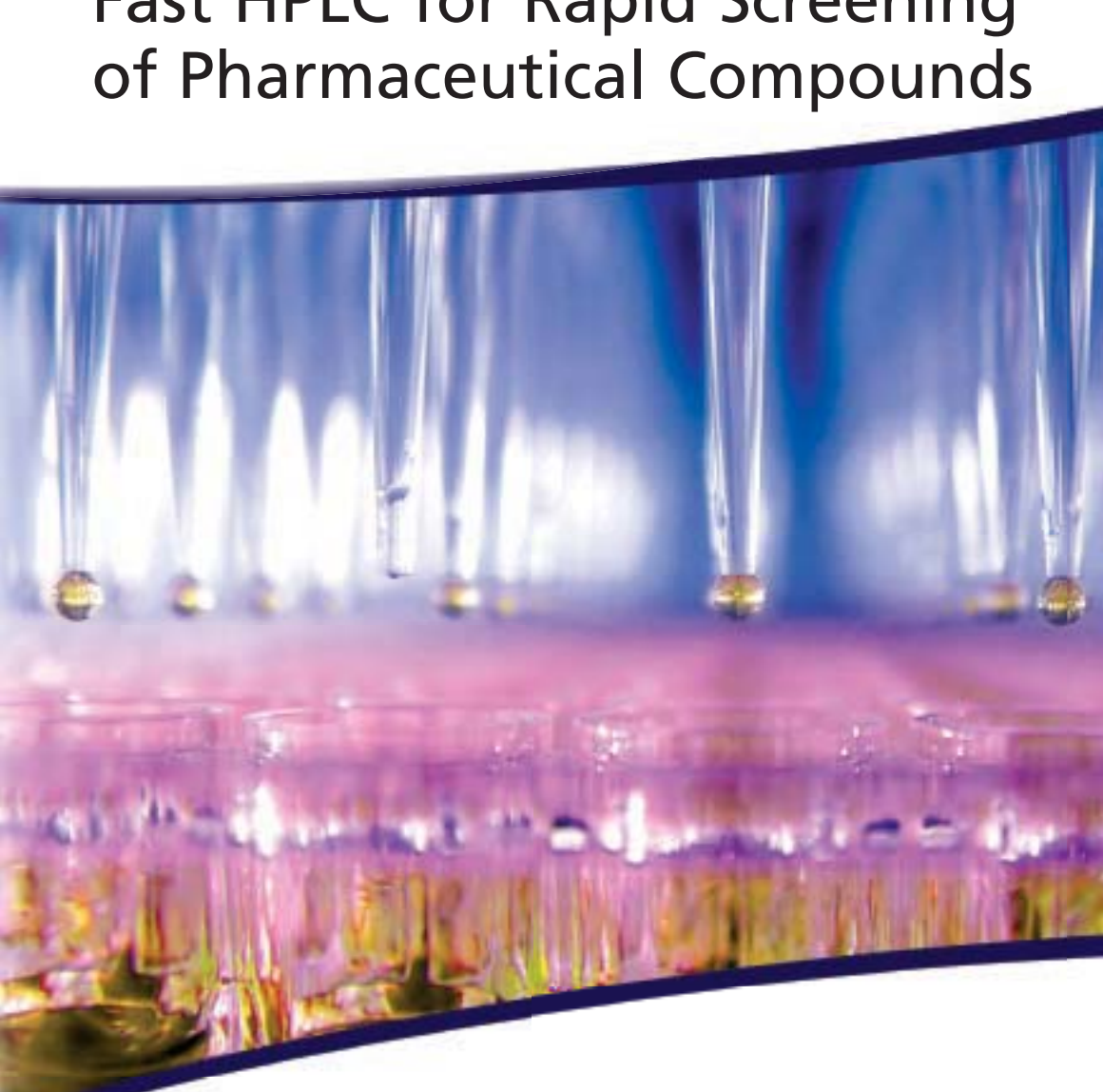


Reporter

Volume 30, March 2008, International



Fast HPLC for Rapid Screening of Pharmaceutical Compounds



An array of samples
for HPLC screening

HPLC/LC

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Reporter

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Kristen Schultz
Product Manager
Sample Preparation
and Purification

Dear Colleague,

Innovation begins as a concept or idea, which is driven by creating value for our customers. This can be in the form of new and improved products and protocols, leading to novel and unique solutions. Our innovation process is guided by assessing current market needs and technologies and combining them with our internal strengths to develop an idea or product perceived as new, creative and unique to the market.

Our innovation success begins with identifying customer-specific application solutions and combining them with our strengths in particle technology and surface chemistry to achieve our goal to:

Accelerate our customers' success by identifying and addressing critical customer needs with proprietary, unique, and valued application solutions. These application solutions must meet real customer needs via proprietary and unique intellectual property and proprietary capabilities such as particle platform technology and surface chemistry.

Implementing this strategy provides rewarding challenges, which have led to new, unique and exciting products to the analytical market. Among them are:

Ascentis® Express HPLC Columns – Based on Fused-Core™ technology, these columns provide fast HPLC with high resolution at backpressures suitable for use in standard HPLC systems. These columns provide sub-2 µm-like performance without the drawback of ultra-high backpressures. This breakthrough in HPLC column performance delivers extreme performance on any LC system (see page 3).

SupelMIP™ – Molecularly imprinted polymers engineered for the highly selective extraction of traceanalytes from complex sample matrices. Each phase is application specific and optimised to reduce ion-suppression and achieve lower detection limits through superior selectivity. Each phase comes with a recommended protocol, thereby minimising method development (see page 11).

MiniTip™ SPE products – Designed for micro-scale extraction and concentration of small molecules and biological macromolecules. The sorbent bed is held in place using a patented high-purity adhesive allowing the tube to act as a solid phase extraction medium for your work. Currently available in C18 or carbon, with other phases available very soon (see page 14).

We are committed to developing innovative solutions driven by our customers' inputs and specific market needs. You will find these products in this issue as well as future issues of The Reporter, and also on our website at sigma-aldrich.com/chromatography Put our staff to work for you, let us know what we can do for you today.

Regards,

Kristen Schultz
Product Manager – Sample Preparation and Purification

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Fast HPLC Using Fused-Core Particle Technology for Rapid Screening of Pharmaceutical Compounds

Hillel Brandes, Craig Aurand and Wayne K. Way wayne.way@sial.com

Introduction

HPLC is critical to the discovery, development and eventual commercialisation of pharmaceutical products. HPLC is the benchmark analytical method in the pharmaceutical industry due to its ability to score such high marks in analytical validation characteristics including accuracy, precision, limit of detection, specificity, linearity and range, and ruggedness. No other analytical techniques can consistently score high in all characteristics on compounds and matrices that are of interest to the pharmaceutical industry.

A major benefit of the Fused-Core particle is the small diffusion path

Furthermore, it has been generally accepted that a typical HPLC analysis takes 15–30 minutes, with some taking as long as an hour. When multiplied by the number of samples to be analysed either in discovery or product release, the total instrument time required is staggering. This overwhelming amount of instrument time has resulted in a growing number of instruments, around-the-clock analysis, and a push for faster methods.

Fast HPLC, using short columns (3–10 cm) packed with small particles (<3 μm) and high flow rates has recently become an effective means to reduce analysis time. This is primarily due to the improved quality of sub-3 μm particle columns and the introduction of new instrumentation to meet the requirements of higher column backpressure and low instrument dispersion. The reasons for using sub-3 μm particle columns in fast HPLC are evident by examining Van Deemter plots for various particle sizes. The smaller particles yield lower HETP or higher efficiency per unit length. Furthermore, the optimum flow rate is higher for smaller particles. Smaller particle columns have less efficiency loss at high flow rates because mass transfer is less sensitive to velocity changes as illustrated by “flatter” Van Deemter plots.

Unfortunately, column backpressure increases at a greater rate than column efficiency as you decrease particle size. This increase in backpressure is so great for sub-2 μm particle columns that they are practically unusable using standard HPLC systems, such as Agilent® 1100. For this reason, a particle with high efficiency plus low backpressure would be a more suitable candidate for Fast HPLC.

Fused-Core Particle Technology Delivers High Efficiency at Low Backpressure

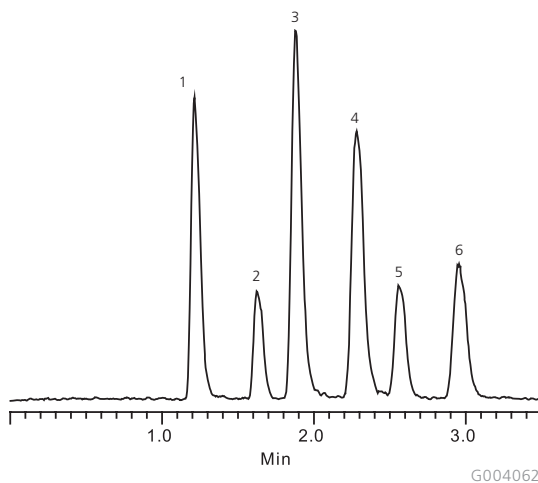
Ascentis Express columns provide a breakthrough in fast HPLC performance. Based on Fused-Core particle technology, Ascentis Express provides the high-efficiency-based benefits of sub-2 μm particles but at much lower backpressure. Due to the high efficiencies at low back pressures, Ascentis Express can provide Fast HPLC chromatography that was previously unattainable on commercial LC systems.

The Fused-Core particle consists of a 1.7 μm solid core and a 0.5 μm porous shell. A major benefit of the Fused-Core particle is the small diffusion path (0.5 μm) compared to conventional fully porous particles. The shorter diffusion path reduces axial dispersion of solutes and minimises peak broadening. In fact, Ascentis Express columns are able to achieve efficiencies similar to that obtained with sub-2 μm particle columns, even though the backpressures are only 50% of that achieved under similar conditions with sub-2 μm particles. This means that Ascentis Express can turn almost any LC system into a fast HPLC workhorse for your lab.

Fast HPLC of Pharmaceutical Compounds

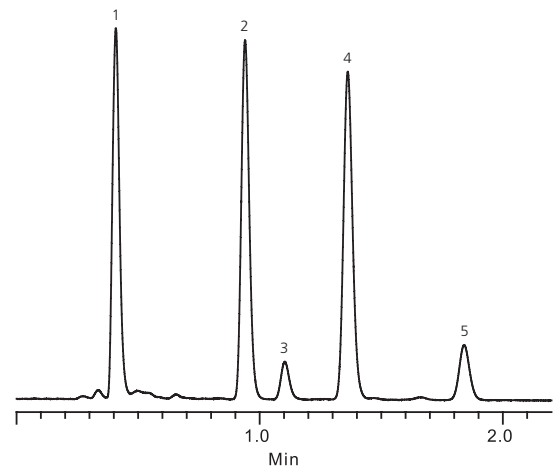
Shown in Figures 1–3 on page 4 are the chromatograms for the separation of three sets of closely related pharmaceutical compounds. These examples include both basic and neutral as well as polar and non-polar compounds. While each example utilises 2.1 mm I.D. columns, three different flow rates and three unique mobile phase conditions are presented to demonstrate the versatility of fast HPLC with Fused-Core particle columns.



Figure 1 TCAs sur Ascentis Express

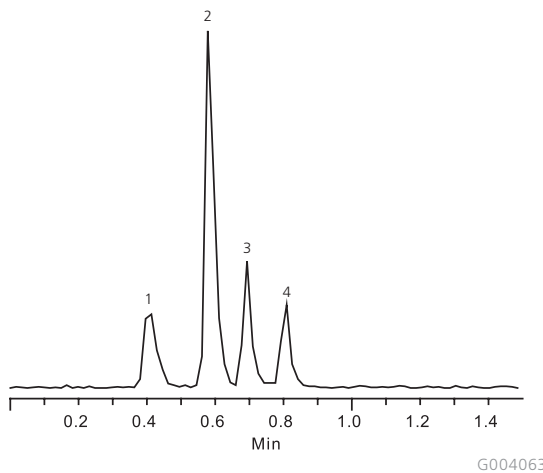
Column: Ascentis Express C18, 10 cm x 2.1 mm ID (53823-U)
Instrument: Jasco X-LC
Mobile phase A: 100 mM ammonium acetate (pH 7.0; titrated with ammonium hydroxide)
Mobile phase B: water
Mobile phase C: methanol
Online mixing: A:B:C = 10:30:60
Flow rate: 0.3 mL/min
Temp.: 55 °C
Det.: Thermo LCQ Advantage; ESI(+), m/z 250-320
Injection: 1 µL

1. Nordoxepin	4. Doxepin
2. Desipramine	5. Norclomipramine
3. Nortriptyline	6. Imipramine

Figure 3 Stéroïdes sur Ascentis Express

Column: Ascentis Express C18, 10 cm x 2.1 mm ID (53823-U)
Instrument: Jasco X-LC
Mobile phase: 55:45 water:acetonitrile
Flow rate: 0.6 mL/min
Temp.: ambient
Det.: 200 nm
Injection: 1 µL

1. Estriol
2. b-Estradiol
3. Impurity
4. Estrone
5. Estrone dégradant

Figure 2 β-Bloquants sur Ascentis Express

Column: Ascentis Express C18, 5 cm x 2.1 mm ID (53822-U)
Instrument: Agilent 1100
Mobile phase A: 0.1% acetic acid in water
Mobile phase B: 0.1% acetic acid in acetonitrile
Online mixing: A:B = 74:26
Flow rate: 0.2 mL/min
Temp.: 35 °C
Det.: ABI 3200 QT; ESI(+), MS/MS
Injection: 1 µL

1. Atenolol	3. Timolol
2. Pindolol	4. Metoprolol

Shown in **Figure 1** is the separation of six tricyclic anti-depressants (TCAs). The separation of these closely related compounds was performed under isocratic mobile phase conditions with mass spectrometric (MS) detection. Baseline resolution was achieved with a total separation time of 3 minutes demonstrating not only the potential speed of the Ascentis Express columns but also the resolving power. Note the MS compatible mobile phase and flow rate. Furthermore, the use of 2.1 mm I.D. columns provides a reduction in solvent consumption compared to typical flow rates for 4.6 mm I.D. or monolithic columns.

Data in **Figure 2** further illustrates the speed in which closely related compounds can be resolved using the Fused-Core particle. In this example, four β-blockers are resolved in less than one minute under isocratic conditions utilising MS detection. While a 10 cm column was utilised for the TCAs separation, a 5 cm column was used for the β-blockers example.

The separation of three steroids as well as a related impurity and degradant is shown in **Figure 3**. A high mobile phase flow rate of 0.6 mL/min was utilised and is suitable for Ascentis Express columns due to the Van Deemter curve associated with these columns. Isocratic mobile phase conditions were utilised as well as UV detection at 200 nm, a common detection wavelength

for impurity profiling. Again, baseline resolution was achieved for all compounds with a total runtime of less than two minutes. It should be noted that the isocratic conditions used in these examples further enhances sample throughput versus gradient conditions due to no need for column re-equilibration. With a backpressure of just 4500 psi, this analysis could be performed on almost any HPLC system. A similar separation was attempted using a sub-2 μm particle column but was not possible given the same instrument constraints put on the Ascentis Express column.

Conclusions

Fast HPLC is increasingly becoming a reality for all phases of pharmaceutical product development from discovery to production. Ascentis Express HPLC columns provide the high efficiency and low backpressure that make it a suitable candidate for fast HPLC using conventional instruments such as an Agilent 1100. These columns also provide proportionate performance improvements on new, ultra-high pressure systems compared to sub-2 μm particles because the higher efficiency per unit backpressure characteristic of the innovative Fused-Core particle is a fundamental technology advance.

Featured Products

ID (mm)	Length (cm)	Ascentis Express C 18	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

Did you know?

Selecting the Right Buffer

A partial list of common buffers and their corresponding useful pH range is supplied. Perhaps the most common buffer in HPLC is the phosphate ion, although, with the growth of LC-MS, volatile buffers such as TFA, acetate, formate and ammonia are becoming more frequently used. Remember, the purpose of a buffer in the mobile phase is to inhibit a pH change in the mobile phase after the introduction of a sample. When developing a method, it is important to select a mobile phase with a final pH at least one pH unit away from any analytes pK value. As a rule of thumb, one should work within a ± 1 pH unit of the buffer pKa. Typical buffer concentrations for HPLC tend to be 10–100 millimolar level.

Guidelines for Preparing Mobile Phases

It should be understood that slight variations in pH and buffer concentration could have a dramatic effect on the chromatographic process; consistent and specific techniques should be a regular practice in the preparation of mobile phases. A common practice is to place a sufficient amount of pure water into a volumetric flask and add an accurate amount of buffer. The pH of the solution should be adjusted, if necessary. Then dilute to final volume of water prior to adding or blending of organic solvents. Add a volumetrically measured amount of organic solvent to obtain the final mobile phase. Thorough blending, degassing and filtering prior to use is also recommended.

To view a listing of suitable HPLC and LC-MS additives and solvents, visit sigma-aldrich.com/lc-ms-solvents

Buffer	pKa @ 25 °C	Useful pH Range
Trifluoroacetic acid (TFA)	0.5	<1.5
Phosphate 1	2.1	1.1 – 3.1
Formate	3.8	2.8 – 4.8
Acetate	4.8	3.8 – 5.8
Phosphate 2	7.2	6.2 – 8.2
Ammonia	9.2	8.2 – 10.2
Phosphate 3	12.3	11.3 – 13.3

Chiral HPLC Separations Using Astec CHIROBIOTIC™ TAG Stationary Phases

Tom Beesley tom.beesley@sial.com

Broad chiral selectivity, easy method development protocols, good resolution, fast analysis time, robustness and reproducibility are all desired attributes of a superior chiral stationary phase (CSP). Astec CHIROBIOTIC CSPs offer many of these attributes for a wide variety of chiral compounds.

CHIROBIOTIC stationary phases are manufactured by linking various macrocyclic glycopeptides through multiple covalent bonds to silica surfaces. To date, vancomycin (V/V2), teicoplanin (T/T2) and ristocetin (R) glycopeptides have been commercialised. The difference in V/V2 and T/T2 pairs is the technology used in the bonding process. The alternative attachment to the surface often provides complementary differences in chiral selectivity. An additional CSP has been developed whereby the sugar moiety of teicoplanin glycopeptide has been removed prior to bonding. This is referred to as teicoplanin aglycone or TAG.

The CHIROBIOTIC TAG CSP has proven especially unique in the CHIROBIOTIC series. TAG has demonstrated effectiveness in resolving the enantiomers of underivatised amino acids with simple mobile phases like methanol/water with excellent selectivity. TAG has been applied to chiral amino acid separations of serine, isoleucine and many other underivatised amino acids. Separation conditions are often appropriate for LC-MS analyses. **Figure 1** shows an excellent separation of serine enantiomers using a very simple, LC-MS compatible mobile phase. CHIROBIOTIC TAG has not only shown improved separation over other CSPs, it provides unique selectivity. TAG is the only known CSP to separate carnitine (gamma amino acid) and all of its analogs. See **Figure 2**.

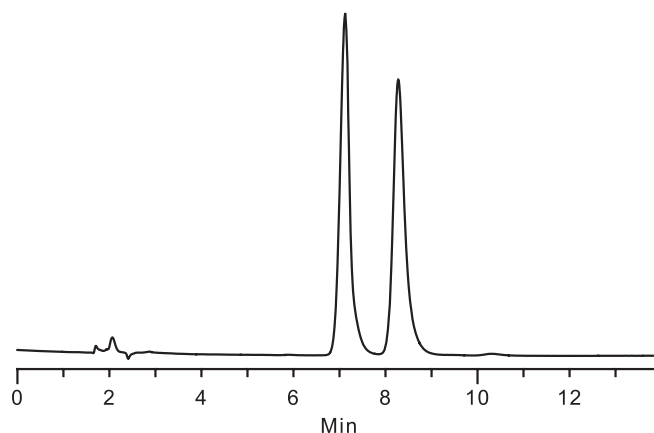
Astec CHIROBIOTIC CSPs offer many desirable attributes for chiral separations. Alternative bonding strategies as in the V/V2 and T/T2 series as well as modifications of the glycopeptide itself as in the T/TAG series show unique and complementary selectivity towards a wide variety of chiral compounds. Selectivity of TAG towards amino acid enantiomers using simple, LC-MS compatible mobile phases as shown in this report is just a sample of what the CHIROBIOTIC series of CSPs can accomplish.

Featured Products

Description	Cat. No.
Astec CHIROBIOTIC TAG, 25 cm x 4.6 mm, 5 µm	14024AST
Astec CHIROBIOTIC TAG, 15 cm x 4.6 mm, 5 µm	14023AST
Astec CHIROBIOTIC TAG, 15 cm x 2.1 mm, 5 µm	14019AST

For LC-MS solvents, see page 9.

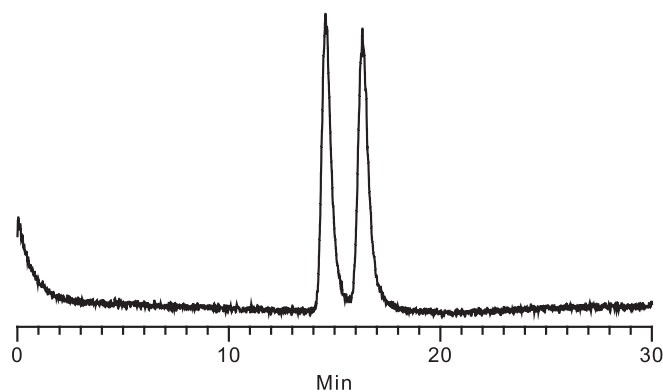
Figure 1 Separation of Serine Enantiomers using CHIROBIOTIC TAG



G004065

Column: Astec CHIROBIOTIC TAG, 25 cm x 4.6 mm I.D., 5 µm particles (14024AST)
 Mobile phase: 30:70, water:acetonitrile
 Flow rate: 1 mL/min.
 Temp.: ambient
 Det.: UV, 210 nm
 Injection: 5 mL
 Sample: 5 mg/mL in 50:50 water:methanol

Figure 2 LC-MS Chromatogram of Carnitine



G004061

Column: Astec CHIROBIOTIC TAG, 25 cm x 4.6 mm I.D., 5 µm particles (14024AST)
 Mobile phase: 25:75, 5 mM ammonium acetate, pH 6 with concentrated acetic acid: methanol
 Flow rate: 0.6 mL/min, split to the MS
 Temp.: 35 °C
 Det.: MS, ESI(+) in Selected Ion Recording (SIR) mode
 Injection: 5 µL
 Sample: 1 mg/mL in 25:75

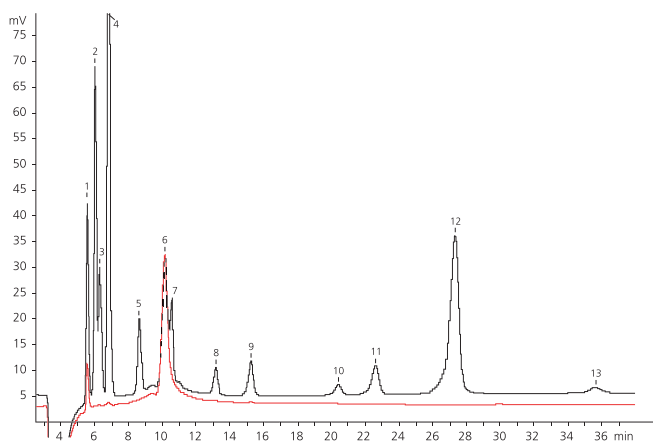
High-Purity Water for Ion Chromatography

Nicole Amann nicole.amann@sial.com

Water is an omnipresent variable in many chemical analyses, whether it is used as a reagent, eluent mobile phase, cleaning agent, sample solvent or reference material. Its composition must be well characterised and controlled in order to obtain sensitive, precise, accurate and error-free results and maintain instrument performance. To maintain the purity from the high-quality production, storage containers must be chosen that do not leach contaminants and are impermeable to atmospheric gases.

Ion chromatography (IC) is an area where the quality of the water is especially important because it is used as an eluent for ppm- to ppb- and sometimes even ppt-level determinations of anions and cations. Researchers at Sigma-Aldrich's Fluka brand have developed a quality grade of water specifically for sensitive IC applications. This IC-grade water is suitable for trace analysis of anions, cations and also some organics that are typically analysed by IC. To ensure long-term quality, it is supplied in special storage containers that have been proven in extended storage tests (Figure 1).

Figure 1 Anion chromatogram from a 4-month leaching test of the 2.5 L HDPE bottle



r2201527
r2221327

The black line shows the anions and organics in the concentration of the specification limits. The peaks are: (1) fluoride, (5) chloride, (7) nitrite, (8) bromide, (9) nitrate, (10) phosphate, (11) sulphate, (13) iodide with 1 µg/kg each (2) formiate, (3) glycolate, (4) formiate and (13) oxalate with 10 µg/kg each (6) system peak from carbonate eluent

The importance of water quality is tested according to national and international norms that have been developed for the specification of purified water for different applications. ISO 3696:1987, for example, specifies three different types of water. Type 1 is the highest analytical grade for high-purity analysis like HPLC or IC trace analysis and is largely free of dissolved impurities (inorganic, organic), colloidal impurities, specified for conductivity, UV absorption at 254 nm and SiO₂ content. ASTM D1193-91 also specifies high-purity water but also uses TOC, resistance, sodium and chloride levels to measure quality. Also, trace analysis by IC according to ASTM D5542-94, for example, requires high-purity water due to the low detection levels. At the very minimum, type 1 water is necessary to provide satisfactory results for trace analysis, as today's instruments are sensitive to even ppt-levels of analytes and contaminants.

Sigma-Aldrich has long supplied a broad range of high-quality water products tailored around the specific needs of the myriad analytical chemistry applications. In keeping with this tradition, we now offer an IC-grade of water produced at our Buchs, Switzerland facility, using various purifying stages and highly sensitive online-analyses. The product packaging has been designed to ensure that the quality of the water will be maintained when it is put into use by the consumer.

Specifications for Fluka Brand IC-Grade Water

Anion Traces

- Bromide, chloride, fluoride, iodide, nitrate, nitrite, phosphate, sulphate: ≤ 1 µg/kg each

Cation Traces

- Al, Ba, Bi, Cd, Co, Cr, Cu, Fe, Li, Mg, Mn, Mo, Ni, Pb, Sr, Zn: ≤ 5 µg/kg each; Ca, K, Na: ≤ 10 µg/kg each; NH₄⁺: ≤ 50 µg/kg

Organic Traces

- Acetate, formate, glycolate, oxalate: ≤ 10 µg/kg each

Conductivity

- ≤ 2 µS/cm

Featured Product

Description	Pkg. Size.	Cat. No.
Water for Ion Chromatography	2.5 L and 5 L	00612
Packaging: HDPE bottle with 45 mm thread		



High-Purity CHROMASOLV® Solvents, Blends and Additives for LC-MS Analysis

Shyam Verma shyam.verma@sial.com

LC-MS is a preferred analytical tool for chromatographic separation and detection due to its high sensitivity and specificity. A high-level performance is required in identification and quantification of complex compounds. It is, therefore, necessary to use purified and well-specified solvents. Using pure solvents, blends and additives can help to improve detection for a complex spectral analysis and keep the instrument source clean.

LC-MS CHROMASOLV Solvents:

- Water
- Acetonitrile
- Methanol
- 2-Propanol
- Ethyl acetate

Presence of contaminants in the solvent can clog the inlet filter of a Nano-LC instrument. High-purity LC-MS CHROMASOLV solvents offered by Sigma-Aldrich ensure clean system lines and a stable, low-gradient baseline. Results of a suitability test for LC-MS CHROMASOLV solvents based on the reserpine specification test measured in LC-MS CHROMASOLV methanol shows a clean signal with no other clusters of peaks (Figure 1).

Low metal ion content (especially sodium and potassium) is an important specification for LC-MS solvents as these metal ions tend to form dominating clusters with many compounds, like peptides, and pose problems in downstream analysis. For example, the normal ionisation product with electro spray for human gastrin peptide (M=2097) is the protonated, double-charged molecular ion with $m/z = 1049.8$ $[M+2H]^+$. When it is dissolved in 0.2% formic acid water solution with a very low amount of sodium and potassium (<0.1 ppm), only few metal ion clusters appear. The molecule mass peak is sharp and further fragmented to yield amino acid sequence (Figure 2). The observed clusters are in a relatively low abundance.

Figure 1 Reserpine Test: reserpine spectrum measured in Methanol LC-MS CHROMASOLV; no signals should be greater than $[M+H] = 609$ (100 ppb reserpine; ESI, positive mode)

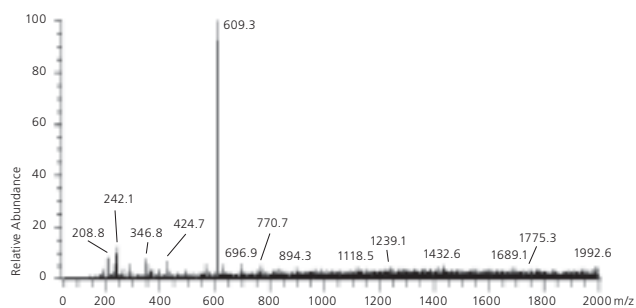


Figure 2 ESI Mass Spectrum of Human Gastrin in LC-MS CHROMASOLV Water

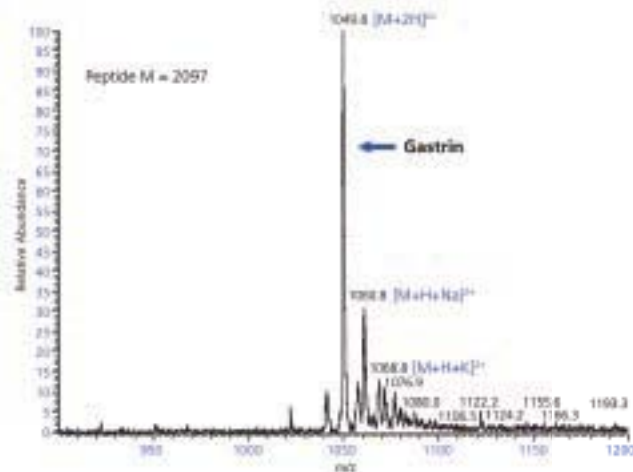
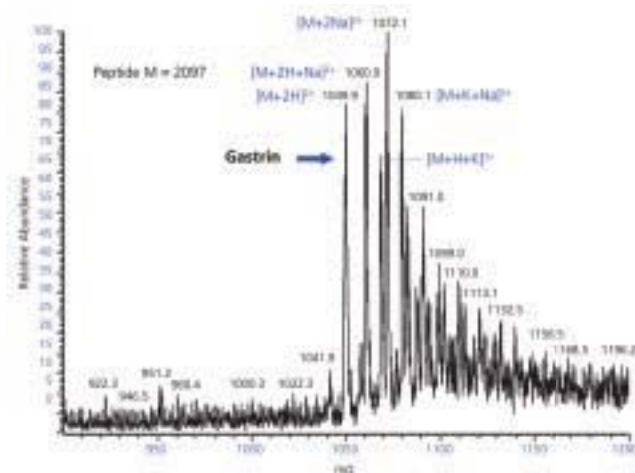


Figure 3 ESI Mass Spectrum of Human Gastrin Dissolved in Water with a Sodium and Potassium Content > 10 ppm



However, when the metal ion content is high (10 ppm), the clusters formed with these metal ions become dominating (Figure 3) and make it difficult to identify true peaks.

LC-MS CHROMASOLV Solvent Blends

Use of highly specified solvents spiked with ultra pure salts and acids minimise the background and artefacts in LC-MS analysis. The most common additives include trifluoroacetic acid (TFA), formic acid, acetic acid and ammonium acetate. Sigma-Aldrich offers a line of pre-mixed solvents ready to use.

Mobile Phase Additives for LC-MS

Buffers and other ionic additives are often added to LC-MS mobile phases. These additives offer many benefits, such as pH control, low adsorption, improved peak shape, selectivity or recovery, and optimised ionisation at the MS interface. Some of the most popular LC-MS mobile phase additives are formic acid, acetic acid, ammonium acetate and TFA.

LC-MS Flush Solution

Flushing the LC-MS system at regular intervals allows a stable baseline. A commonly employed flush solution is 50% isopropanol in water. It is known to effectively solubilise both hydrophilic and moderately hydrophobic contaminants. CHROMASOLV flush solutions provide the required purity.

Featured Products

Description	Pkg. Size	Cat. No.
Water LC-MS CHROMASOLV	1 L	39253
Acetonitrile LC-MS CHROMASOLV	1 L, 6x1 L, 2.5 L, 4x2.5 L	34967
Methanol LC-MS CHROMASOLV	1 L, 6x1 L, 2.5 L, 4x2.5 L	34966
2-Propanol LC-MS CHROMASOLV	1 L, 6x1 L, 2.5 L, 4x2.5 L	34965
Ethyl acetate LC-MS CHROMASOLV	1 L, 2.5 L	34972

Related Product


Description	Pkg. Size	Cat. No.
CHROMASOLV LC-MS Flush Solution (water/2-propanol 50/50, v/v)	1 L	34689


Related Information

For more information about the LC-MS CHROMASOLV product line and suitable additives not only for LC-MS, visit sigma-aldrich.com/lc-ms-solvents

Analytical Videos on sigma-aldrich.com/videos


We are pleased to announce the launch of analytical videos on our website. Through this interactive medium, we hope to provide a better understanding regarding the use and capabilities of our products and technologies.


On many of our web pages, you will find “movie strip” icons like these  which are links to the pages where


the videos reside. To view a video, click on the video camera icon. 

To see the complete listing of available videos in one location, go to sigma-aldrich.com/videos

Video topics currently available are: Karl Fischer (HYDRANAL®) Reagents, Solid Phase Microextraction (SPME), and VersaFlash high-throughput flash purification.

 **Karl Fischer Reagents:** Learn about the use of HYDRANAL through 7 instructional videos that feature volumetric and coulometric titration, the use of ovens, and standardisation of titer.

 **SPME:** 10 SPME videos illustrate the use, benefits, versatility and portability of our unique, patented sampling technique.

 **VersaFlash:** This set of 12 videos explores the innovative aspects of our high throughput flash purification system to provide a better understanding of capabilities available to a medicinal chemistry laboratory.

Coming soon! We will be adding HPLC presentations to this list.

We welcome your comments and suggestions via the on-line feedback form on any of our video pages.

sigma-aldrich.com/videos

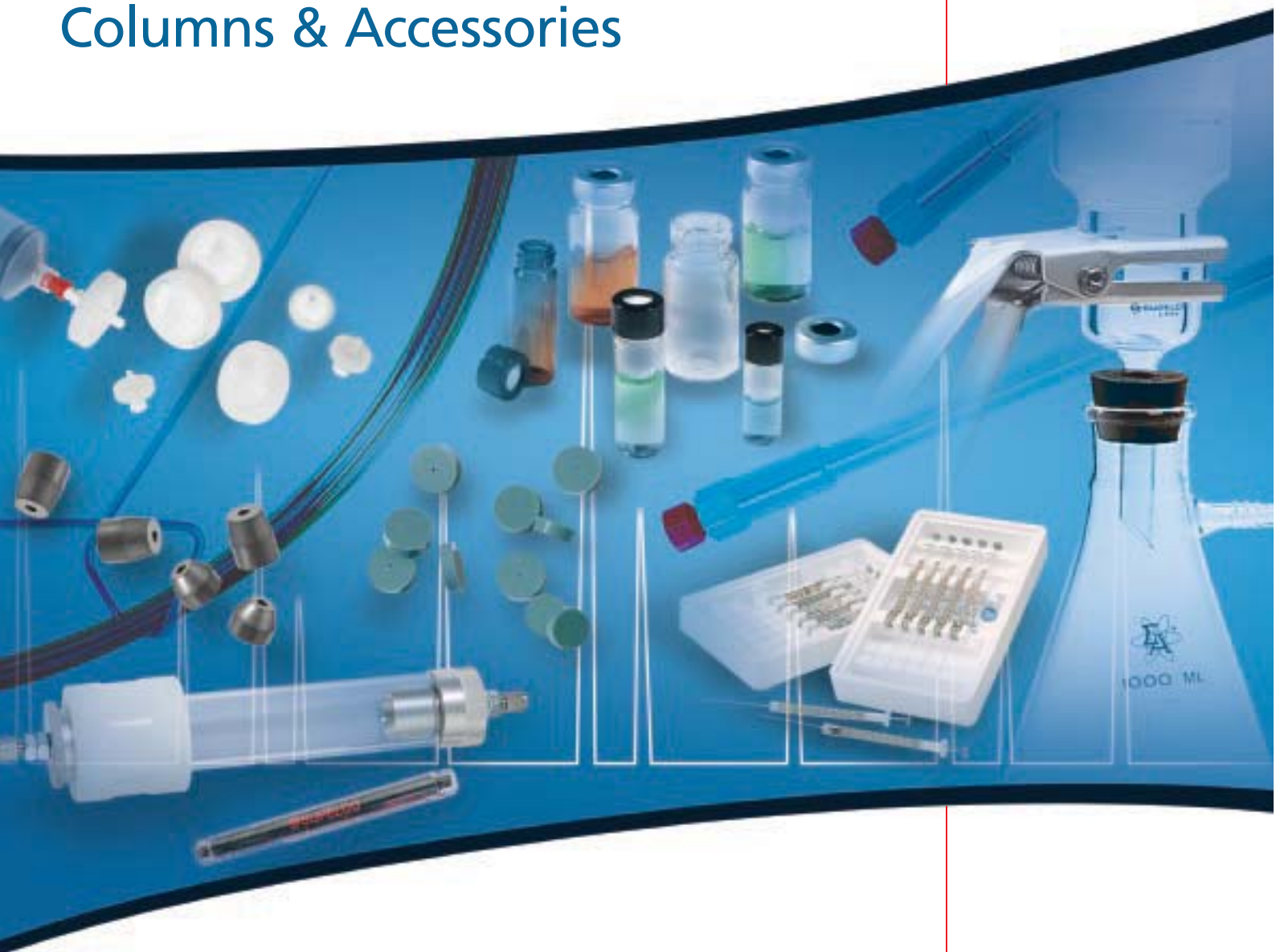


Product specific page with video files



The Complete Line

Columns & Accessories



Supelco has what you need.

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- Solvents
- Valves/Injectors
- Tubing
- Connectors/Tees
- Filters/Frits
- Sample Loops
- Vials / Syringes
- Septa
- Ferrules

For more information visit sigma-aldrich.com/supelco

The Extraction of Tobacco-Specific Nitrosamines Using Molecularly Imprinted Polymer SPE

Brian Boyd, David Lundberg, Sanja Kronauer, Anna-Karin Wihlborg¹ and An Trinh² an.trinh@sial.com

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²Supelco, 595 N. Harrison Rd., Bellefonte, PA, 16823, USA

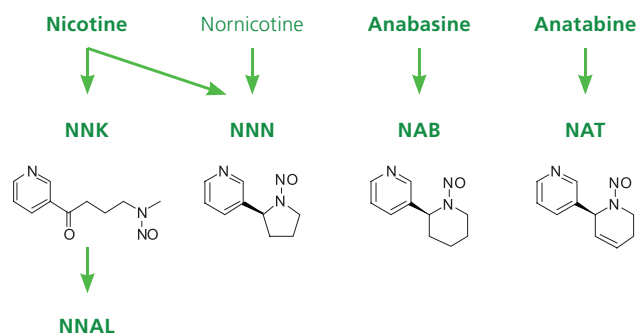
Introduction

Tobacco-specific nitrosamines (TSNAs) are created through the burning, curing and fermentation of tobacco leaf and can be found in chewing, smoking, and snuff tobacco. They are believed to play a significant role as causes of cancer in people who use tobacco products and are found on the US Surgeon General's list of carcinogens from 1989 (1). The most carcinogenic of the commonly occurring TSNAs are 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and N'-nitrosornicotine (NNN). Because TSNAs are only found in tobacco products, their characterisation is invaluable in the study of tobacco's cancerous nature. The monitoring of humans for exposure to tobacco smoke (active or passive) is an important clinical test – NNK is found in tobacco smoke in significant amounts (2).

The extraction and quantitation of TSNAs in urine is a useful biomarker when assessing a subject's exposure to tobacco smoke. TSNAs are not only found in smokers but in non-smokers (via second-hand smoke) as well. Because TSNAs are detected in urine at low ppt levels, a highly specific and sensitive assay is required. Although extraction and analysis protocols have been previously developed, many of them require extensive and time-consuming sample preparation (3). Also, such low detection limits (ppt) are not attainable using conventional SPE phases and MS/MS detection (4). A summary of the pathway of formation of TSNAs from tobacco is outlined in **Figure 1**. Since there are several TSNAs formed from precursors present in tobacco, an analytical method that is "class selective" for TSNAs is appealing. The SupelMIP SPE-TSNA is designed to offer class-selective binding sites for the 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), N'-nitrosornicotine (NNN), N'-nitrosoanabasine (NAB), and N'-nitrosoanatabine (NAT) molecules.

In this article we will show the performance of the extraction of the four different TSNAs from urine samples using a class-selective molecularly imprinted polymer SPE phase.

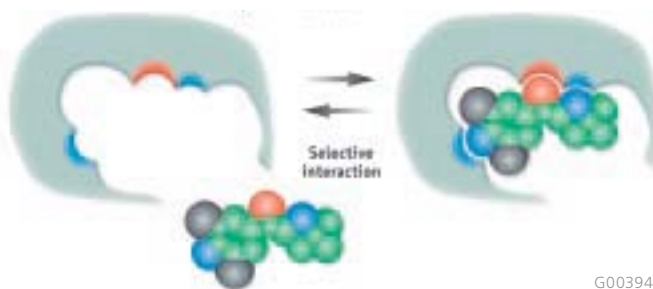
Figure 1 Formation of TSNAs in Tobacco



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 2 Illustration of a Selective MIP Cavity



Extraction and Analysis of TSNAs (NNN, NNK, NAB and NAT) Using SupelMIP SPE-TSNAs

In this study, NNN, NNK, NAB and NAT were extracted from urine using SupelMIP SPE-TSNAs via the extraction procedure described in **Table 1**. Analysis of the resulting eluate was conducted by LC-MS-MS using the procedure (5) described in **Table 2**.

Lower Limits of Quantitation in Urine

Using the procedure described, trace levels of TSNAs were determined in spiked urine, and lower limits of quantitation (LLOQ) values were determined by measuring the signal-to-noise ratio for each analyte response. Using the SupelMIP SPE protocol and LC-MS-MS conditions described in this report for analysing urine samples, the limits of detection were 5.1 pg/mL for NNN, 3.8 pg/mL for NNK, 4.7 pg/mL for NAB and 6.2 pg/mL for NAT.

The limits of quantification were 16.8 pg/mL for NNN, 12.7 pg/mL for NNK, 15.8 pg/mL for NAB and 20.8 pg/mL for NAT. **Figures 3 and 4** (pg. 13) show a typical chromatogram of an extracted urine sample spiked with 100 pg/mL of each TSNA, and the chromatograms for the corresponding blank urine samples, respectively.



Table 1 SupelMIP Extraction Procedure for TSNA_s

Sample Pre-Treatment:	
Adjust sample pH to 5.5 with acetic acid. Add 1 ng/mL NNK-d ₃ , NNN-d ₄ , NAB-d ₄ and NAT-d ₄ internal standard.	
SPE Procedure:	
1. Condition and equilibrate MIP phase with 1 mL methanol, 1 mL DI water. (Do not allow the cartridge to dry during conditioning.)	
2. Load 1 mL pre-treated urine sample.	
3. Wash (elute interferences) using the following wash scheme:	
<ul style="list-style-type: none"> • 1 mL 10 mM ammonium acetate, pH 5.5 • Apply full vacuum through cartridge for 10 min. to remove residual moisture from cartridge. • 1 mL heptane (selective removal of hydrophobic interferences) • Apply full vacuum through cartridge for 5 min. to remove residual solvent. 	
4. Elute TSNA _s with 2 x 1 mL 10% methanol in dichloromethane. Apply a gentle vacuum between each fraction.	
5. Evaporate and reconstitute with 100 µL LC mobile phase prior to analysis.	

Table 2 LC-MS-MS Conditions for TSNA_s

Column:	Ascentis C18, 5 cm x 3 mm I.D., 3 µm particles (581307-U)									
Instrument:	API 3200 MS/MS									
Injection vol.:	5 µL									
Flow:	0.5 mL/min.									
Mobile phase:	(A) 10 mM ammonium formate, pH 6.1, pH (adjusted with acetic acid); and (B) acetonitrile									
Gradient:	Time (min.)	% A	% B							
	0.0	90	10							
	1.0	90	10							
	4.0	60	40							
	5.0	30	70							
	6.0	30	70							
	6.1	90	10							
	9.0	90	10							
Flow rate:	0.5 mL/min.									
Detection: (MS/MS)	Analyte	Rt (min)	Q1	Q3	Time (ms)	DP	EP	CEP	CE	CXP
	NNN	2.9	178.20	148.10	100	22	5.0	10.0	14	5.0
	NNK	3.5	208.10	122.0	100	25	5.0	10.0	10	5.0
	NAT	4.0	190.10	160.20	100	20	5.0	10.0	14	5.0
	NAB	4.2	4.2	192.20	100	30	5.0	10.0	17	5.0
	NNK-d ₃	3.5	211.30	122.10	100	25	5.0	10.0	18	5.0
	NAB-d ₄	4.2	196.00	166.00	50	60	20	10.0	18	12.0
	NAT-d ₄	4.0	194.00	164.00	50	60	20	10.0	16	11.0
	NNN-d ₄	2.9	182.00	152.00	50	60	20	10.0	16	10.0
Ion mode:	Positive									
Ion source:	TurboSpray									
Ion spray voltage:	5500 V									
Source temp.:	500 °C									
Curtain gas:	30 psi									
Collision gas:	5 psi									
Gas 1:	40 psi									
Gas 2:	30 psi									

Recovery

Eight different urine samples were spiked to a level of 100 pg/mL with TSNA_s. These samples were extracted by the procedure described in **Table 1**. The intermediate precision was established by testing the performance of the method using three different urine samples, three different analysts, three separate lots/batches of SupelMIP TSNA_s and on two different LC-MS-MS instruments. The average recovery and variation (%RSD) of all the samples tested were $97.8 \pm 8.1\%$, $103.1 \pm 6.2\%$, $106.1 \pm 11.6\%$, $107.4 \pm 18.6\%$ for NNK, NNN, NAB, and NAT, respectively (raw data not shown).

It should be noted that during the analysis only the NNK-d₃ internal standard was available in the lab. The response from NNK-d₃ was used for the remaining three TSNA_s also. Further improvements are possible for NNN, NAB and NAT if the equivalent internal standard is used.

Ion Suppression

Eight blank urine samples were also cleaned up according to the SPE procedure for SupelMIP SPE-TSNA. The resulting extracted eluate from the SupelMIP SPE cartridges were spiked with TSNA_s before evaporation. Standards were prepared in the SupelMIP SPE elution solvent. Samples and standard solutions were evaporated and redissolved in mobile phase prior to analysis and the responses between the post-SPE blank urine spiked samples and standards were compared, and the degree of ion-suppression was calculated. The average suppression due to matrix were 4.5, 2.9, 5.3, and 4.2% for NNK, NNN, NAB, and NAT, respectively (data not shown). In general, effects due to the matrix are low from three different urine samples demonstrating the accuracy of the method.

Conclusions

In this article, we have described a novel method for the extraction of TSNA_s (NNN, NNK, NAT and NAB) from urine using SupelMIP SPE-TSNA. The SupelMIP SPE-TSNA assay described in this report took less than two hours to complete and offered the selectivity necessary to achieve detection limits in urine at the low ppt level. Recovery values for each TSNA are above 90% and ion suppression due to matrix effects is low. This performance allows for accurate determination with the required precision for measurement of TSNA_s in urine using a simple, one-step SPE cleanup step.

Figure 3 MRM Chromatogram of extracted urine sample spiked with 100 pg/mL of NNN (green), NNK (blue), NAT (brown) and NAB (pink).

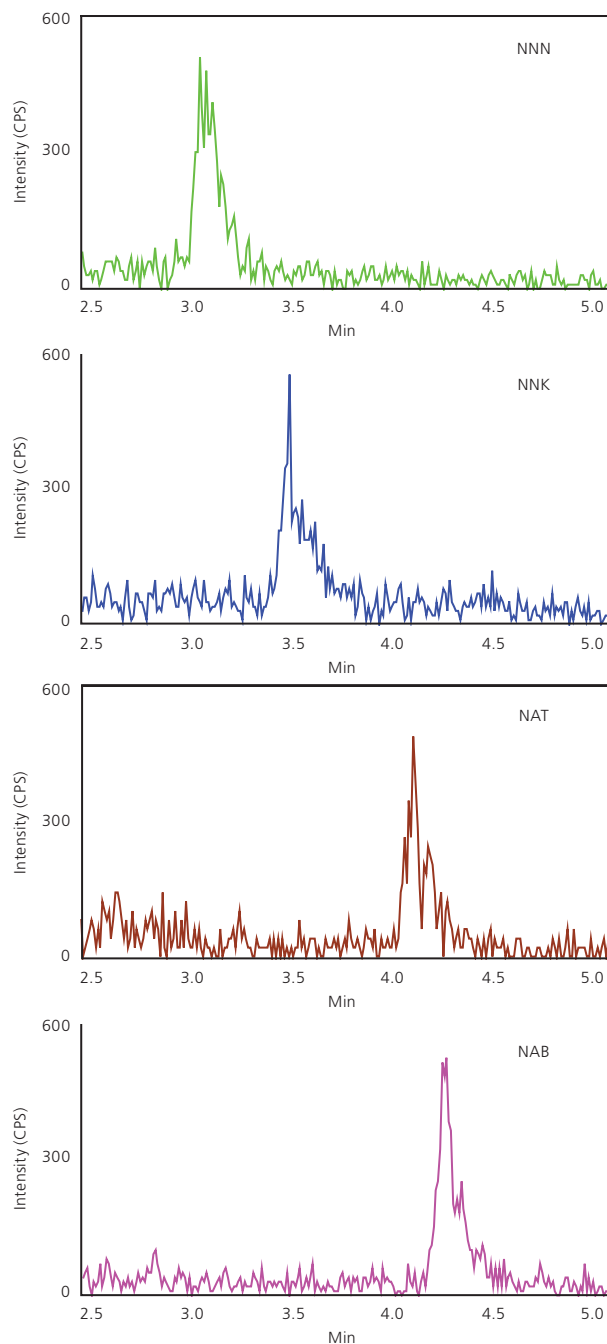
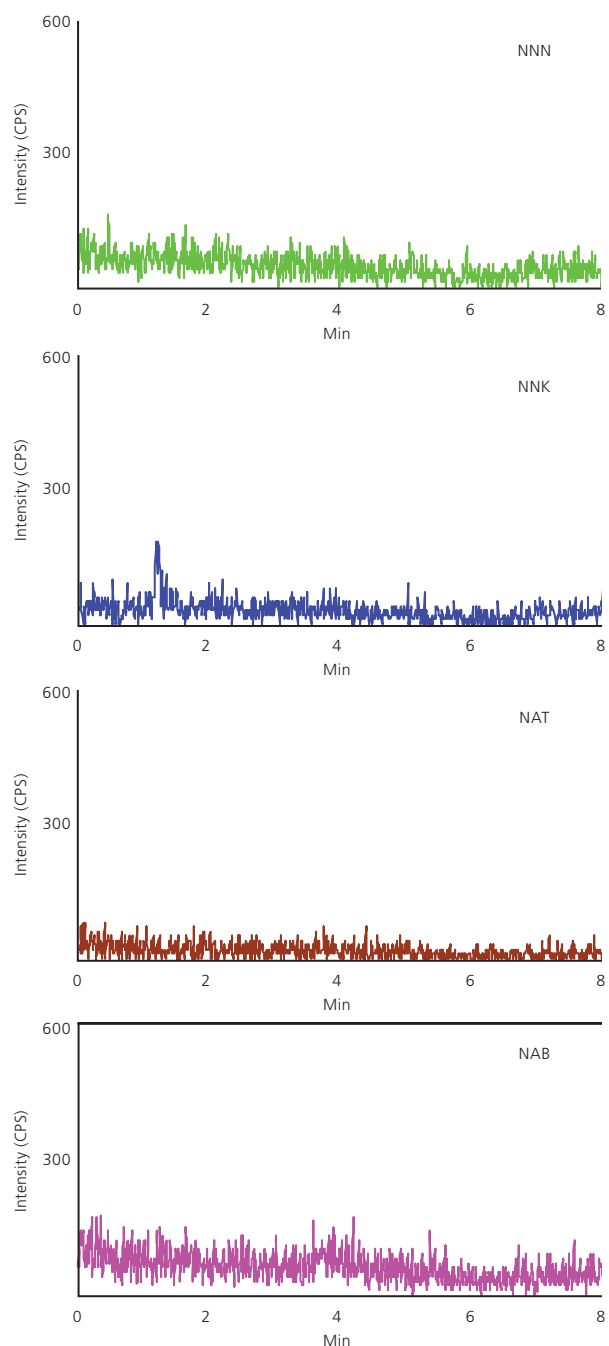


Figure 4 MRM Chromatogram of extracted blank urine sample. NNN (green), NNK (blue), NAT (brown) and NAB (pink).



Related Information

For more information on the extraction of TSNA, request the electronic format of T407117 (KAI), and for general information on SPE and SupelMIP SPE, please visit sigma-aldrich.com/spe and sigma-aldrich.com/supelmip respectively.

References

1] U.S. Department of Health and Human Services, "Reducing the Health Consequences of Smoking: 25 Years of Progress. A report of the Surgeon General, 1989".

- 2] Hecht, SS. Biochemistry, biology, and carcinogenicity of tobacco-specific N-nitrosamines. *Chem Res Toxicol* 1998; 11:559–603.
- 3] Stepanov, I and Hecht, SS. Tobacco-Specific Nitrosamines and Their Pyridine-N-glucuronides in the Urine of Smokers and Smokeless Tobacco Users. *Cancer Epidemiol Biomarkers Prev* 2005; 14(4), 885–891.
- 4] Yang, J.; Hu, Y.; Cai, J. B.; Zhu, X. L.; Su, Q. D.; Hu, Y. Q.; Liang, F. X. Selective hair analysis of nicotine by molecular imprinted solid-phase extraction: An application for evaluating tobacco smoke exposure. *Food and Chemical Toxicology* 2007; 45, 896–903.
- 5] SupelMIP SPE-TSNAs Instruction Sheet T706031, www.sigma-aldrich.com/supelmip



Discovery® Ag-Ion SPE Tubes

Silver-Ion SPE for the extraction and fractionation of cis & trans fatty acids

Retention:

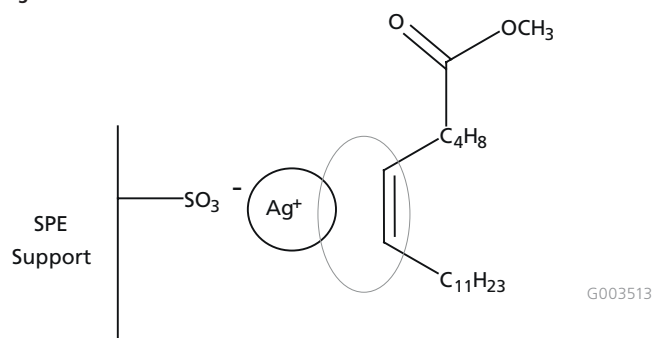
Mechanism: Normal-phase

Sample Matrix:

Compatibility: Organic solvents, oils and lipids

- Developed for the fractionation of FAMES based on degree of unsaturation and for the resolution of cis/trans isomers.
- Silver counter-ions are anchored onto a SCX support using a proprietary procedure to offer optimal resolution, performance and capacity.
- Each lot is tested and quality controlled for cis/trans FAME resolution.

Figure 1



Charge-transfer complex between Ag⁺ and unsaturated bond

Description	Qty.	Cat. No.
750 mg/6 mL	30	54225-U
750 mg/1 mL reversible cartridge	10	54226-U

MiniTip SPE – Microscale Extraction

The MiniTip SPE product line is designed for the micro-scale extraction, concentration, and concentration of small molecules and biological macromolecules. These 10 µL pipette tips contain a sorbent bed bonded at the working end of the tip, using a patented high-purity adhesive. The bed acts as a solid phase extraction medium to adsorb molecules of interest from the sample matrix. Subsequently, the concentrated, desalted analytes are eluted for downstream analysis.

MiniTip C18 Micropipette Tips are designed to extract, concentrate and/or purify macromolecules (proteins, peptides and other biological molecules) through hydrophobic interaction, and can be used for sample preparation/cleanup prior to analysis using ESI-MS, MALDI-TOF-MS and other mass spectrometric/chromatographic methods.

MiniTip Carbon Micropipette Tips are designed for the extraction, desalting and purification of oligosaccharides and glycoproteins prior to ESI-MS, MALDI-TOF-MS and other mass spectrometric/chromatographic methods.

Table 1

Description	MiniTip C18	MiniTip Carbon
	C18 bonded on spherical silica, endcapped	Graphitised Carbon Adsorbent
Particle size	50–60 µm	50–60 µm
Pore size	200 Å	175 Å
Binding capacity (per tip)	17 µg Insulin, Chain B, Oxidised 18 µg β-Amyloid 7.6 µg Bradykinin, Fragment 1–7	1.2 µg maltahexose
Cat. no. (pk. 96)	TPSC18	54227-U



G001231

VersaFlash High Throughput Flash Purification

Truly Unique

VersaFlash is one of the most simple and cost-effective disposable cartridge flash chromatography systems, yet it has capabilities that most flash systems do not have.

- Spherical Particles – provide improved efficiency and higher sample loading capacities
- Easy & Quick Scale-Up – from 1 g up to 190 g samples
- Fast Cartridge Change-Out – saves time between samples
- Stackable Cartridges – allow one-step bimodal purifications & easy solid sample loading
- Pre-Compressed Cartridges – no additional compression equipment needed
- Versatile Modular System – compatible with existing flash hardware
- Flexible Flow Direction – Rev-Elution saves solvent
- Various Sample Loading Options

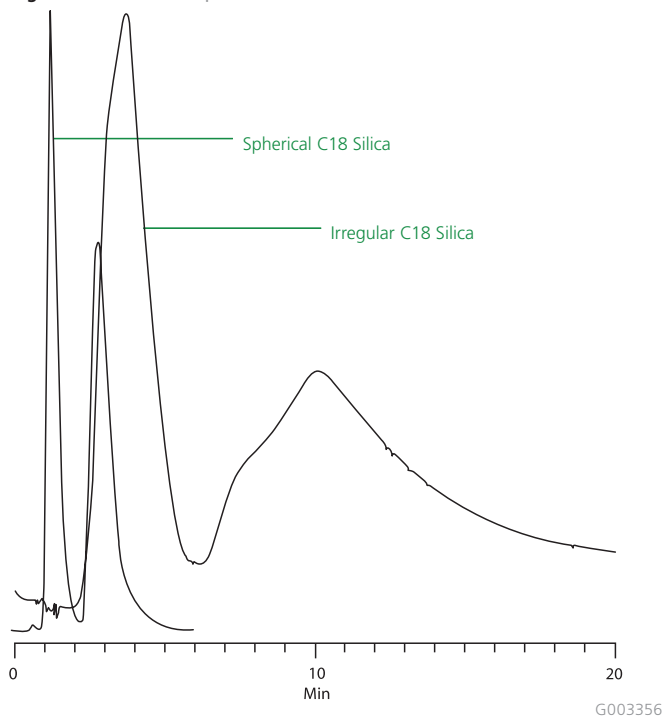


Expand Flash Capabilities with Spherical Silica

- Quicker results by shorter run times
- Decreased solvent consumption
- Smaller, more concentrated fractions, demanding less rotary evaporator time
- Higher capacity spherical silica
- Lower pressure drop allows higher flow rates

The benefits of spherical silica are shown in **Figure 1**. To use the efficient VersaPak® cartridges with spherical silica on your existing flash system, simply use the VersaFlash station as your cartridge holder for your current set up or inquire about adapters for your system. Adapters for Teledyne Isco and other instruments are available from stock!

Figure 1 Benefits of Spherical Silica



NEW! VersaFlash Demonstration CD

The new VersaFlash demonstration CD shows short video demonstrations of our flash chromatography system with pre-packed flash cartridges. The CD includes video clips of the many versatile features of the VersaFlash system including scalability, quick cartridge change-out, cartridge stacking, Rev-Elution and other unique features. The CD also includes a copy of the VersaFlash brochure, which further explains the benefits of spherical silica and gives an overview of the entire product line.

For your free copy of the Supelco VersaFlash Demonstration CD, call your local Sigma-Aldrich office and request IZI, or select IZI on the attached postcard.



NEW! Capillary Column for Fast Omega-3 and Omega-6 FAME Analyses

Leonard M. Sidisky, Katherine K. Stenerson, Greg A. Baney, and Michael D. Buchanan len.sidisky@sial.com

Introduction

As a result of changes in nutritional labelling laws and consumers' desire to have "healthier fat" in their diet, the analysis of the trans fat and the omega-3 and -6 fatty acid content of food products has become a very active area of research for many food companies. Trans fatty acids are currently noted as being a "bad fat" to have in a diet, as studies have linked their nutritional contribution to be similar to saturated fats and their possible role in coronary heart disease. The omega-3 and -6 fatty acids are important, as increased consumption of omega-3 fatty acids has been linked with reducing coronary heart disease. They also play an important role in brain development in babies.

An article in a previous issue of The Reporter discussed the fast analysis of trans fats (1). This article will focus on the fast analysis of omega-3 and -6 fatty acids.

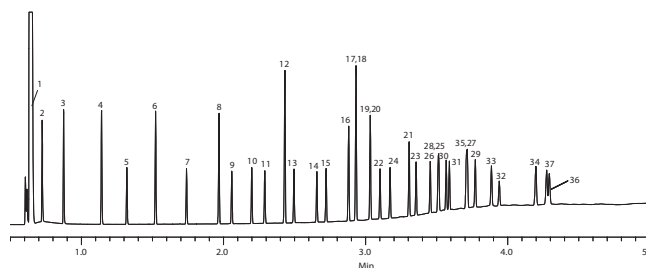
The Need for a Fast GC Column

The analysis of food products containing the omega-3 and -6 fatty acids is typically performed according to the Association of Official Analytical Chemists (AOAC) Method 991.39 (2). This method uses a polar, wax-type capillary column of 30 metres in length for the analysis of fatty acids as their corresponding methyl esters (FAMES). The analysis is complex and requires approximately 30 to 40 minutes to complete. Recently, a new version Omegawax™ capillary column was developed at Supelco. This column has the ability to significantly reduce the time required to perform the analysis of omega-3 and -6 FAMES. Column length and internal diameter have been reduced, allowing for a shorter analysis time while still maintaining resolution of key FAMES.

37-Component FAME Mix

Figure 1 demonstrates the analysis of the Supelco 37-Component FAME mix on the new 15 m x 0.10 mm I.D., 0.10 µm Omegawax 100 capillary column. The 37-Component mix is designed to mimic many types of fatty acid-containing samples, including grains, dairy, fish oils and vegetable oils. This standard can be used for the qualitative and quantitative analysis of fatty acid content in a wide variety of foods. As is typical for a polyethylene glycol-type stationary phase, the Omegawax column elutes the FAMES according to carbon chain length and degree of unsaturation, with minimal carbon chain length overlap. The elution pattern is saturated, monoene, diene, and then triene, of a carbon chain length. The analysis time is less than 5 minutes.

Figure 1 Supelco 37-Component FAME Mix



Column: Omegawax 100, 15 m x 0.10 mm I.D., 0.10 µm (23399-U)
 Oven: 140 °C, 40 °C/min. to 280 °C (2 min.)
 Inj.: 250 °C
 Det.: FID, 260 °C
 Carrier gas: hydrogen, 50 cm/sec constant
 Injection: 0.2 µL, 200:1 split
 Liner: 4 mm I.D., split, cup design
 Sample: Supelco 37-Component FAME Mix (47885-U)

G003886

1. Butyric Acid Methyl Ester (C4:0) at 4 wt %
2. Caproic Acid Methyl Ester (C6:0) at 4 wt %
3. Caprylic Acid Methyl Ester (C8:0) at 4 wt %
4. Capric Acid Methyl Ester (C10:0) at 4 wt %
5. Undecanoic Acid Methyl Ester (C11:0) at 2 wt %
6. Lauric Acid Methyl Ester (C12:0) at 4 wt %
7. Tridecanoic Acid Methyl Ester (C13:0) at 2 wt %
8. Myristic Acid Methyl Ester (C14:0) at 4 wt %
9. Myristoleic Acid Methyl Ester (C14:1) at 2 wt %
10. Pentadecanoic Acid Methyl Ester (C15:0) at 2 wt %
11. cis-10-Pentadecenoic Acid Methyl Ester (C15:1) at 2 wt %
12. Palmitic Acid Methyl Ester (C16:0) at 6 wt %
13. Palmitoleic Acid Methyl Ester (C16:1) at 2 wt %
14. Heptadecanoic Acid Methyl Ester (C17:0) at 2 wt %
15. cis-10-Heptadecenoic Acid Methyl Ester (C17:1) at 2 wt %
16. Stearic Acid Methyl Ester (C18:0) at 4 wt %
17. Oleic Acid Methyl Ester (C18:1n9c) at 4 wt %
18. Elaidic Acid Methyl Ester (C18:1n9t) at 2 wt %
19. Linoleic Acid Methyl Ester (C18:2n6c) at 2 wt %
20. Linolelaidic Acid Methyl Ester (C18:2n6t) at 2 wt %
21. Arachidic Acid Methyl Ester (C20:0) at 4 wt %
22. g-Linolenic Acid Methyl Ester (C18:3n6) at 2 wt %
23. cis-11-Eicosenoic Acid Methyl Ester (C20:1) at 2 wt %
24. Linolenic Acid Methyl Ester (C18:3n3) at 2 wt %
25. Heneicosanoic Acid Methyl Ester (C21:0) at 2 wt %
26. cis-11,14-Eicosadienoic Acid Methyl Ester (C20:2) at 2 wt %
27. Behenic Acid Methyl Ester (C22:0) at 4 wt %
28. cis-8,11,14-Eicosatrienoic Acid Methyl Ester (C20:3n6) at 2 wt %
29. Erucic Acid Methyl Ester (C22:1n9) at 2 wt %
30. cis-11,14,17-Eicosatrienoic Acid Methyl Ester (C20:3n3) at 2 wt %
31. Arachidonic Acid Methyl Ester (C20:4n6) at 2 wt %
32. Tricosanoic Acid Methyl Ester (C23:0) at 2 wt %
33. cis-13,16-Docosadienoic Acid Methyl Ester (C22:2) at 2 wt %
34. Lignoceric Acid Methyl Ester (C24:0) at 4 wt %
35. cis-5,8,11,14,17-Eicosapentaenoic Acid Methyl Ester (C20:5n3) at 2 wt %
36. Nervonic Acid Methyl Ester (C24:1) at 2 wt %
37. cis-4,7,10,13,16,19-Docosahexaenoic Acid Methyl Ester (C22:6n3) at 2 wt %

Under these conditions, minimal resolution of the cis and trans isomers is achieved on this column as shown by the resolution of methyl oleate (peak 17) from methyl elaidate (peak 18) and methyl linoleate (peak 19) from methyl linolelaidic (peak 20). In both cases, the cis isomer elutes prior to the trans isomer. For detailed cis-trans isomer resolution, a polar biscyanopropyl phase, such as the SP-2560, is required.

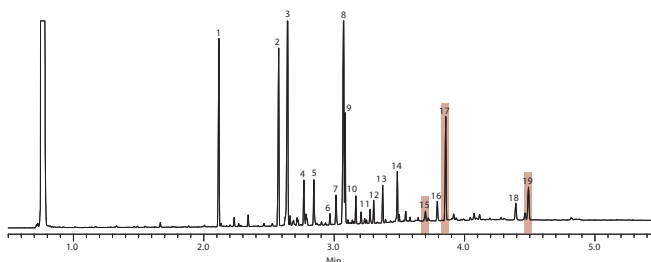
Polyunsaturated Fatty Acid (PUFA) FAME Mixes

Figures 2–4 illustrate the separation of FAME isomers from several PUFA standards. These standards are derived from different natural sources (such as marine or animal) and reflect the ratios of the omega-3 and -6 fatty acids found in “real-world” samples. These standards can be used to identify the retention times of these key fatty acids for subsequent qualitative analysis. The analysis time required for each of these mixes was under 5 minutes. Note the ability of this column to resolve the important omega-3 PUFAs C20:5n3 (EPA) and C22:6n3 (DHA) as well as the important omega-6 PUFA C20:4n6 (peaks highlighted with brown).

Conclusion

Supelco recognises the need of food chemists to achieve faster analysis times and greater throughput in order to meet the demands of today’s stricter food labelling requirements. The new Omegawax 100 capillary column provides analysts with one of the tools necessary to satisfy this need, without sacrificing the quality of the chromatography.

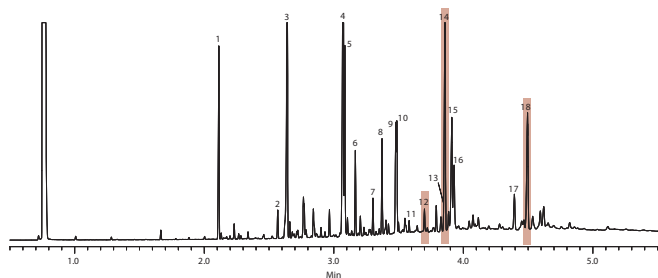
Figure 3 Menhaden Oil FAMES



Column: Omegawax 100, 15 m x 0.10 mm I.D., 0.10 μ m (23399-U)
 Oven: 140 $^{\circ}$ C, 40 $^{\circ}$ C/min. to 280 $^{\circ}$ C (2 min.)
 Inj.: 250 $^{\circ}$ C
 Det.: FID, 280 $^{\circ}$ C
 Carrier gas: hydrogen, 50 cm/sec constant
 Injection: 0.2 μ L, 200:1 split
 Liner: 4 mm I.D., split, cup design
 Sample: PUFA No. III – Menhaden Oil (47085-U), diluted to 50 mg/mL in methylene chloride

1. C14:0	11. C18:3n4
2. C16:0	12. C18:3n3
3. C16:1n7	13. C18:4n3
4. C16:2n4	14. C20:1n9
5. C16:3n4	15. C20:4n6
6. C16:4n1	16. C20:4n3
7. C18:0	17. C20:5n3
8. C18:1n9	18. C22:5n3
9. C18:1n7	19. C22:6n3
10. C18:2n6	

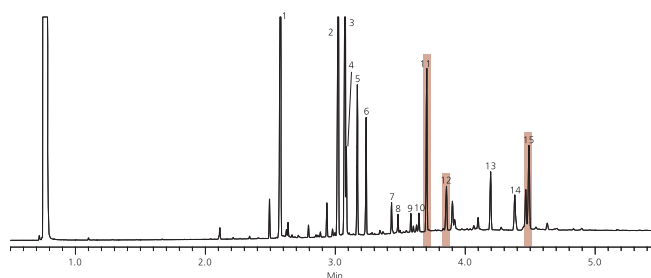
Figure 2 Marine Source FAMES



Column: Omegawax 100, 15 m x 0.10 mm I.D., 0.10 μ m (23399-U)
 Oven: 140 $^{\circ}$ C, 40 $^{\circ}$ C/min. to 280 $^{\circ}$ C (2 min.)
 Inj.: 250 $^{\circ}$ C
 Det.: FID, 280 $^{\circ}$ C
 Carrier gas: hydrogen, 50 cm/sec constant
 Injection: 0.2 μ L, 200:1 split
 Liner: 4 mm I.D., split, cup design
 Sample: PUFA No. I – Marine Source (47033), diluted to 50 mg/mL in methylene chloride

1. C14:0	10. C20:1n9
2. C16:0	11. C20:1n7
3. C16:1n7	12. C20:4n6
4. C18:1n9	13. C20:4n3
5. C18:1n7	14. C20:5n3
6. C18:2n6	15. C22:1n11
7. C18:3n3	16. C22:1n9
8. C18:4n3	17. C22:5n3
9. C20:1n11	18. C22:6n3

Figure 4 Animal Source FAMES



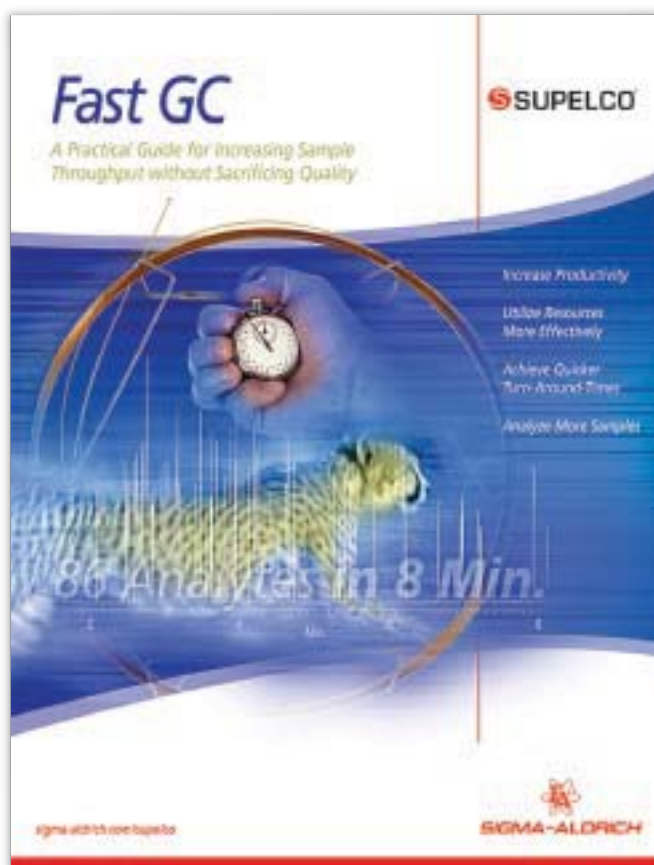
Column: Omegawax 100, 15 m x 0.10 mm I.D., 0.10 μ m (23399-U)
 Oven: 140 $^{\circ}$ C, 40 $^{\circ}$ C/min. to 280 $^{\circ}$ C (2 min.)
 Inj.: 250 $^{\circ}$ C
 Det.: FID, 280 $^{\circ}$ C
 Carrier gas: hydrogen, 50 cm/sec constant
 Injection: 0.2 μ L, 200:1 split
 Liner: 4 mm I.D., split, cup design
 Sample: PUFA No. II – Animal Source (47015-U), diluted to 50 mg/mL in methylene chloride

1. C16:0	10. C20:3n6
2. C18:0	11. C20:4n6
3. C18:1n9	12. C20:5n3
4. C18:1n7	13. C22:5n6
5. C18:2n6	14. C22:5n3
6. C18:3n6	15. C22:6n3
7. C20:0	
8. C20:1n9	
9. C20:2n9	

(continued on page 18)

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 Supelco Technical Service at EurTechServ@sial.com.



Featured Products

Description	Cat. No.
Omegawax 100, 15 m x 0.10 mm I.D., 0.10 μ m	23399-U
Supelco 37-Component FAME Mix 10 mg/mL (total wt.) in methylene chloride, 1 mL See Figure 1 for a list of components	47885-U
PUFA No. I (Marine Source) 100 mg (total wt.) See Figure 2 for a list of components	47033
PUFA No. II (Animal Source) 100 mg (total wt.) See Figure 4 for a list of components	47015-U
PUFA No. III (Menhaden Oil) 100 mg (total wt.) See Figure 3 for a list of components	47085-U

Related Products

Description	Cat. No.
Omegawax 250, 30 m x 0.25 mm I.D., 0.25 μ m	24136
Omegawax 320, 30 m x 0.32 mm I.D., 0.25 μ m	24152
Omegawax 530, 30 m x 0.53 mm I.D., 0.50 μ m	25374
BCI3-Methanol, 12% (w/w) 20 x 1 mL	33353
20 x 2 mL	33089-U
400 mL	33033
BF3-Methanol, 10% (w/w) 20 x 1 mL	33356
19 x 2 mL	33020-U
10 x 5 mL	33040-U
400 mL	33021

Related Information

For more information on the analysis of FAMES, request Bulletin 855, T110855 (AYC) or visit sigma-aldrich.com/fame. For more information, see page 14 for fractionation of cis & trans fatty acids by SPE.

References

- 1] M.D. Buchanan, Supelco The Reporter, Aug 2007; Vol. 25.4: 3–4.
- 2] Official Methods of Analysis of AOAC International, 17th edition, Revision 1 (2002).

Generate High Purity Gas with Reliability and Safety

Robert F. Wallace and Michael D. Buchanan bob.wallace@sial.com

Laboratory gas generators are a great alternative to gas cylinders. In addition to being a much more sensible source of gas from a cost standpoint, generators are safer, more aesthetically pleasing, take up less space, and do not require the labour needed to move bulky cylinders around the lab. Gas generators do not require switching systems or long runs of tubing to, or through, exterior walls. They just do their job – quietly, safely, year after year. Supelco offers gas generators from both Parker® and domnick hunter®. Additionally, high-quality air compressors from Jun-Air® and critical particle and oil filters from Norgren® are offered.

Economical

Some gas chromatographers are unsure whether there is an economic advantage to replacing gas cylinders with gas generators. It is simple to compare the costs of using gas cylinders to the cost of purchasing and operating gas generators. In many situations, the change to a gas generator will result in large savings over time.

Table 1 Example Payback Time for Four GCs, Each with Dual FIDs
A total of 240 mL/min. of hydrogen is required (equates to 4,455 cubic feet of hydrogen per year)

Gas Cylinders/Year:	23 (each contains 196 cubic feet of gas)
Cost/Cylinder:	\$110 (includes rental fees)
Cost of Gas/Year:	\$2,530
Cost of Gas/Month:	\$211
Cost of Generator:	\$8,661 (Parker Model H2PEM-260)
Payback Time:	41 months (3.4 years)
Savings/Year After Payback:	\$2,530

Note: Gas cylinder price and rental fees are based on costs at Bellefonte, PA, USA and are used for illustrative purposes. Your costs will probably differ.

To determine the costs of gas cylinders versus gas generators, estimate the total volume of gas needed for a year. For an accurate comparison, include other costs associated with gas cylinders, such as cylinder rental fees and direct labor for handling cylinders. **Table 1** summarises the time required to recover the purchase cost of a hydrogen generator (payback time). The savings result because gas generators operate for many years after the payback time, essentially generating free gas. To determine the number of months until payback, divide the monthly cost of using gas cylinders into the total cost of purchasing a gas generator. In most situations, the use of a gas generator often results in savings in the thousands after just a few years.

Because helium generators are not available, helium cylinders supplying carrier gas cannot simply be replaced with a gas generator. However, a switch from expensive helium to hydrogen as a carrier gas will allow the helium cylinders to be replaced with a hydrogen generator that will pay for itself in a very short time. In addition to the economy, hydrogen has a greater flow range over which efficiency is high and generally appears to be a better carrier gas for capillary GC.

Safe

Safety plays a major role in justifying hydrogen generators, but also plays a role in decisions concerning other gas generators. The explosive nature of hydrogen along with the many other safety issues associated with high-pressure gas cylinders should be considered.

- If a leak develops in a gas line, the entire contents of a gas cylinder will be emptied into the laboratory space. If the gas is hydrogen, it may result in an explosive mixture.
- Every time a gas cylinder valve is opened, a regulator can fail, releasing full pressure into the lines. Very few plumbing systems will withstand the 2000 to 3000 psi of pressure that would be instantly introduced into the system.
- Handling heavy gas cylinders may lead to back injuries. If a gas cylinder is dropped, it may crush a worker's hands or feet.

All of these scenarios lead to a dangerous situation for laboratory workers. The use of gas generators can help avoid these potential problems. The safety benefits that gas generators have over gas cylinders are:

- A minimal volume of gas is stored
- The gas that is stored is at a relatively low pressure
- The risk of injury from moving heavy cylinders is eliminated

Convenient

Gas generators are more convenient and reliable than gas cylinders. Their use eliminates shutting down operations during gas cylinder change-out, shuttling one gas cylinder into place while moving another out of the way, continuously handling orders and invoices, and delivery delays waiting for gas cylinders to arrive. Additionally, gas generators are more aesthetically pleasing than big, bulky gas cylinders.

! Related Information

For more information on gas generators, request the Gas Generator Brochure, T407110 (JXP), or visit our website, sigma-aldrich.com/gc



Quality Pesticide Standards Available from Sigma-Aldrich

Vicki Yearick vicki.yearick@sial.com

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To complement our PESTANAL grade standards, we stock several popular Chem Service brand pesticide and pesticide metabolite standards. We can also source many others for you in a timely manner, making it convenient to order all your chromatography supplies from Sigma-Aldrich. Each Chem Service standards grade pesticide, with the exception of technical grade, is shipped with documentation. Expiration dates are provided on the label for your convenience.

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Featured Products

Description	Concentration	Pkg. Size	Cat. No.
Neat and Single Component Solutions			
4,4'-DDT solution PESTANAL, analytical standard	100 ng/μL in methanol	2 mL	36662
Atrazine solution PESTANAL, analytical standard	100 ng/μL in methanol	2 mL	36665
Azadirachtin		10 mg	PS2075
Cypermethrin PESTANAL, analytical standard, (mixture of isomers), ≥95% (HPLC)		100 mg	36128
Famoxadone solution PESTANAL, analytical standard	100 ng/μL in acetonitrile	2 mL	33495
Fipronil PESTANAL, analytical standard		100 mg	46451
Glufosinate-ammonium PESTANAL, analytical standard, ≥95.0%		100 mg	45520
Glyphosate PESTANAL, analytical standard		250 mg	45521
Isofenphos-methyl PESTANAL, analytical standard		50 mg	33436
Malathion		500 mg	PS86
o,p'-DDT		50 mg	PS698
Oxydemeton-methyl		50 mg	PS641
Paraoxon		100 mg	PS610
trans-Permethrin solution PESTANAL, analytical standard	10 ng/μL in cyclohexane	2 mL	36893
Calibration Mixtures			
TCL Pesticides Mix – 2000 μg/mL each component in hexane:toluene (50:50)		1 x 1 mL	48913
Aldrin	4,4'-DDD	β-Endosulfan	Heptachlor
α-BHC	4,4'-DDE	Endosulfan sulfate	Heptachlor epoxide
β-BHC	4,4'-DDT	Endrin	Methoxychlor
Lindane	Dieldrin	Endrin aldehyde	
δ-BHC	α-Endosulfan	Endrin ketone	
CLP Organochlorine Pesticides Mix – 2000 μg/mL each component in hexane:toluene (50:50)		1 x 1 mL	47426-U
Aldrin	α-Chlordane	Dieldrin	Endrin aldehyde
α-BHC	β-Chlordane	α-Endosulfan	Endrin ketone
β-BHC	4,4'-DDD	β-Endosulfan	Heptachlor
Lindane	4,4'-DDE	Endosulfan sulfate	Heptachlor epoxide isomer B
δ-BHC	4,4'-DDT	Endrin	Methoxychlor
Triazine Pesticides Standard Mix – 100 μg/mL each component in methanol *		1 x 1 mL	48392
Ametryn	Prometryn	Propazine	Terbutryn
Atrazine	Prometon	Simazine	
Organophosphorus Pesticides Mix A – 2000 μg/mL each component in hexane:acetone (9:1)		1 x 1 mL	48391
Chlorpyrifos (Dursban)	Disulfoton	Fenchlorvos (Ronnel)	Methyl parathion
Dichlorvos	Ethoprophos (MOCAP)	Guthion	Prothiofos (Tokuthion)

* For a highly selective sample clean-up of triazines, molecularly imprinted polymer SPE is available. Visit sigma-aldrich.com/supelmip for details.

Manufacturing and testing information is available for each Supelco brand calibration mixture in the form of a data packet. Each data packet documents the rigorous analytical methods we use to verify raw material identity and purity, and provides certification as to purity and final concentration accuracy. Data packets can be requested free of charge with a standard order.

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concerns with you and make suggestions where needed to improve the quality of your purchase. If you are interested in a customised pesticide standard, please feel free to contact us by email at customstandards@sial.com, or use the quote request form on our website sigma-aldrich.com/standards

Examples of the many neat, single and multi-component pesticide and pesticide metabolite standards available through Sigma-Aldrich are listed on the previous page. To view all our standards, please visit sigma-aldrich.com/standards

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DEA Drug Reference Materials Available from Sigma-Aldrich

Vicki Yearick vicki.yearick@sial.com

Sigma-Aldrich offers more than 2000 drug compounds as reference materials for use by toxicologists, forensic scientists, and medical researchers. The large collection of drug compounds includes controlled substances as defined by the Single Convention On Narcotic Drugs Treaty of 1961. This international treaty serves as the foundation for global drug control. Under this treaty, controlled substances are further classified as Schedule I, II, III, IV or V drugs. This classification is based on the degree to which dependency or drug-induced physical and/or psychological impairment may occur (refer to **Table 1**).

Table 1 Definitions for Schedule I – V Drugs

<p>Schedule I Drugs</p> <ul style="list-style-type: none"> - High potential for abuse - No currently accepted medical use in the U.S. - Lack of accepted safety for use of the drug under medical supervision
<p>Schedule II Drugs</p> <ul style="list-style-type: none"> - High potential for abuse - Currently accepted medical use in the U.S. - Abuse may lead to severe psychological or physical dependence
<p>Schedule III Drugs</p> <ul style="list-style-type: none"> - Potential for abuse less than schedule I and II drugs - Currently accepted medical use in the U.S. - Abuse may lead to moderate or low physical dependence or high psychological dependence
<p>Schedule IV Drugs</p> <ul style="list-style-type: none"> - Potential for abuse less than schedule III drugs - Currently accepted medical use in the U.S. - Abuse may lead to limited physical or psychological dependence relative to schedule III substances
<p>Schedule V Drugs</p> <ul style="list-style-type: none"> - Low potential for abuse relative to schedule IV substances - Currently accepted medical use in the U.S. - Abuse may lead to limited physical or psychological dependence relative to schedule IV substances

In the United States, the US Drug Enforcement Administration (DEA) regulates manufacturing, importation and sale of these controlled substances. DEA drug compounds available from Sigma-Aldrich include the true illicit drugs like heroin and cocaine, therapeutic drugs that are commonly abused like the benzodiazepines, and drug metabolites. All drug compounds are of the highest quality possible to meet the needs of forensic investigators and medical personnel.

Packaging and Documentation

DEA drug compounds are offered as neat and single component solutions, in labelled and non-labelled forms. Neat compounds are available in a variety of milligram and gram packaging configuration, with larger packages available upon request. These pure compounds are shipped with a data sheet that describes the drug's physical properties and provides the purity of the drug lot.

Single component solutions are prepared from carefully analysed pure solids and packaged in flame-sealed ampules. A certificate of analysis is supplied with every purchase and is also easily accessible at sigma-aldrich.com

Ordering Information

US Customers

Orders for neat controlled substances must be placed in writing and are available only to those licensed by the DEA. Telephone orders will not be accepted. For information on obtaining a DEA licence, contact the DEA at 800-882-9539 or visit www.deadiversions.usdoj.gov

Orders for Schedule I and II neat drugs will also require the submission of DEA Form 222. Instructions on how to properly complete and submit the form can be found at sigma-aldrich.com or by calling 800-521-8956, ext. 2595. If you do not know the drug's schedule number, please visit our website where information on the drug's classification can be found under the product listing.

Single component drug solutions prepared in methanol are considered exempt. No DEA licence or Form 222 need be submitted to purchase these materials.

Outside of the US

For DEA sales outside of the USA, you may be required to submit an import licence or import permit issued by your government agency that corresponds to the US Drug Enforcement Administration. Contact your local Sigma-Aldrich office if you are not sure which documents are required.

Below is a sampling of the many exempt single component DEA drug solutions available through Sigma-Aldrich. Please visit sigma-aldrich.com to view all our DEA drug products.

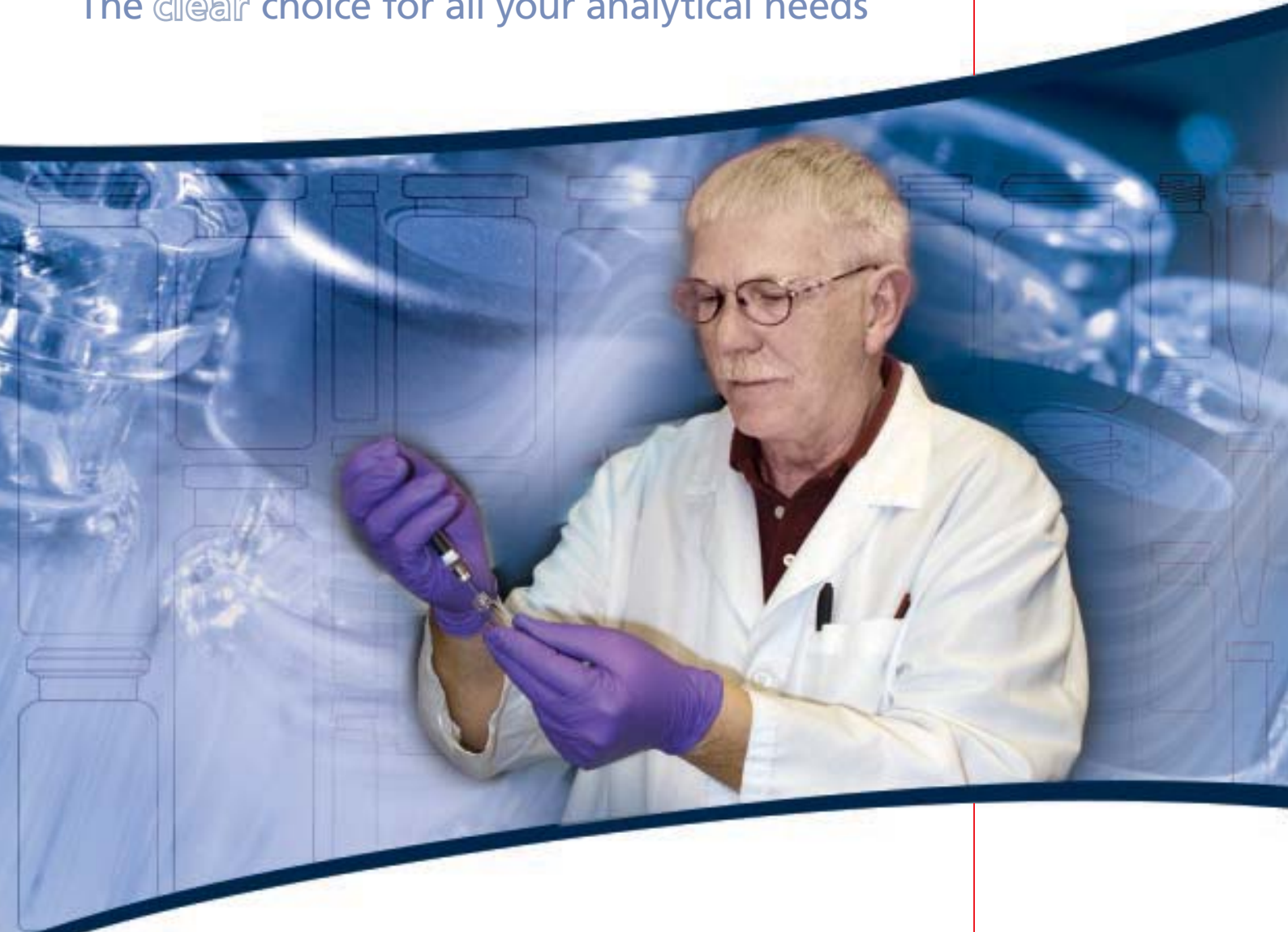
Featured Products

Description	Concentration	Cat. No.
Benzoylcegonine	1000 µg/mL	B8900
Cannabinol	1000 µg/mL	C6520
Cocaine hydrochloride	1000 µg/mL	C1528
Codeine	1000 µg/mL	C1653
D-Amphetamine sulphate	1000 µg/mL	A3278
Diazepam	1000 µg/mL	D9900
Fenfluramine hydrochloride	1000 µg/mL	F1884
Morphine sulphate	1000 µg/mL	M9524
(-)-Nicotine	1000 µg/mL	N5511
Oxycodone hydrochloride	1000 µg/mL	O2628
Phencyclidine	1000 µg/mL	610305
Secobarbital	1000 µg/mL	S4006

All solutions are prepared in methanol. Pkg size is 1 mL.

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