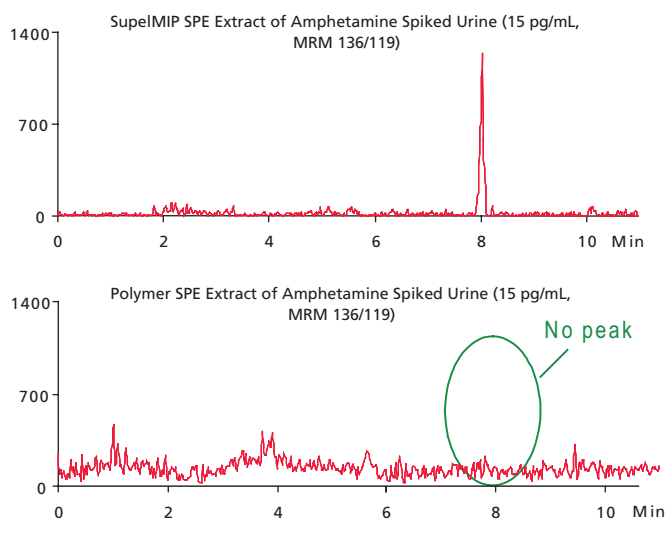
Column:	Ascentis C18, 15 cm x 2.1 mm, 5 µm particles, (581304-U)		
Instrument:	Shimadzu LC-20/Applied Biosystems/MDS SCIEX API3200		
Mobile phase:	Solvent A – DI Water + 0.05% TFA Solvent B – Acetonitrile + 0.05% TFA		
Gradient:	Initial: 90% A – 10% B 7 min 70% A – 30% B 10–11 min 10% A – 90% B 11.2 min 90% A – 10% B		
Temp.:	Ambient		
Flow rate:	0.2 mL/min		
Ion mode:	Positive		
Ion source:	TurboSpray		
Ion spray voltage:	5500 V		
Source temp.:	600°C		
Collision gas:	6 psi		
Inj.:	20 µL		
Det.:	MS-MS		
MRM transition and retention times:	Compound	Rt (min)	Q1/Q3
	Amphetamine	7.8	136/119 and 136/91
	Methamphetamine	8.3	150/119 and 150/91
	Methamphetamine D8	8.3	158/124 and 158/93
	Phentermine	8.7	150/133 and 150/91
	MDA	8.0	180/163 and 180/105
	MDMA	8.5	194/163 and 194/105
	MDMA D5	8.5	199/165 and 199/136
	MDEA	9.2	208/163 and 108/105

Table 3 LLOQ for SupelMIP SPE vs. Conventional Hydrophilic Polymer SPE

	SupelMIP (pg/mL)	Conventional Hydrophilic Polymer SPE (pg/mL)
Methamphetamine	6.6	52
Amphetamine	7.3	138
Phentermine	1.5	141
MDA	4.3	261
MDMA	3.0	56
MDEA	2.5	52

Table 4 % Ion Suppression of SupelMIP SPE vs. Hydrophilic Polymer SPE

ng/mL	SupelMIP		Conventional Hydrophilic Polymer SPE	
	10	100	10	100
Methamphetamine	0%	3%	31%	12%
Amphetamine	5%	10%	33%	28%
Phentermine	3%	2%	36%	22%
MDA	30%	23%	51%	48%
MDMA	5%	13%	45%	36%
MDEA	10%	4%	65%	52%

Figure 3 Amphetamine Spiked Urine Samples (15 pg/mL) cleaned up with SupelMIP SPE vs. Conventional Hydrophilic Polymer SPE

The standard calibration curves were compared to the matrix-matched samples for both methods, without using internal standard corrections. Table 4 summarises the percentage of ion suppression at 10 and 100 ng/mL for the SupelMIP SPE and the hydrophilic polymer SPE method. From the table, ion suppression is significantly less for the SupelMIP SPE.

Conclusion

In this report, we demonstrated the utility of a molecularly imprinted polymer SPE (SupelMIP) method designed for the class-selective extraction of amphetamine and related drugs from urine. When compared to a recently published conventional hydrophilic polymer SPE method, the SupelMIP method offers reduced ion-suppression and achieved lower LOQs/LODs by an order of magnitude. The highly selective SupelMIP method also offered increased recovery and reproducibility for better sensitivity, precision and accuracy.

References

- 1] The Combat Meth Act of 2005. Available at: http://www.deadiversion.usdoj.gov/meth/q_a.htm
- 2] European Monitoring Centre for Drugs and Drug Addiction, Annual Report 2005. Available at: <http://www.emcdda.eu.int/>
- 3] Walsh, Forensic Science International, 2007. In Press.
- 4] Woodworth et al., Clinical Chemistry 52, No. 4, 2006.
- 5] M.-R. Fuh, T.-Y. Wu and T.-Y. Lin, Talanta, 2006, 68:987-991.

Introductory offer

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Related Information

For more information on the extraction of amphetamines, request the electronic format of T407148 (KDN) on the attached postcard. Please provide an email address.

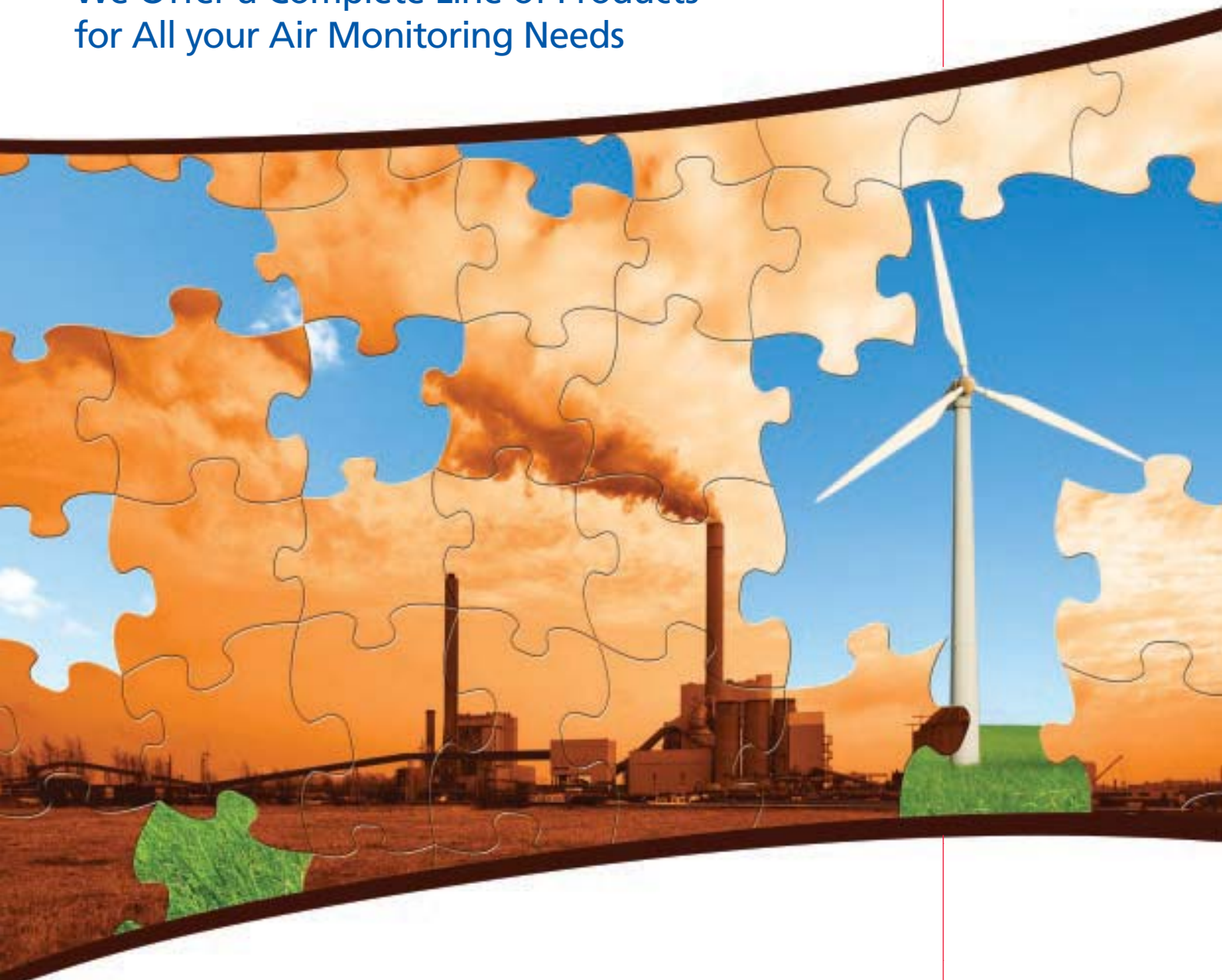
For general information on SPE and SupelMIP SPE, please visit: sigma-aldrich.com/spe and sigma-aldrich.com/supelmip respectively.

Please also see the article on p.21 in this Reporter edition for more SupelMIP products and standards.

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Fast GC Analyses of Volatiles

Michael D. Buchanan mike.buchanan@sial.com

Introduction

The primary aim of Fast GC is to maintain (compared to conventional GC) sufficient resolving power in a shorter time. Basically, Fast GC is accomplished by using a short column (reduces analysis time) with a narrow I.D. (offsets the loss of efficiency of the shorter column) while manipulating specific operating parameters, such as linear velocity and oven temperature ramp rates (1). The use of Fast GC has previously been demonstrated for several applications (2–7). In this space, Fast GC will be demonstrated for the analysis of volatiles using purge and trap (P&T).

Options for Decreasing Analysis Time for P&T Methods

The overwhelming number of samples that must be analysed for the presence of volatiles is due to the ease with which many of these compounds are able to migrate through the environment, because of their water-soluble nature. Many regulatory agencies require constant monitoring for volatiles, resulting in heavy sample loads with short turnaround times. Therefore, laboratories are constantly looking for ways to reduce analysis times. Several options currently exist for decreasing analysis time.

1. Use two P&Ts for each GC. Synchronise so while the GC is analysing the sample from one P&T, that P&T is in bake mode and the other P&T is purging the next sample. When the GC is ready, a sample is also ready for desorption so that the GC is never idle.
2. Use a P&T model that employs super-high flow rates (i.e. 400 mL/min.) during the bake mode so that it is ready to purge the next sample sooner.
3. Convert the existing conventional GC method to a Fast GC method. Note that this option can be used with the current equipment found in most laboratories, or in combination with either (or both) option(s) listed above.

Converting Conventional GC to Fast GC for Waste Water Volatiles

Converting a conventional GC method to a Fast GC method is not as simple as just changing to a smaller I.D. column. Column dimensions, linear velocity, and oven temperature ramp rates must be optimised together. Changing only one parameter may decrease analysis time (desirable), but will likely cause a loss of resolution (undesirable). It is also critical to account for the reduced sample capacity of the smaller I.D. column.

A method for the analysis of volatiles from waste water samples (US EPA Method 624, commonly performed in the United States) on the SPB™-624 column was selected to illustrate the change from conventional GC to Fast GC. The optimised chromatogram obtained using conventional GC is shown in Figure 1. The analysis time is <18 minutes, peak shapes are good, and the mass spectrometer (MS) is able to mass resolve all analytes.

The column dimensions were changed to Fast GC dimensions, and then linear velocity and oven temperature ramp rates were optimised to produce the chromatogram shown in Figure 2 (parameters which were changed are highlighted). While analysis time is reduced to <10 minutes and the MS is still able to mass resolve all analytes, peak shape overall is not good. Why the poor chromatography? The reduced sample capacity of the smaller I.D. column was not accounted for.

The primary aim of Fast GC is to maintain (compared to conventional GC) sufficient resolving power in a shorter time

The capacity of the 0.18 mm I.D. column is significantly less than the 0.25 mm I.D. column, leading to sample overload. To alleviate this problem, the mass of sample reaching the column must be reduced. Diluting the sample, decreasing the injection volume, or increasing the split ratio are options to achieve this. The first two options are not as compatible with P&T methods as the third. Therefore, the split ratio was increased from 30:1 to 100:1, and then the linear velocity was optimised to achieve the chromatogram shown in Figure 3 (parameters which were changed are highlighted). Now, the short analysis time (<10 minutes) and mass resolution of all analytes is accompanied with improved peak shape of all analytes. This represents a vast improvement in analysis time (from over 17 minutes to under 10 minutes) compared to the conventional GC chromatogram in Figure 1.

Did you know?

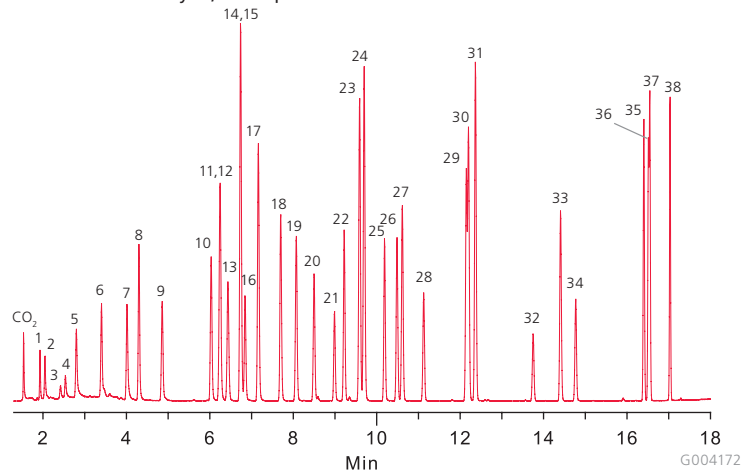
Supelco currently offers a total of **nineteen columns in Fast GC dimensions**, covering twelve popular phases (SPB-624, VOCOL™, SLB™-5ms, Equity®-1701, TCEP, SP™-2560, Omega-wax™ 100, SUPELCOWAX™ 10, Equity-1, SPB-1, Equity-5, and SPB-5). If increasing sample throughput is your goal, consider a change to a Supelco Fast GC column.

(continued on page 18)



Figure 1 Waste Water Volatiles on the SPB-624

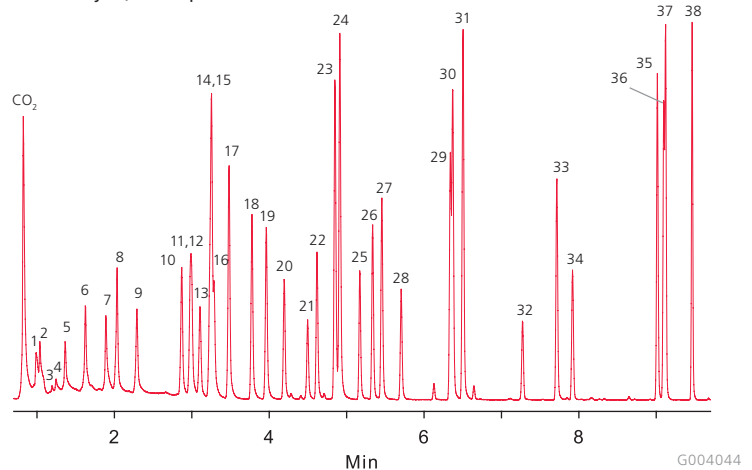
Sample/matrix:	each analyte at 50 ppb in 5 mL water
Purge trap:	VOCARB® 3000 "K" (24940-U)
Purge:	40 mL/min. at 25°C for 11 min.
Dry purge:	2 min.
Desorption pre-heat:	205°C
Desorption temp.:	210°C for 2 min.
Desorption flow:	40 mL/min.
Bake.:	260°C for 10 min.
Transfer line/ valve temp.:	110°C
Column:	SPB-624, 30 m x 0.25 mm I.D., 1.4 µm (24255)
Oven:	40°C (2 min.), 7°C/min. to 135°C, 30°C/min. to 230°C (3 min.)
Inj.:	150°C
MSD interface:	200°C
Scan range:	m/z = 35-400
Carrier gas:	helium, 1.1 mL/min.
Injection:	30:1 split
Liner:	0.75 mm I.D. SPME

Conventional GC Analysis, 30:1 Split

G004172

Figure 2 Waste Water Volatiles on the SPB-624

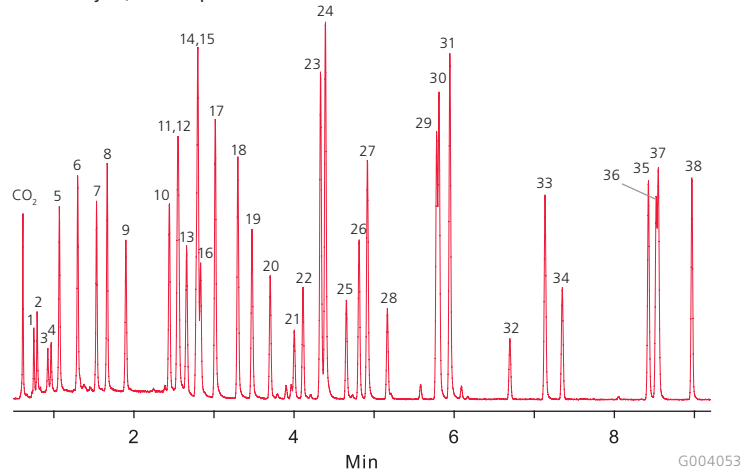
Sample/matrix:	each analyte at 50 ppb in 5 mL water
Purge trap:	VOCARB® 3000 "K" (24940-U)
Purge:	40 mL/min. at 25°C for 11 min.
Dry purge:	2 min.
Desorption pre-heat:	205°C
Desorption temp.:	210°C for 2 min.
Desorption flow:	40 mL/min.
Bake.:	260°C for 10 min.
Transfer line/ valve temp.:	110°C
Column:	SPB-624, 20 m x 0.18 mm I.D., 1.0 µm (28662-U)
Oven:	40°C (1 min.), 11°C/min. to 125°C, 35°C/min. to 230°C (2 min.)
Inj.:	150°C
MSD interface:	200°C
Scan range:	m/z = 35-400
Carrier gas:	helium, 1.2 mL/min.
Injection:	30:1 split
Liner:	0.75 mm I.D. SPME

Fast GC Analysis, 30:1 Split

G004044

Figure 3 Waste Water Volatiles on the SPB-624

Sample/matrix:	each analyte at 50 ppb in 5 mL water
Purge trap:	VOCARB 3000 "K" (24940-U)
Purge:	40 mL/min. at 25°C for 11 min.
Dry purge:	2 min.
Desorption pre-heat:	205°C
Desorption temp.:	210°C for 2 min.
Desorption flow:	150 mL/min.
Bake.:	260°C for 10 min.
Transfer line/ valve temp.:	110°C
Column:	SPB-624, 20 m x 0.18 mm I.D., 1.0 µm (28662-U)
Oven:	40°C (1 min.), 11°C/min. to 125°C, 35°C/min. to 230°C (2 min.)
Inj.:	150°C
MSD interface:	200°C
Scan range:	m/z = 35-400
Carrier gas:	helium, 1.5 mL/min.
Injection:	100:1 split
Liner:	0.75 mm I.D. SPME

Fast GC Analysis, 100:1 Split

G004053

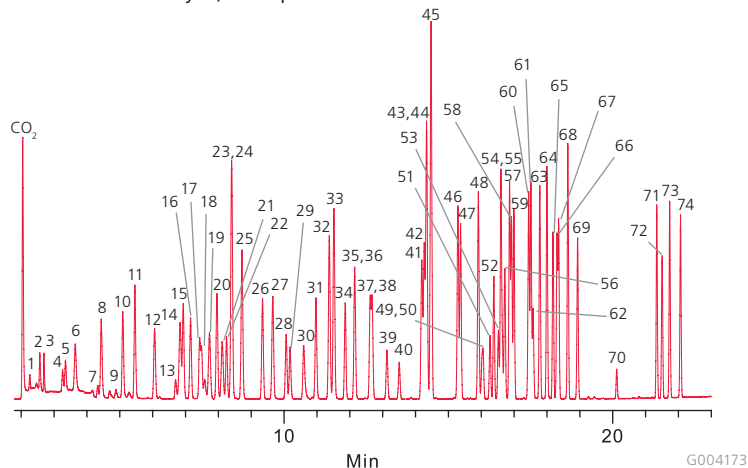
Peak IDs for Figures 1–3

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| 1. Chloromethane | 11. Dibromofluoromethane (surr.) | 21. 2-Chloroethyl vinyl ether | 31. Ethylbenzene |
| 2. Vinyl chloride | 12. 1,1,1-Trichloroethane | 22. cis-1,3-Dichloropropene | 32. Bromoform |
| 3. Bromomethane | 13. Carbon tetrachloride | 23. Toluene-d8 (surr.) | 33. 4-Bromofluorobenzene (surr.) |
| 4. Chloroethane | 14. 1,2-Dichloroethane-d4 (surr.) | 24. Toluene | 34. 1,1,2,2-Tetrachloroethane |
| 5. Trichlorofluoromethane | 15. Benzene | 25. trans-1,3-Dichloropropene | 35. 1,3-Dichlorobenzene |
| 6. 1,1-Dichloroethene | 16. 1,2-Dichloroethane | 26. 1,1,2-Trichloroethane | 36. 1,4-Dichlorobenzene-d4 (I.S.) |
| 7. Methylene chloride | 17. Fluorobenzene (I.S.) | 27. Tetrachloroethane | 37. 1,4-Dichlorobenzene |
| 8. trans-1,2-Dichloroethene | 18. Trichloroethene | 28. Dibromochloromethane | 38. 1,2-Dichlorobenzene |
| 9. 1,1-Dichloroethane | 19. 1,2-Dichloropropane | 29. Chlorobenzene-d5 (I.S.) | |
| 10. Chloroform | 20. Bromodichloromethane | 30. Chlorobenzene | |

Figure 4 Solid Waste Volatiles on the VOCOL

Sample/matrix:	each analyte at 50 ppb in 5 mL water
Purge trap:	VOCARB 3000 "K" (24940-U)
Purge:	40 mL/min. at 25°C for 11 min.
Dry purge:	2 min.
Desorption pre-heat:	205°C
Desorption temp.:	210°C for 2 min.
Desorption flow:	40 mL/min.
Bake:	260°C for 10 min.
Transfer line/ valve temp.:	110°C
Column:	VOCOL, 30 m x 0.25 mm I.D., 1.5 µm (24205-U)
Oven:	40°C (2 min.), 7°C/min. to 125°C, 12°C/min. to 220°C (5 min.)
Inj.:	150°C
MSD interface:	200°C
Scan range:	m/z = 35-400
Carrier gas:	helium, 0.7 mL/min.
Injection:	30:1 split
Liner:	0.75 mm I.D. SPME

Conventional GC Analysis, 30:1 Split

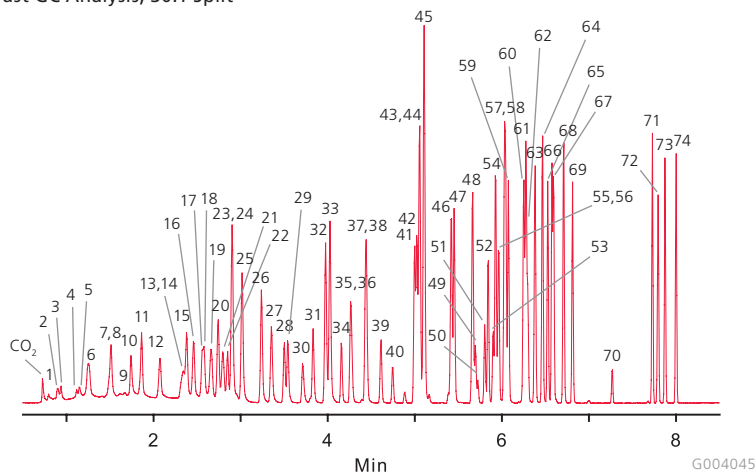


G004173

Figure 5 Solid Waste Volatiles on the VOCOL

Sample/matrix:	each analyte at 50 ppb in 5 mL water
Purge trap:	VOCARB 3000 "K" (24940-U)
Purge:	40 mL/min. at 25°C for 11 min.
Dry purge:	1 min.
Desorption pre-heat:	205°C
Desorption temp.:	210°C for 1 min.
Desorption flow:	46 mL/min.
Bake.:	260°C for 10 min.
Transfer line/ valve temp.:	110°C
Column:	VOCOL, 20 m x 0.18 mm I.D., 1.0 µm (28463-U)
Oven:	40°C (0.8 min.), 19°C/min. to 125°C, 32°C/min. to 220°C (1 min.)
Inj.:	150°C
MSD interface:	220°C
Scan range:	m/z = 35-400
Carrier gas:	helium, 1.4 mL/min.
Injection:	30:1 split
Liner:	0.75 mm I.D. SPME

Fast GC Analysis, 30:1 Split

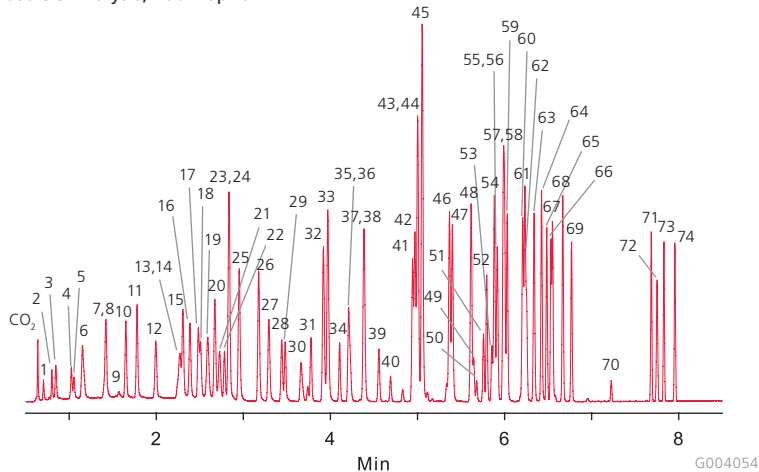


G004045

Figure 6 Solid Waste Volatiles on the VOCOL

Sample/matrix:	each analyte at 50 ppb in 5 mL water
Purge trap:	VOCARB 3000 "K" (24940-U)
Purge:	40 mL/min. at 25°C for 11 min.
Dry purge:	1 min.
Desorption pre-heat:	205°C
Desorption temp.:	210°C for 1 min.
Desorption flow:	150 mL/min.
Bake.:	260°C for 10 min.
Transfer line/ valve temp.:	110°C
Column:	VOCOL, 20 m x 0.18 mm I.D., 1.0 µm (28463-U)
Oven:	40°C (0.8 min.), 19°C/min. to 125°C, 32°C/min. to 220°C (1 min.)
Inj.:	150°C
MSD interface:	220°C
Scan range:	m/z = 35-400
Carrier gas:	helium, 1.5 mL/min.
Injection:	100:1 split
Liner:	0.75 mm I.D. SPME

Fast GC Analysis, 100:1 Split



G004054

Peak IDs for Figures 4 – 6

1. Dichlorofluoromethane	11. trans-1,2-Dichloroethene	21. Carbon tetrachloride	32. Toluene-d8 (surr.)	43. Ethylbenzene	54. n-Propylbenzene	65. 1,3-Dichlorobenzene
2. Chloromethane	12. 1,1-Dichloroethane	22. 1,2-Dichloroethane-d4 (surr.)	33. Toluene	44. 1,1,1,2-Tetrachloroethane	55. Bromobenzene	66. 1,4-Dichlorobenzene-d4 (I.S.)
3. Vinyl chloride	13. 2-Butanone	23. 1,2-Dichloroethane	34. trans-1,3-Dichloropropene	45. m-Xylene & p-Xylene	56. trans-1,4-Dichloro-2-butene	67. 1,4-Dichlorobenzene
4. Bromomethane	14. 2,2-Dichloropropane	24. Benzene	35. 1,1,2-Trichloroethane	46. o-Xylene	57. 1,3,5-Trimethylbenzene	68. Butylbenzene
5. Chloroethane	15. cis-1,2-Dichloroethene	25. Fluorobenzene (I.S.)	36. 2-Hexanone	47. Styrene	58. o-Chlorotoluene	69. 1,2-Dichlorobenzene
6. Trichlorofluoromethane	16. Chloroform	26. Trichloroethene	37. 1,3-Dichloropropane	48. Isopropylbenzene	59. p-Chlorotoluene	70. 1,2-Dibromo-3-chloropropane
7. Acetone	17. Bromochloromethane	27. 1,2-Dichloropropane	38. Tetrachloroethene	49. Bromoform	60. tert-Butylbenzene	71. 1,2,4-Trichlorobenzene
8. 1,1-Dichloroethene	18. Dibromofluoromethane	28. Bromodichloromethane	39. Dibromochloromethane	50. cis-1,4-Dichloro-2-butene	61. 1,2,4-Trimethylbenzene	72. Hexachlorobutadiene
9. Iodomethane	19. 1,1,1-Trichloroethane	29. Dibromomethane	40. 1,2-Dibromomethane	51. 1,1,2,2-Tetrachloroethane	62. Pentachloroethane	73. Naphthalene
10. Methylene chloride	20. 1,1-Dichloropropene	30. 4-methyl-2-pentanone	41. Chlorobenzene-d5 (I.S.)	52. 4-Bromofluorobenzene (surr.)	63. sec-Butylbenzene	74. 1,2,3-Trichlorobenzene
		31. cis-1,3-Dichloropropene	42. Chlorobenzene	53. 1,2,3-Trichloropropane	64. p-Isopropyltoluene	

(continued from page 15)

Converting Conventional GC to Fast GC for Solid Waste Volatiles

Fast GC is also compatible with more complex samples. A method for the analysis of volatiles from solid waste samples (US EPA Method 8260, also commonly performed in the United States) was selected as an example, this time using the VOCOL column. The optimised chromatogram obtained using conventional GC is shown in Figure 4. The analysis time is <23 minutes.

The column dimensions were changed to Fast GC dimensions, and then conditions were optimised to produce the chromatogram shown in Figure 5 (parameters which were changed are highlighted). While analysis time is reduced to <9 minutes, the shapes of the first several peaks are not good due to the lower capacity of the 0.18 mm I.D. column. Therefore, the split ratio was increased from 30:1 to 100:1, and then the linear velocity was optimised to achieve the chromatogram shown in Figure 6 (parameters which were changed are highlighted). Again, the short analysis time (<9 minutes) is accompanied with improved peak shapes. This represents a vast improvement in analysis time (from over 22 minutes to under 9 minutes) compared to the conventional GC chromatogram in Figure 4.

Fast GC of Hazardous Waste Site Volatiles

A method for the analysis of volatiles from hazardous waste site samples (US EPA Method OLM04.2 VOA) was selected to show the selectivity differences between the SPB-624 column and the VOCOL column. Optimised Fast GC chromatograms are shown in Figure 7 (SPB-624) and Figure 8 (VOCOL). Both show quick analysis times and good shapes of all peaks. Note the change in elution order for several peaks (10/11, 18/19, 31/32, 37/38, 47/48 and 49/50) due to the selectivity difference between the two columns.

Conclusion

Converting methods from conventional GC to Fast GC can result in decreased costs (fewer people and/or instruments are needed) and increased revenue (more samples can be processed). However, care must be taken to ensure that all Fast GC method parameters are optimised together. Changing only one may decrease analysis time (desirable), but will likely cause a loss of resolution (undesirable). With any Fast GC method, the reduced sample capacity of the smaller I.D. column must be accounted for so that unacceptable chromatography is not created.

Fast GC methods can be used with complex samples, and with any column, regardless of its selectivity. Furthermore, Fast GC is compatible with the current equipment found in most laboratories, and also with newer equipment that is designed for speed.

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Featured Products

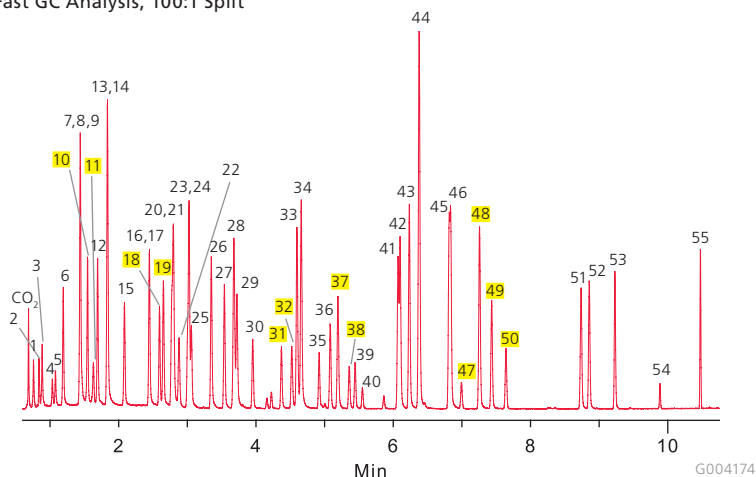
Description	Cat. No.
SPB-624 Fused Silica Columns	
20 m x 0.18 mm I.D., 1.0 µm	28662-U
30 m x 0.25 mm I.D., 1.4 µm	24255
VOCOL Fused Silica Columns	
20 m x 0.18 mm I.D., 1.0 µm	28463-U
30 m x 0.25 mm I.D., 1.5 µm	24205-U
VOCARB 3000 "K" Purge Traps	
Fits OI Analytical Eclipse 4660 / 4560	24940-U

Related Products

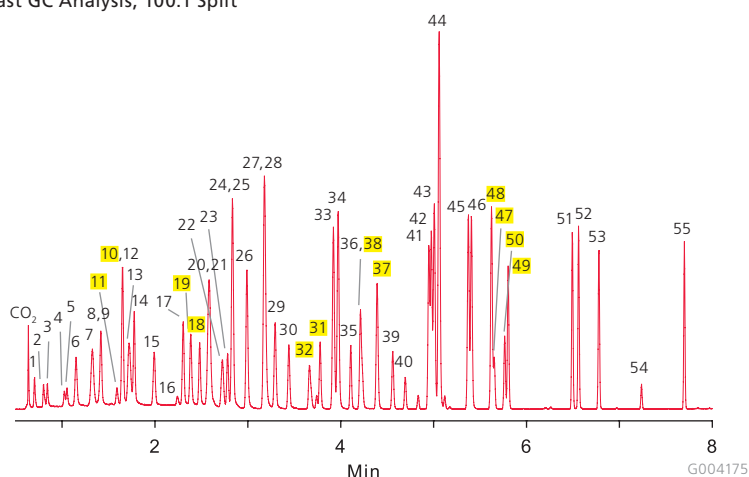
Description	Cat. No.
SPB-624 Fused Silica Columns	
60 m x 0.25 mm I.D., 1.4 µm	24256
30 m x 0.32 mm I.D., 1.8 µm	23323-U
60 m x 0.32 mm I.D., 1.8 µm	24251
30 m x 0.53 mm I.D., 3.0 µm	25430
60 m x 0.53 mm I.D., 3.0 µm	28663-U
75 m x 0.53 mm I.D., 3.0 µm	25432
105 m x 0.53 mm I.D., 3.0 µm	28664-U
VOCOL Fused Silica Columns	
10 m x 0.20 mm I.D., 1.2 µm	24129-U
60 m x 0.25 mm I.D., 1.5 µm	24154
30 m x 0.32 mm I.D., 1.8 µm	28464-U
60 m x 0.32 mm I.D., 1.8 µm	24217-U
60 m x 0.32 mm I.D., 3.0 µm	24157
30 m x 0.53 mm I.D., 3.0 µm	25320-U
60 m x 0.53 mm I.D., 3.0 µm	25381
105 m x 0.53 mm I.D., 3.0 µm	25358
60 m x 0.75 mm I.D., 1.5 µm	23313-U
VOCARB 3000 "K" Purge Traps	
Fits Tekmar Velocity XPT / 2000 / 4000	21066-U
Fits Tekmar 3000 / 3100	24920-U
Fits OI Analytical 4460	21131-U
Fits Dynatech "Dyna" Models	21085-U
Fits CDS Peak Master	21159

Figure 7 Hazardous Waste Site Volatiles on the SPB-624

Sample/matrix:	each analyte at 50 ppb in 5 mL water
Purge trap:	VOCARB 3000 "K" (24940-U)
Purge:	40 mL/min. at 25°C for 11 min.
Dry purge:	2 min.
Desorption pre-heat:	205°C
Desorption temp.:	210°C for 2 min.
Desorption flow:	124 mL/min.
Bake.:	260°C for 10 min.
Transfer line/ valve temp.:	110°C
Column:	SPB-624, 20 m x 0.18 mm I.D., 1.0 µm (28662-U)
Oven:	40°C (1 min.), 11°C/min. to 125°C, 35°C/min. to 230°C (2 min.)
Inj.:	150°C
MSD interface:	200°C
Scan range:	m/z = 35-400
Carrier gas:	helium, 1.2 mL/min.
Injection:	100:1 split
Liner:	0.75 mm I.D. SPME

Fast GC Analysis, 100:1 Split**Figure 8** Hazardous Waste Site Volatiles on the VOCOL

Sample/matrix:	each analyte at 50 ppb in 5 mL water
Purge trap:	VOCARB 3000 "K" (24940-U)
Purge:	40 mL/min. at 25°C for 11 min.
Dry purge:	2 min.
Desorption pre-heat:	205°C
Desorption temp.:	210°C for 2 min.
Desorption flow:	150 mL/min.
Bake.:	260°C for 10 min.
Transfer line/ valve temp.:	110°C
Column:	VOCOL, 20 m x 0.18 mm I.D., 1.0 µm (28463-U)
Oven:	40°C (0.8 min.), 19°C/min. to 125°C, 32°C/min. to 220°C (1 min.)
Inj.:	150°C
MSD interface:	200°C
Scan range:	m/z = 35-400
Carrier gas:	helium, 1.4 mL/min.
Injection:	100:1 split
Liner:	0.75 mm I.D. SPME

Fast GC Analysis, 100:1 Split**Peak IDs for Figures 7–8**

1. Dichlorofluoromethane	9. Acetone	19. Chloroform	29. 1,2-Dichloropropane	39. Dibromochloromethane	49. 4-Bromofluorobenzene (surr.)
2. Chloromethane	10. Carbon disulfide	20. 1,1,1-Trichloroethane	30. Bromodichloromethane	40. 1,2-Dibromomethane	50. 1,1,2,2-Tetrachloroethane
3. Vinyl chloride	11. Methyl acetate	21. Cyclohexane	31. cis-1,3-Dichloropropene	41. Chlorobenzene-d5 (I.S.)	51. 1,3-Dichlorobenzene
4. Bromomethane	12. Methylene chloride	22. Carbon tetrachloride	32. 4-Methyl-2-pentanone	42. Chlorobenzene	52. 1,4-Dichlorobenzene
5. Chloroethane	13. Methyl-tert-butyl ether	23. 1,2-Dichloroethane-d4 (surr.)	33. Toluene-d8 (surr.)	43. Ethylbenzene	53. 1,2-Dichlorobenzene
6. Trichlorofluoromethane	14. trans-1,2-Dichloroethane	24. Benzene	34. Toluene	44. m-Xylene & p-Xylene	54. 1,2-Dibromo-3-chloropropane
7. 1,1,2-Trichloro-1,2,2-trifluoroethane	15. 1,1-Dichloroethane	25. 1,2-Dichloroethane	35. trans-1,3-Dichloropropene	45. o-Xylene	55. 1,2,4-Trichlorobenzene
8. 1,1-Dichloroethene	16. 2-Butanone	26. 1,4-Difluorobenzene (I.S.)	36. 1,1,2-Trichloroethane	46. Styrene	
	17. cis-1,2-Dichloroethene	27. Trichloroethene	37. Tetrachloroethene	47. Bromoform	
	18. Bromochloromethane (I.S.)	28. Methylcyclohexane	38. 2-Hexanone	48. Isopropylbenzene	

Did you know?

The 2007 brochure "Fast GC: A Practical Guide for Increasing Sample Throughput without Sacrificing Quality" (T407096 JTW) contains valuable information concerning Fast GC principles that is not covered in this article. Included are practical considerations, theoretical discussions, a listing of columns in Fast GC dimensions, twenty-six chromatograms, a listing of related products designed to maximise performance, plus a list of literature for additional reading. Request a copy of this brochure on the attached postcard or contact the local Supelco Technical Service at EurTechServ@sial.com

**Related Information**

The Supelco "Purge-and-Trap System Guide" (T197916 BIN) contains both theory as well as troubleshooting information. Request a copy of this bulletin on the attached postcard or contact Supelco Technical Service at EurTechServ@sial.com (Available in electronic form only. Please provide email address.)

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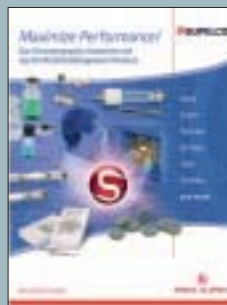
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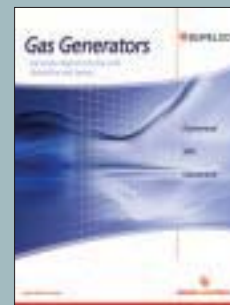
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Trace Analysis of Triazines, Beta-Agonists, Chloramphenicol and Clenbuterol

Fluka standards for SupelMIP™ SPE phases for highly sensitive LC-MS analysis

Nicole Amann, Product Manager Analytical Standards nicole.amann@sial.com

Collaborating with MIP Technologies AB, in 2007 Supelco launched SupelMIP™, a unique, new, highly selective solid phase extraction (SPE) particle platform. Since this introduction, users have benefited from SupelMIP™ particles' high degree of selectivity that gives enhanced sensitivity and MS-compatibility of samples extracted with them.

How MIPs achieve high analyte selectivity

The underpinning of the high analyte selectivity of MIP (molecularly imprinted polymer) particles is, simply put, a lock-and-key mechanism for a particular compound or group of structurally related compounds. MIP particles are synthesised in the presence of a templating molecule, which is a structural mimetic of the analyte of interest. Post-synthesis, the templating molecules are removed from the particles, leaving behind cavities that are sterically and chemically complementary to the analyte of interest (**Figure 1**). When samples are extracted using the corresponding MIP particles, analytes enter the cavities and are held by multiple molecular interactions (e.g. ionic, H-bonding, hydrophobic). Other components of the sample, including matrix constituents, are also likely to non-specifically adsorb to the MIP particle. However, only the target analyte is adsorbed strongly enough to the particle, because of the multiple molecular interactions, to withstand the rigorous washing that removes unwanted sample components. After the wash steps, the analytes of interest are eluted in a highly purified form from the particles using specific elution conditions.

SupelMIP™ particles have two important parts that work in synergy to achieve low-level analysis:

- The particle – Designed to hold onto a particular compound or group of structurally related compounds by virtue of chemically and structurally selective cavities created with a templating mimetic molecule
- The extraction protocol – Developed and optimised to provide clean preps of a particular analyte or class from the specified matrix

The extraction protocol, meaning the sequence of eluants and drying steps, is as important to success as the particle itself. This implies another benefit: there is no time-consuming method development when using SupelMIP™ particles. By simply following the complete instructions included with each SupelMIP™ product, analysts can realise low-level detection of the target analyte from complex matrices.

Figure 1 Visual depiction of the MIP cavity



G003942



Standards and isotopically labeled standards – Enhancing analytical precision and accuracy

Chemical standards are important components to a successful analysis, whether the internal standards or labelled standards in isotope dilution experiments. Sigma-Aldrich analytical brands Supelco and Fluka combine to offer both the high-quality extraction and separation devices and the necessary chemical standards to achieve your analytical objectives.

Addressing today's challenging extractions

SupelMIPs™ have been developed to extract several groups of frequently monitored or regulated substances. We have placed particular emphasis on food, environmental and veterinary analysis, because of their human health implications and regulatory demands. Besides the need for low-level analyte quantification, these samples present a challenge in the matrix complexity. MIPs were developed when the current clean-up procedure, if one existed, was lacking in some important characteristic, like sensitivity, recovery, complexity, or ease-of-use. Four of these analyses will be described briefly here. The complete list of SupelMIPs™ and support literature can be seen at sigma-aldrich.com/supelmip

Chloramphenicol

A broad-spectrum antibiotic, chloramphenicol is still widely used to treat livestock, especially in developing countries, in spite of its being known to cause aplastic anemia and possibly cancer in humans [1]. For this reason, there is a zero tolerance for its presence in food products. SupelMIP™ Chloramphenicol SPE cartridges have been successfully applied to extract chloramphenicol from milk [2,3]. Compared to a hydrophilic polymer SPE particle, which required extensive sample pre-treatment, the SupelMIP™ particle and the pre-defined extraction method require fewer steps and result in higher recovery and better signal-to-noise ratio (Figure 2). Various Fluka-brand chloramphenicol standards, including deuterated chloramphenicol, complement this analysis.

Clenbuterol

Clenbuterol is a β -agonist (β 2-adrenergic receptor agonists) used in livestock animals because of its growth-promoting properties. It is also abused by some body-builders and athletes. Although it has adverse effects on human health and is currently banned, outbreaks of clenbuterol-induced food poisoning occur from its illegal use [4]. SupelMIP™ Clenbuterol cartridges are designed to extract this compound from urine [5]. Compared to procedures using conventional hydrophilic polymer SPE particles, the SupelMIP™ cartridge and the optimised extraction method provide lower matrix background and higher recovery (Figure 3). A Fluka-brand deuterated clenbuterol standard permits accurate quantification.

β -Agonists

In addition to clenbuterol, there are other members of the β -agonists family. Because the extraction of β -agonists is often difficult and time-consuming, our customers asked for MIP-based solution that was both simple to use and selective for the entire β -agonist family. We responded by developing SupelMIP™ Beta-agonists and a simple extraction procedure to quantify β -agonists at low levels in

food, robustly and with lower cost than other methods [6]. There is little or no loss of sensitivity in LC-MS/MS due to contamination by interfering compounds. The product is selective for brombuterol, clenbuterol, formoterol, isoxuprine, mapenterol, ractopamine, ritaline, salbutamol, salmeterol, terbutaline, tulobuterol, zilpaterol and other β -agonists. SupelMIP™ Beta-agonists cartridges, combined with the Fluka-brand standards, are perfect for the extraction and quantification of these compounds in urine.

Triazine pesticides

Triazines herbicides are widely used in agriculture to control weeds. However, the triazines are harmful to humans and other animals. They are strictly regulated and therefore require sensitive and reliable analytical methods. SupelMIP™ Triazine 10 cartridges have been developed to isolate triazines and their metabolites from water, soil and food products [7]. They permit fast and robust extraction at low levels (ppb) from industrial wastewater, environmental samples of drinking and river water, as well as food samples. Class-selective, SupelMIP™ Triazine10 shows excellent selectivity and high recovery of low levels of atrazine, simazine, propazine, cyanazine, sebutylazine, deisopropylatrazine, deethylatrazine, deethylterbutylazine, prometon and hydroxyterbutylazine from water. Fluka-brand standards are available to permit quantitative analysis.

For more information on the above-mentioned analyses and SupelMIP™ in general, including the comprehensive SupelMIP™ brochure, please use the enclosed reply card, contact our technical service or visit our dedicated website: sigma-aldrich.com/supelmip

Figure 2 Mass spectrum of full ion chromatograms (3.65 – 4.00 min.) of the SupelMIP™ SPE extract (A) and the hydrophilic polymer SPE extract (B)

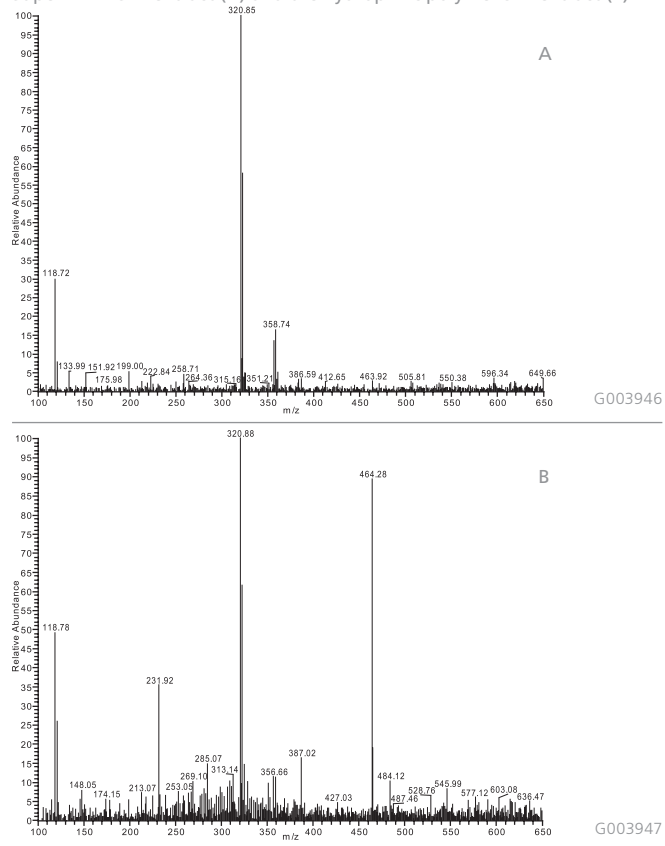
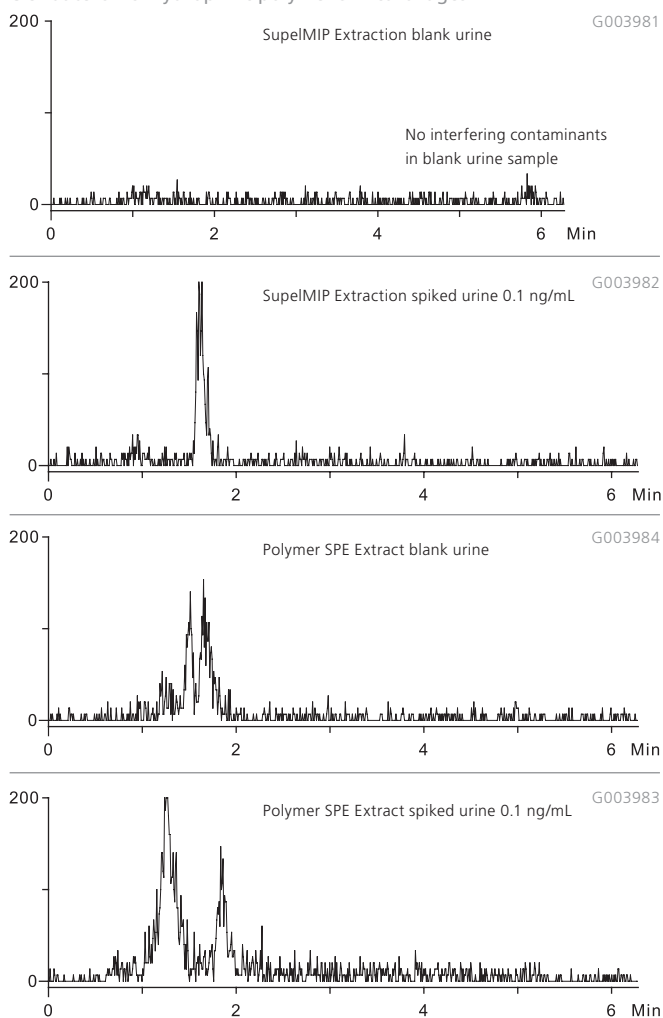


Figure 3 Clenbuterol-spiked urine samples extracted with SupelMIP™–Clenbuterol vs. hydrophilic polymer SPE cartridges



Spike Level (ng/mL)	% Recovery from Urine	
	SupelMIP SPE Clenbuterol	Hydrophilic Polymer SPE
0.1	99%	8%
0.5	75%	66%
1.0	75%	69%

Table 1 SupelMIP™ SPE Cartridges

Cat. No.	Brand	Description	Package Size
53210-U	Supelco	SupelMIP™ Chloramphenicol 25 mg sorbent mass / 10 mL cartridge volume	50 cartridges
53209-U	Supelco	SupelMIP™ Chloramphenicol 25 mg sorbent mass / 3 mL cartridge volume	50 cartridges
53201-U	Supelco	SupelMIP™ Clenbuterol 25 mg sorbent mass / 10 mL cartridge volume	50 cartridges
53202-U	Supelco	SupelMIP™ Beta agonist (class selective) 25 mg sorbent mass / 10 mL cartridge volume	50 cartridges
53225-U	Supelco	SupelMIP™ Beta agonist (class selective) 25 mg sorbent mass / 3 mL cartridge volume	50 cartridges
53208-U	Supelco	SupelMIP™ Triazine 10 (class selective) 25 mg sorbent mass / 10 mL cartridge volume	50 cartridges

Table 2 Standards

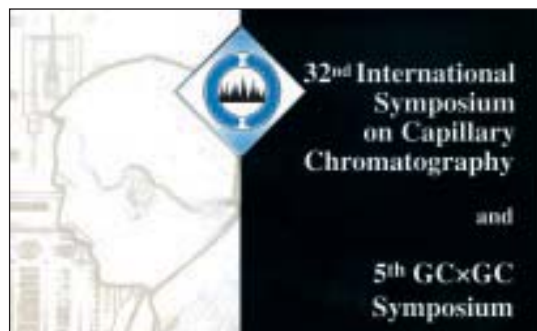
Cat. No.	Brand	Description	Package Size
Chloramphenicol			
46110	Fluka	Chloramphenicol	250 mg
34005	Fluka	m-Chloramphenicol	10 mg
46109	Fluka	Chloramphenicol palmitate	250 mg
41724	Fluka	Chloramphenicol-d5	1 mg
β-Agonists			
06613	Fluka	Atenolol-d7	2 mg
94972	Fluka	Brombuterol hydrochloride	10 mg
53787	Fluka	Carazolol	10 mg
06246	Fluka	Clenbuterol-d9	2 mg
49358	Fluka	Mapenterol hydrochloride	5 mg
34198	Fluka	Ractopamine hydrochloride	100 mg
46725	Fluka	Salbutamol	100 mg
46732	Fluka	Salbutamol hemisulfate salt	250 mg
53541	Fluka	Tulobuterol hydrochloride	10 mg
Triazine Herbicides			
45330	Fluka	Atrazine	250 mg
36665	Fluka	Atrazine solution, 100 ng/μL in methanol	2 mL
36629	Fluka	Atrazine-desethyl	250 mg
36628	Fluka	Atrazine-desisopropyl	250 mg
45407	Fluka	Cyanazine	250 mg
45834	Fluka	Cyanazine solution, 100 ng/μL in acetonitrile	2 mL
45635	Fluka	Prometon	250 mg
45640	Fluka	Propazine	250 mg
36587	Fluka	Propazine solution, 100 ng/μL in methanol	2 mL
36785	Fluka	Sebuthylazin	250 mg
45659	Fluka	Simazine	250 mg
45678	Fluka	Terbutylazin	250 mg
36589	Fluka	Terbutylazin solution, 100 ng/μL in methanol	2 mL
36769	Fluka	Terbutylazin-desethyl	250 mg
46019	Fluka	Terbutylazine-2-hydroxy	100 mg

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32nd International Symposium on Capillary Chromatography and 5th GCxGC Symposium



Announcement

32nd International Symposium on Capillary Chromatography
Palazzo dei Congressi – Riva del Garda – Italy
May 26–30, 2008

The 32nd International Symposium on Capillary Chromatography (ISCC), the premier meeting for pressure and electro-driven microcolumn separations and related techniques, will be held **May 27–30, 2008 in the Congress Centre, Riva del Garda, Italy.**

Join us in celebrating this Silver Jubilee (Riva 1983–2008).

The programme will include plenary lectures, keynote lectures by young scientists, prominently featured poster presentations, an instrument exhibition displaying the latest instrumental innovations, and a highly attractive social programme offering opportunities to meet with world-renowned scientists.

The 5th GCxGC Symposium will be organised jointly with the ISCC meeting to allow scientists to attend both meetings. The symposium will start with a short course on Sunday May 25 covering the fundamentals and applications of comprehensive GCxGC and with a plenary session on Monday May 26. Keynote lectures and posters will be included in the programme of the ISCC meeting.

Researchers in all areas relevant to the subjects of the symposia are invited to submit abstracts. As traditional for the Riva meetings, the majority of presentations will be in poster format and the scientific committee will select contributions for oral presentation.

Exhibitors and sponsors are encouraged to participate by submitting abstracts, reserving booth space, and becoming a sponsor. Please keep visiting our website for new information as it becomes available. Looking forward to meeting you in Riva de Garda,

Prof. Dr. P. Sandra
Chairman RIVA 2008

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