

A Technical Newsletter for Analytical & Chromatography

The Reporter

Issue 26, May 2007, International

Ascentis[®] Express
HPLC Columns

SUPELCO[®]

HPLC/LC

Introducing Ascentis Express..... 3

HPLC Separation of Fluorinated
Pharmaceutical Compounds on
Ascentis Columns 6

Simple and Fast Methods for Chiral
Amino Acids and Peptides on
CHIROBIOTIC[™] Stationary Phases..... 9

Sample Handling

Selective Extraction of Chloramphenicol
Using SupelMIP[™]..... 11

radiello[®] - Diffusive Sampler with Sampling
Rates Close to Active Sampling 14

GC

Alcohols Gas Chromatography
Separation in Spirits..... 15

The Use of Derivatization Reagents
for GC..... 17

Extend the Lifetime of Your Capillary
Columns With Guard Columns and
Butt Connectors..... 19

Standards & Reagents

Explosive Calibration Standards for Site
Assessment and Remediation..... 21

New! Ginsenoside Certified Reference
Materials..... 22

Versa Vial[™] Autosampler Vials..... 23

Super-Rugged

Hyper-Fast

HD-Resolution

NEU Ascentis[®] Express
– Fused-Core[™] Particle Technology Doubles
the Speed and Sensitivity of HPLC

sigma-aldrich.com/supelco



SIGMA-ALDRICH

NEW Ascentis® Express – Fused-Core™ Particle Technology Doubles the Speed and Sensitivity of HPLC

Dear Colleague,



During a break at a recent chromatography users group meeting, I struck up a conversation with a woman who directs a contract testing laboratory. After exchanging introductions, she began describing a dilemma she faced: to be competitive in business she needed to increase the number of samples her lab processes per day, but she wasn't sure if retooling – investing in expensive ultra-high pressure systems and their dedicated columns – was the right way to go. She also worried about the transferability of methods developed on these special, and currently rare, systems.

There is no denying that the need for analytical speed has sparked interest in ultra-high pressure LC, carried out using special pumps and hardware, and columns packed with sub-2 μm particles. But, is ultra-high pressure really necessary? Is there a way that HPLC users can get the resolution and sensitivity they need, along with the speed of sub-2 μm particles, but use the HPLC and LC-MS systems they already have in place?

The answer is yes. Yes, you can have the speed, efficiency and sensitivity of sub-2 μm particles but at pressures that permit their operation on any LC system. This significant paradigm shift has come about because of a new particle platform developed by HPLC pioneer Jack Kirkland and termed Fused-Core™ technology.

To bring this innovation and its dramatic benefits to our customers, we have incorporated this new particle platform into our Ascentis line of premier HPLC columns. Ascentis Express, as we have called this new column, is introduced in the article that appears on page 3. Although its name reflects its speed advantage, speed is by no means its only advantage. Indeed, Ascentis Express delivers unmatched efficiency and sensitivity – on existing LC systems. We will continue to develop the Ascentis Express story in subsequent issues of The Reporter this year.

HPLC is a mature technology and users have become accustomed to incremental improvements in column technology. However, I can't overstate the revolutionary nature of Ascentis Express. Imagine a particle that produces HPLC columns with twice the efficiency of 3 μm particles and half the backpressure sub-2 μm particles, and it can be used on your existing HPLC and LC-MS equipment.

Lest you come away thinking that Ascentis Express is only as good in terms of speed and efficiency as sub-2 μm particles, here is a surprise: Since they have the same efficiency per unit length at half the backpressure, you can achieve twice the speed (for the same total efficiency) by increasing flow rate, or twice the efficiency (for the same pressure) by using longer columns on Ascentis Express. We call it the "kinetic advantage" and the article on page 3 gives the full explanation.

I hope you find the articles in this issue of The Reporter both interesting and useful. As for me, I'm going to call that lab director and tell her that her dilemma may be solved!

Sincerely,

Dr. Klaus Herick
European Sales Development Manager, HPLC

The Reporter is published 5 times per year by Sigma-Aldrich
MarCom Europe, Industriestrasse 25, CH-9471 Buchs SG, Switzerland
Publisher: Sigma-Aldrich Marketing Communications Europe
Publication Director: Ingo Haag, PhD
Editor: Isabell Davies-Falch

Ascentis Express: High Speed, Efficiency and Sensitivity HPLC Separations with any LC System

Wayne K. Way, Ph.D.,
wway@sial.com

Designed for high speed and high resolution, Ascentis Express has all the efficiency benefits of sub-2 μ m particles, but without the high column backpressure that restricts the use of sub-2 μ m particles to ultra-high pressure instruments.

Exceeding the performance of other "fast" HPLC particles

Designed to deliver speed and resolution on all LC systems, Ascentis Express meets and exceeds the benefits of competitive particles, including 3 μ m and sub-2 μ m particles. Under the same conditions and using the same dimensions, Ascentis Express columns generate half the backpressure of sub-2 μ m particles and nearly twice the efficiency of 3 μ m particles with only slightly higher backpressure.

Ascentis Express provides extreme performance on any HPLC, LC-MS or UPLC™ or other ultra-high pressure LC system:

- Hyper-Fast
- High Definition "HD"-Resolution
- Super Sensitive
- Super Rugged

Compared to 3 μ m particles:

Advantage: Double the efficiency. Ascentis Express columns have nearly twice the column efficiency of 3 μ m particles.

Compared to sub-2 μ m particles:

Advantage: Ascentis Express columns can be run successfully on conventional, mid-pressure and ultra high pressure HPLC and LC-MS instruments.

Advantage: Double the column length. Longer Ascentis Express columns can be used, giving additional resolving power.

Advantage: Double the flow rate. Run Ascentis Express column at higher flow rates for faster analyses.

The particle platform innovations behind Ascentis Express

Like most modern HPLC particles, Ascentis Express particles are high surface area spheres made from highly pure silica gel. The total particle diameter is 2.7 μ m. However, here the comparison ends. What sets apart Ascentis Express from conventional HPLC particles is the patent pending Fused-Core technology. Ascentis Express

particles comprise a solid 1.7 μ m diameter silica core which is encapsulated in a 0.5 μ m thick layer of porous silica gel (For a scheme of a fused-core particle see page 5).

There are six distinct properties of Ascentis Express particles that account for their high performance and are worth emphasizing:

1. The solid core.

Because of the solid core, analytes cannot diffuse as deeply into the particle, resulting in less band broadening, and hence higher efficiency and sensitivity, compared to totally porous particles of the same diameter (Figure 1).

Figure 1. Higher Efficiency of Ascentis Express Compared to 3 μ m Particles Gives Better Sensitivity

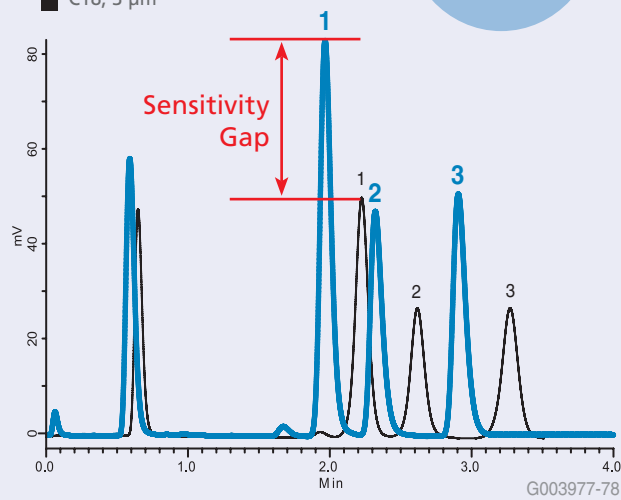
column: Ascentis Express C18, 5 cm x 2.1 mm I.D., 2.7 μ m particles (53822-U) and C18, 5 cm x 2.1 mm I.D., 3 μ m particles
mobile phase: 35:0:65 or 35:4:61, 25 mM dibasic ammonium phosphate (pH 7.0):water:acetonitrile
flow rate: 0.2 mL/min.
temp.: 35 °C
det.: UV at 220 nm
injection: 1 μ L

1. Quinidine
2. Fluoxetine
3. Diphenhydramine

■ Ascentis Express C18

■ C18, 3 μ m

HIGHER
EFFICIENCY
RESULTS IN
HIGHER
SENSITIVITY



2. The 0.5 μ m porous shell surrounding the solid core.

The porous shell gives the particles a surface area comparable to totally porous particles for excellent phase loading and sample capacity.

3. The total particle diameter (2.7 μ m).

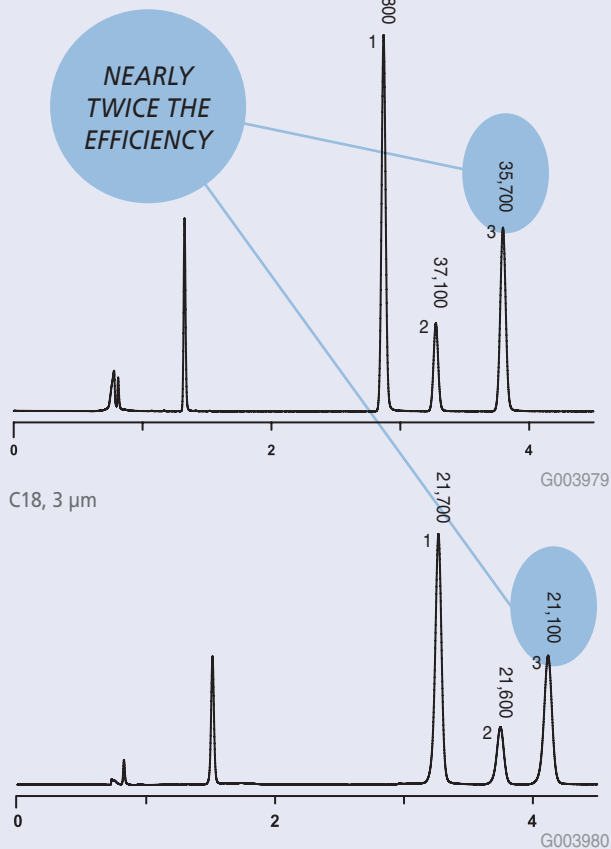
Compared to sub-2 μ m porous particles, Ascentis Express yields half the column backpressure, allowing longer column lengths and faster flow rates.

Compared to 3 μm porous particles, Ascentis Express yields nearly twice the efficiency (Figure2).

Figure 2. HD-Resolution on Ascentis Express Compared to 3 μm Particles

column: Ascentis Express C18, 15 cm x 4.6 mm I.D., 2.7 μm particles (53829-U) and C18, 15 cm x 4.6 mm I.D., 3 μm particles
mobile phase: 35:65 or 27.5:72.5, water:acetonitrile
flow rate: 1.5 mL/min.
temp.: ambient
det.: UV at 220 nm
injection: 2 μL

Ascentis Express C18



Ascentis Express: High speed, high efficiency separations adaptable equally to R&D and routine analysis settings

The recent introduction of UPLC™ and other ultra-high pressure LC systems addressed the need for high throughput separations. However, speed is not the only important criteria: the need for more sensitivity, more resolution and improved ruggedness of the technique has led to a continual stream of new LC and LC-MS instruments. Coupled with the large installed base of conventional HPLC instruments for QA/QC and other routine analyses, this result is that most laboratories have a mixture of instruments, old and new. Whereas columns packed with sub-2 μm particles have to be run on ultra-high pressure instruments, Ascentis Express columns can be run on any LC system. Methods developed on Ascentis Express can be readily and reliably transferred from R&D to routine analysis labs, whether across the building or across the world.

We hope this article has sparked an interest in Ascentis Express and the benefits it can bring to your laboratory. Subsequent Reporter articles will develop the Ascentis Express message by focusing on specific features and application areas.

Ascentis Express Properties

- Ultra-pure, Type B Silica
- 1.7 μm solid core particle with 0.5 μm porous silica shell (effective 2.7 μm)
- 150 m^2/gram surface area (comparable to $\sim 225 \text{m}^2/\text{g}$ porous particle)
- 90 \AA pore size
- Monomeric bonding chemistry and maximized endcapping
- pH Range: 2 – 9
- Maximum Pressure: 9,000 psi (600 bar)

4. The tight particle size distribution.

Compared to both sub-2 μm and 3 μm particles, Ascentis Express provides longer column lifetime because the tight particle size distribution allows us to use larger pore size frits (2 μm vs. 0.5 μm) which are less susceptible to fouling.

5. The high particle density

By virtue of the solid core, Ascentis Express particles yield a more densely packed bed for added stability and long column lifetime.

6. The high purity, type-B silica

Excellent peak shape on Ascentis Express is ensured because of the absence of highly adsorptive active sites, including metal ions and certain types of free silanol groups.

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	3	53805-U	53844-U
3	5	53811-U	53848-U
3	7.5	53812-U	53849-U
3	10	53814-U	53852-U
3	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

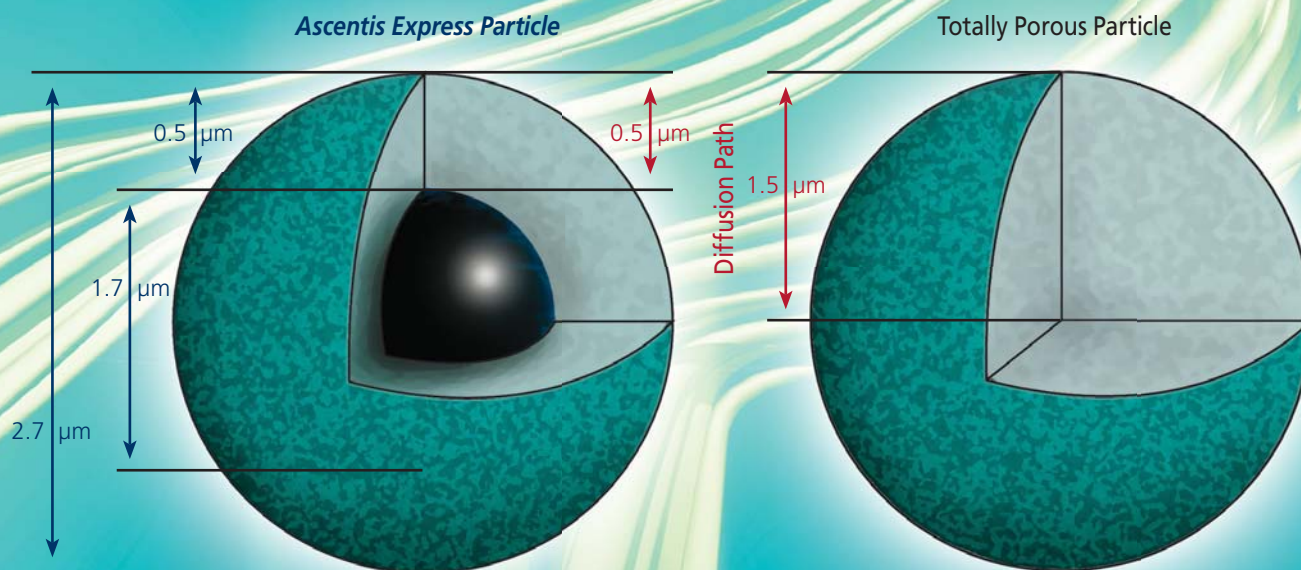
Fused-Core is a trademark of Advanced Materials Technology, Inc.

UPLC is a trademark of Waters Corp.

A Breakthrough in HPLC Column Technology

High Speed and Efficiencies with Low Backpressures

Fused-Core Structure of Ascentis Express Compared to Totally Porous Particles



Ascentis[®] Express
HPLC Columns

Ascentis Express columns provide a breakthrough in HPLC column performance.

Based on Fused-Core™ particle technology, Ascentis Express provides the benefits of high speed and high efficiencies of sub- $2\ \mu\text{m}$ particles at much lower backpressures. Due to the high efficiencies at low backpressures, Ascentis Express can benefit both conventional HPLC users as well as UPLC™ or other ultra pressure system users.

Ascentis Express Extreme Performance benefits include:

- Double the efficiencies of conventional $3\ \mu\text{m}$ particles
- Equal efficiencies of sub- $2\ \mu\text{m}$ columns at half of the backpressure
- Rugged design capable of high pressure operation

For more information on Ascentis Express HPLC Columns, email us at EurTechServ@sial.com

HPLC Separation of Fluorinated Pharmaceutical Compounds on Supelco Ascentis Columns

Choices in stationary phase selectivity provide optimal retention and resolution of fluorinated corticosteroids, fluorinated pyrimidine nucleosides and floxacins

Jacynth A. M. McKenzie and Wayne K. Way

wway@sial.com

Introduction

Fluorinated compounds possess distinct characteristics that confer desirable physico-chemical properties compared to their non-fluorinated analogs. Their abundance in pharmaceuticals, chemicals, consumer products and as environmental pollutants dictates the need for a reliable analytical method. Ascentis HPLC columns, by virtue of their efficiency and choices in stationary phase selectivity, are ideally suited for the analysis of fluorinated compounds.

The isostere replacement of the H in a C-H bond with a fluorine atom confers unique biological, chemical and physical properties on the molecule

Current Interest in Fluorinated Compounds

Fluorinated compounds and their H-containing analogs are isosteres because both atoms possess the same number of valence electrons. Isosteres are studied based on the premise that similarities in properties of elements within vertical groups of the Periodic Table are due to identical valence electronic configurations. The isostere replacement of the H in a C-H bond with a fluorine atom confers unique biological, chemical and physical properties on the molecule. Researchers in many areas of chemistry and biochemistry exploit these differences. Fluorinated compounds are abundant in consumer products, including coatings, lubricants, plastics, cleaners, firefighting foams, food containers, cosmetics, medical devices and pharmaceuticals (1). As a consequence, they pass into the environment prompting the need for their analysis in soil, water and biota, including animals and humans (2, 3).

Pharmaceutical Implications of Fluorination

Fluorine isostere replacement imparts different characteristics onto the drug substance. For example, it may alter lipophilicity which in turn affects absorption, uptake, distribution and excretion of the drug.

Fluorination also affects efficacy, potency, duration of action and toxicity.

A prime example of this is uracil, a natural component of RNA, and 5-fluorouracil, which is lethal if incorporated into RNA and is widely used in the treatment of cancer (4). Fluorinated compounds also are more resistant to metabolic oxidation and enzymatic cleavage, giving them better stability against microbial degradation than their non-fluorinated analogs in some cases (5, 6).

Fluorination is also used in pharmaceutical mechanistic studies. For example, Tang et al. (7) used α -fluorinated analogs as mechanistic probes in valproic acid-induced hepatotoxicity. The substitution of a fluorine atom at the α -position to the carboxylic acid group creates a derivative that contains a chemically and enzymatically inert carbon center that is not hepatotoxic.

Finally, the halogenation profile has also been used in the forensic investigation of illicit drug trafficking. For example, depending on the source, methamphetamine may contain varying degrees of I, Br and F, among other elements. (8).

The HPLC Analysis of Fluorinated Drug Substances

The prevalence of fluorinated compounds in medicine, industry and the environment requires a reliable analytical method. A detailed review of modern detection and identification methods that are specific for fluorine- and other halogen-containing compounds was written by Brede and Pedersen-Bjergaard (9). In every reference cited here, HPLC and LC/MS were the primary means for separation, identification and quantification of fluorinated compounds. HPLC is especially suited to pharmaceutical compounds because it can accommodate polar, water-soluble, thermally-labile compounds and the biological matrixes in which they are often found. HPLC also has the ability to



resolve compounds that have subtle differences in molecular structure, like isostere analogs and metabolites.

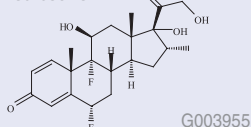
The line of Ascentis HPLC columns from Supelco offers the necessary resolution, selectivity and detector compatibility to analyze fluorinated compounds. Three examples are presented: fluorinated corticosteroids, fluorinated pyrimidine nucleosides and floxacins.

Fluorinated Corticosteroids

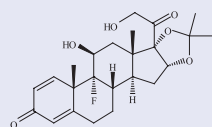
The compounds flumethasone, triamcinolone acetonide, clobetasol propionate and fluticasone propionate are topical corticosteroids used to help relieve redness, swelling, itching and discomfort of many skin problems. They share the underlying cortisone structure. The four

Structures for Figure 1

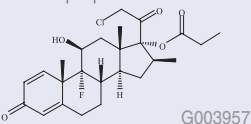
Flumethasone



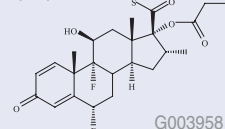
Triamcinolone acetonide



Clobetasol propionate

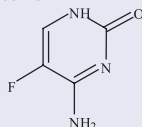


Fluticasone propionate

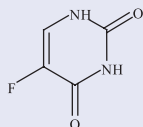


Structures for Figure 2

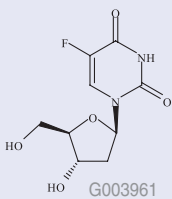
5-Fluorocytosine



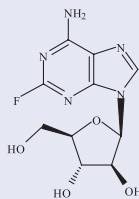
5-Fluorouracil



Floxuridine



Fudarabine

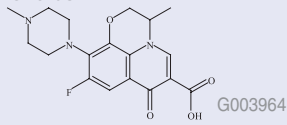


Trifluridine

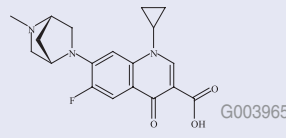


Structures for Figure 3

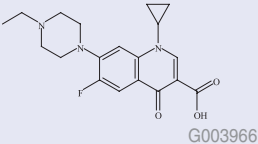
Ofloxacin



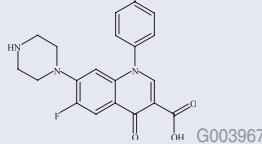
Danofloxacin



Enrofloxacin



Sarafloxacin



compounds are shown in Figure 1 separated on an Ascentis C18 column using a simple gradient of acetonitrile in water.

Fluorinated Pyrimidine Nucleosides

These compounds find primary indication for the treatment of cancer and viral infections. Their polar nature, compared to the corticosteroids, makes them ideally suited to separation on a polar-embedded HPLC phase, like the Ascentis RP-Amide shown in Figure 2.

Figure 1. Separation of Fluorinated Corticosteroids on Ascentis C18

column: Ascentis C18, 5 cm x 4.6 mm I.D., 5 µm particles (581323-U)
 mobile phase: A – water, B – acetonitrile
 gradient: time %B
 0 35
 10 65
 11 35
 flow rate: 1.0 mL/min.
 temp.: 30 °C
 det.: UV at 240 nm
 injection: 5 µL
 sample: as indicated in acetonitrile

1. Flumethasone (50 µg/mL)
2. Triamcinolone acetonide (50 µg/mL)
3. Clobetasol propionate (50 µg/mL)
4. Fluticasone propionate (50 µg/mL)

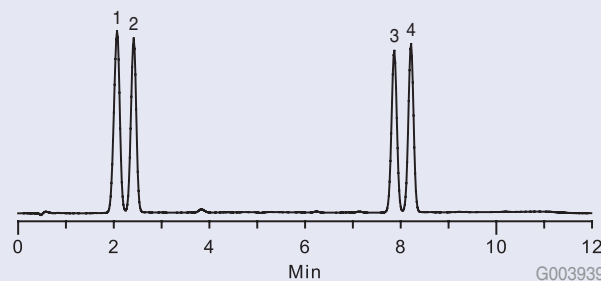
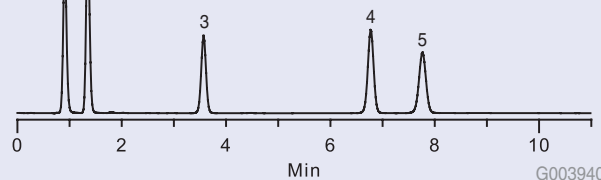


Figure 2. Fluorinated Pyrimidine Nucleosides on Ascentis RP-Amide

column: Ascentis RP-Amide, 5 cm x 4.6 mm I.D., 5 µm particles (565323-U)
 mobile phase: A – water with 0.1 % ammonium formate (pH 3.04 with formic acid), B – acetonitrile
 gradient: time %B
 0 0
 8 8
 10 8
 11 0
 flow rate: 1.0 mL/min
 temp.: 30 °C
 det.: UV at 260 nm
 injection: 5 µL
 sample: as indicated in mobile phase A

1. 5-Fluorocytosine (75 µg/mL)
2. 5-Fluorouracil (50 µg/mL)
3. Floxuridine (50 µg/mL)
4. Fudarabine (50 µg/mL)
5. Trifluridine (60 µg/mL)



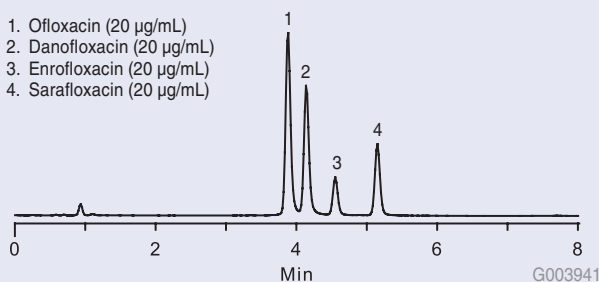
Floxacin

Floxacin are a class of anti-infective drugs. An Ascentis Phenyl column and a gradient of acetonitrile in 1% ammonium formate gave rapid, baseline resolution.

Figure 3. Fluorinated Pyrimidine Nucleosides

column: Ascentis Phenyl, 5 cm x 4.6 mm I.D., 5 µm particles (581615-U)
 mobile phase: A – water with 1 % ammonium formate (pH 3.04 with formic acid), B – acetonitrile
 gradient: time %B
 0 10
 7.5 40
 8 10
 flow rate: 1.0 mL/min.
 temp.: 30 °C
 det.: UV at 294 nm
 injection: 10 µL
 sample: as indicated in mobile phase A

1. Ofloxacin (20 µg/mL)
2. Danofloxacin (20 µg/mL)
3. Enrofloxacin (20 µg/mL)
4. Sarafloxacin (20 µg/mL)



References

1. Kissa, E. Fluorinated Surfactants: Synthesis, Properties, and Applications; Marcel Dekker: New York, 1994; Vol. 50.
2. Shultz, M.; Arofsky, D.; Field, J. Environ. Sci. Technol. 2006, 40, 289 – 295.
3. Dias, A.; Goncalves, C.; Cacú, A.; Santos, L.; Piñeiro, M.; Vega, L.; Coutinho, J.; Marrucho, I. J. Chem. Eng. Data 2005, 50, 1328–1333.
4. Longley, D.; Harkin, D.; Johnston, P. Nat. Rev. Cancer 2003, 3, 330–338.
5. New, A.; Freitas dos Santos, L.; Lo Biundo, G.; Spcc, A. J. Chromatogr. A 2000, 889 177–184.
6. Funabiki, K.; Suzuki, C.; Takamoto, S.; Matsui, M.; Shibata, K. J. Chem. Soc., Perkin Trans. 1997, 2679–2680.
7. Tang, W.; Palaty, J.; Abbott, F. J. Pharmacol. Exp. Ther. 1997, 282, 1163 – 1172.
8. Suzuki, S.; Tsuchihashi, H.; Nakajima, K.; Matsushita, A.; Nagao, T. J. Chromatogr. 1988, 437, 322 – 327.
9. Brede, C.; Pedersen-Bjergaard, S. J. Chromatogr. A, 2004, 1050, 45 – 62.

+ Featured Products

Description	Cat. No.
HPLC Columns	
Ascentis RP-Amide, 5 cm x 4.6 mm I.D., 5 µm particles	565323-U
Ascentis C18, 5 cm x 4.6 mm I.D., 5 µm particles	581323-U
Ascentis Phenyl, 5 cm x 4.6 mm I.D., 5 µm particles	581615-U
Discovery® HS F5, 5 cm x 4.6 mm I.D., 5 µm particles	567513-U
CHROMASOLV® Solvents and Blends	
Ammonium formate, puriss. p.a., for mass spectroscopy, ≤99.0%	25 g, 100 g 70221
Formic acid, puriss. p.a., eluent additive for LC-MS, ~98%	10 x 1 mL, 50 mL 56302
Acetonitrile CHROMASOLV gradient grade for HPLC, ≤99.9%	100 mL, 1 L, 2 L, 4 L 34851

LC-MS CHROMASOLV - The Highest Quality Solvents and Blends

30% off your next order:

One bottle per laboratory only. Please quote promotion code Y74 when ordering. Offer expires on July 15th 2007.

Solvent Blend	Pack size	Cat. No.
Water with 0.1% TFA LC-MS CHROMASOLV	2.5 L	34978-2.5L-R
Acetonitrile with 0.1% TFA LC-MS CHROMASOLV	2.5 L	34976-2.5L-R
Methanol with 0.1% TFA LC-MS CHROMASOLV	2.5 L	34974-2.5L-R

Sigma-Aldrich offers LC-MS CHROMASOLV solvents and pre-blended solutions that are prepared with unsurpassed attention to quality designed for meeting the stringent purity standards. These solvents and blends undergo distinct tests to ensure quality for sensitive LC-MS analysis.

LC-MS CHROMASOLV Solvents and Blends offer:

- Time savings
- Very low level of inorganic and metal ions
- No particles and non-volatile compounds
- Low gradient baseline even with your own optimized protocols



sigma-aldrich.com/lc-ms-solvents

Chiral Amino Acids and Peptides: Simple and Fast Methods Using CHIROBIOTIC Stationary Phases

Denise Wallworth
denise.wallworth@sial.com

Abstract

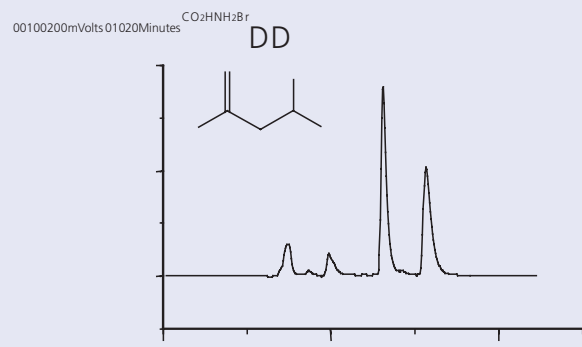
Used as chiral building blocks in enantioselective synthesis, chiral amino acids are also of interest in psychiatric and metabolic diseases. Additionally, pharmaceutical companies are now investigating structurally modified peptides as potential new drug candidates. The unique CHIROBIOTIC chiral stationary phases have largely become the method of choice for the separations of all of these in addition to a wide range of other pharmaceuticals.

Chiral amino acid separations

The separation mechanism utilises an interaction of the free carboxylic acid group of the amino acid with basic sites on the CHIROBIOTIC phases. Because the amino group of the amino acid has little influence, the method can be readily extended to cyclic and any N-blocked amino acid, especially those useful in chiral synthesis such as t-BOC and Fmoc. In contrast, earlier methods for chiral amino acids used a crown ether type of chiral stationary phase with perchloric acid as the mobile phase, requiring a free primary amine. Natural α , β and γ amino acids - aromatic, aliphatic and cyclic - are all separated without derivatisation. An extensive range of synthetic amino acids have also been separated, and an example, developed by Chirotech Technology (part of Dow Pharmaceutical Sciences) is shown in Figure 1.

Figure 1. Separation of a synthetic amino acid using CHIROBIOTIC T

column: CHIROBIOTIC T, 250 x 4.6mm
mobile phase: 50:50 MeOH:Water
flow rate: 0.5 ml/min
temp.: Ambient
det.: UV, 210nm



Method development

One of the key benefits of using CHIROBIOTIC columns for amino acid separations is the very simple mobile phases that are used, all of which are MS and ELSD compatible (1). In many cases, these are methanol or ethanol/water mixes, with formic acid or ammonium acetate buffer added for multifunctional amino acids. To increase peak efficiency (especially for bi-functional amino acids), the addition of formic acid to the mobile phase, or an elevated temperature (35-40 °C) can be used (the latter can also improve solubility).

Biocatalysis & biotransformations

CHIROBIOTIC chiral stationary phases have been shown to be invaluable for monitoring biocatalytic chiral synthesis because no sample preparation is necessary - aqueous reaction mixtures (after a quick spin to remove bacterial cells) are injected directly. And because these chiral phases are capable of detecting small structural differences, all steps in the conversion process can usually be monitored. In the example shown in Figure 2, the starting material plus two intermediates in the reaction are chiral: the method was capable of monitoring all these six enantiomers, plus the final enantiomerically pure product (D-Valine) in a single run. Direct injection has sometimes also been used in chemical catalysis, since CHIROBIOTIC columns are tolerant to all commonly used solvents, including chlorinated organics and acetone.

Neurotransmitter amino acids

Serine has been identified as a possible biomarker for schizophrenia and other psychiatric diseases; monitoring D-serine in plasma requires a highly sensitive method, now possible using LC-MS/MS and CHIROBIOTIC TAG or T (for example, reference (2)).

Peptides

CHIROBIOTIC T has been used for some time for the chiral separation of di- and tri-peptides, again using simple mobile phases such as acetonitrile with ammonium acetate or formate, using temperature as additional optimisation parameter. All four enantiomers of DL-Ala- DL-Val, for instance, separate in 7 minutes on a CHIROBIOTIC T in 50/50 ACN/10 mM NH₄OAc at 35 °C. More recently(3), it was found that much larger peptides could also be analysed using this technology (in this case, CHIROBIOTIC T and T2) and that both chiral and achiral isoforms could be readily separated. For non-chiral peptides, this method also gives

Figure 2. Determination of the conversion and enantiomeric excess of substrate / reaction products in a D-hydantoinase / D-carbamoylase reaction

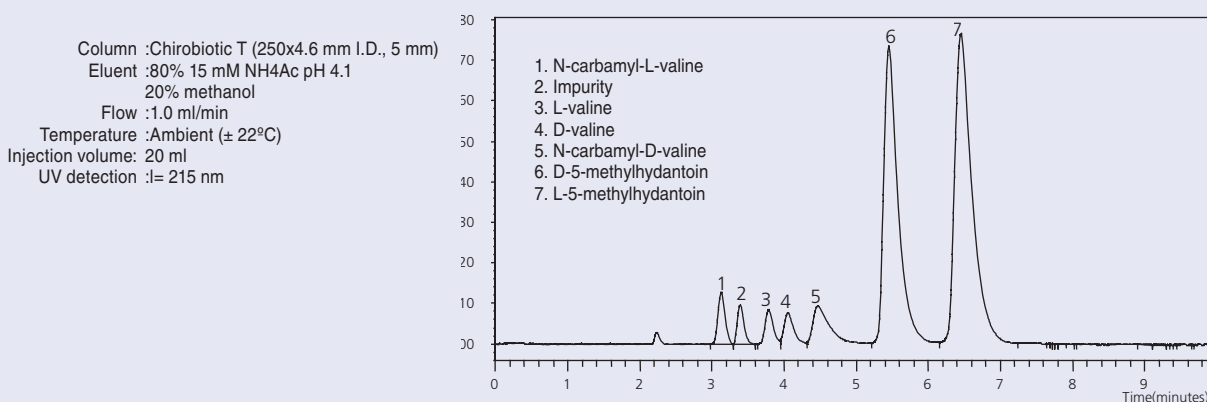
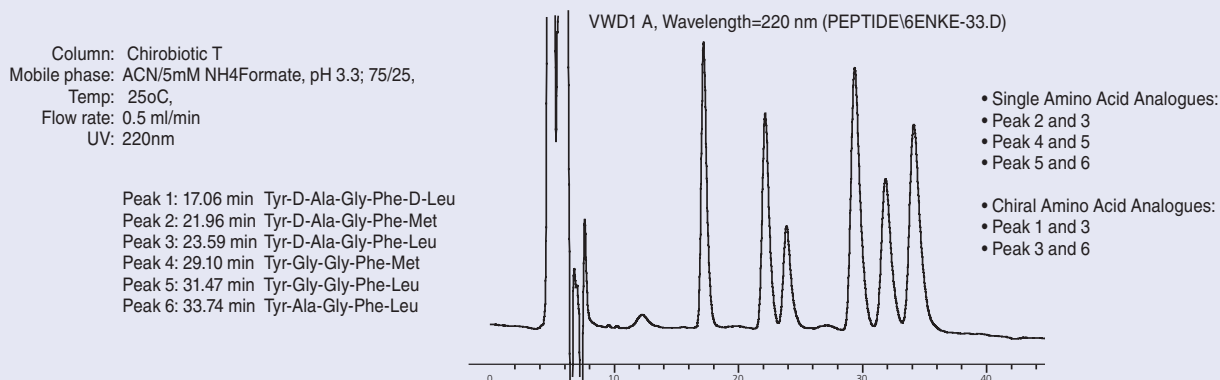


Figure 3. Separation of chiral and achiral Enkephalins in a single run



different selectivity when compared to C18, providing an orthogonal method for checking sample purity. CHIROBIOTIC columns also appear to give higher capacity than C18 for peptide purification. Figure 3 gives an example where both chiral and achiral peptide differences are resolved in a single run. Research is continuing to determine the largest peptide that can be separated by this method – to date, peptides with up to 30 residues have been separated.

Reference

1. M J Desai and D W Armstrong, J Mass Spec , 2004, 39, 177-187.
2. C Gregory, Poster at 16th International Reid Bioanalytical Forum 2005, University of Surrey, Guildford, UK.
3. B Zhang, R Soukup, D W Armstrong, J Chrom A, 2004, 1053, 89-99.

Now available: Amino Acid & Peptide Chiral Separations Handbook (JCJ) - a guide to method development and applications for free and N-blocked amino acids and peptides”

Featured Products

Description	Cat. No.
Astec Chiral HPLC Columns	
CHIROBIOTIC T HPLC Column, 5mm, 250 x 4.6mm	12024AST
CHIROBIOTIC TAG HPLC Column, 5mm, 250 x 4.6mm	16024AST
CHIROBIOTIC T2 HPLC Column, 5mm, 250 x 4.6mm	14024AST

Columns in other lengths and internal diameters are also available

Agilent® of Agilent Technologies
 Ascentis, Chromasolv are registered trademarks of Sigma-Aldrich Co.
 Fuse-Core is a trademark of Advanced Materials Technology, Inc.
 GOW-MAC® of GOW-MAC Instrument Co.
 Supelco, TraceCERT, Supeltext, CHIROBIOTIC, Equity and SUPELCOWAX are TM's of Sigma-Aldrich Co.
 SupelMIPTM is a trademark of Sigma-Aldrich Co.
 UPLC is a trademark of Waters Corp.
 VersaVial are Trademarks of Sigma-Aldrich Co.
 VESPEL® of E. I. duPont de Nemours & Co., Inc.

Selective Extraction of Chloramphenicol Using SupelMIP SPE

Olga Shimelis, An Trinh and Hillel Brandes
atrinh@sial.com

Chloramphenicol is a broad spectrum antibiotic that has recently been determined as a causative agent of aplastic anemia and possible carcinogen in humans. Thus, the EU, US and Canada have banned the use of chloramphenicol in food-producing animals and livestock. Because the drug is still widely available in developing countries and no "safe" residue levels have been determined in food, public health concerns still arise. As of today, a "zero" tolerance level has been established for this antibiotic. It is therefore critical to develop a highly selective and sensitive analytical assay to control and monitor chloramphenicol residues in difficult matrices such as food stuffs.

In this article we discuss the selective use of SupelMIP™ SPE cartridges for the extraction and analysis of chloramphenicol from milk. This method is compared against a conventional hydrophilic polymer-based SPE method obtained from a peer-reviewed journal.

What are Molecular Imprinted Polymers?

Molecular imprinted polymers are a class of highly cross-linked polymer-based molecular recognition elements engineered to bind one target compound or a class of structurally related target compounds with high selectivity. Selectivity is introduced during MIP synthesis in which a template molecule, designed to mimic the analyte, guide the formation of specific cavities or imprints that are sterically and chemically complementary to the target analyte(s). As illustrated in Figure 1, SupelMIPs are prepared by first mixing a template molecule that consists of a structural analog of the analyte(s) of interest with one or more functional monomers. The monomers form spontaneous complexes around the template. Upon complex formation, cross-linking monomers are then added with a suitable porogen (solvent

that aids in the role in pore formation) to drive polymerization. An extensive wash procedure is used to remove the template from the polymer leaving imprints or binding sites that are sterically and chemically complementary to the template.

How is Selectivity Improved Using SupelMIP SPE?

By careful design of the imprinting site, either by molecular modeling, experimental design, or screening methods, the binding cavities can be engineered to offer multiple interactions with the analyte(s) of interest. Multiple non-covalent interaction points between the SupelMIP phase and analyte functional groups allow for stronger and more specific analyte retention. Improved selectivity is then introduced through the use of harsher wash conditions during sample prep methodology. Because extraction selectivity is significantly improved, lower background is observed allowing analysts to achieve lower detection limits.

The Extraction of Chloramphenicol from Milk

In this study, an extraction method using SupelMIP SPE phase was compared against a published method using a conventional hydrophilic polymer SPE phase (1). Table 1 describes the two extraction protocols.

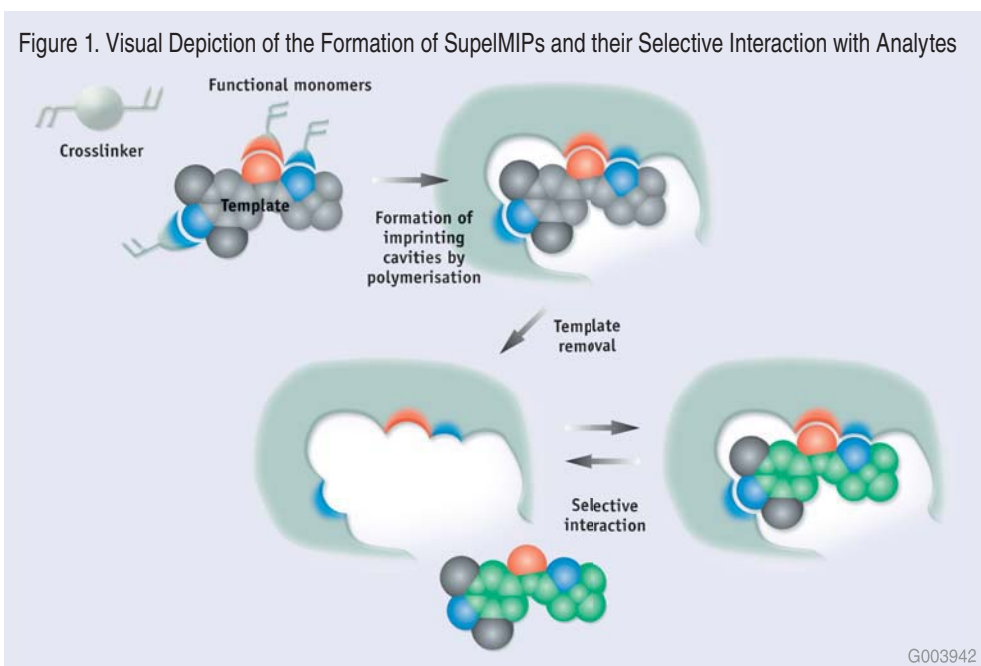


Table 1. Comparison of SupelMIP SPE Method and Conventional Method Using a Hydrophilic Polymer SPE Phase

SupelMIP SPE - Chloramphenicol Method Sample	Published Chloramphenicol Method Using Conventional Hydrophilic Polymer SPE Phase
<p>Pre-Treatment: Whole pasteurized milk (purchased from the local supermarket) was centrifuged for 15 min. at 5k rpm. The aqueous lower layer was spiked with chloramphenicol at the level of 15 ng/mL and 38 ng/mL.</p>	<p>Sample Pre-Treatment: 5 mL of milk was spiked with 40 ng chloramphenicol. Proteins were precipitated by the addition of 15 mL 10% trichloroacetic acid in water. The sample was vortexed and heated for 1 hour at 65 °C. After cooling to room temperature, the mixture was centrifuged for 15 min. at 3K rpm. The supernatant was filtered over glass wool, and the filtrate was rinsed with 10 mL DI water. The pH of the filtrate was adjusted to pH 5 with 0.1 M sodium acetate.</p>
<p>SPE Procedure: SupelMIP SPE – Chloramphenicol, 25 mg/10mL (LRC) (53210-U)</p> <ol style="list-style-type: none"> 1. Condition and equilibrate MIP phase with 1 mL methanol followed by 1 mL DI water. 2. Apply 1 mL of the pre-treated milk sample to the cartridge. 3. Elute interferences using the following wash scheme: 2 x 1 mL MS-grade water 1 mL 5% acetonitrile in 0.5% acetic acid 2 x 1 mL MS-grade water 1 mL 20% acetonitrile in 1% ammonium hydroxide Dry SPE tubes for 15 min. under gentle vacuum 3 x 1 mL dichloromethane Dry SPE tubes for 1 min. under gentle vacuum 4. Elute chloramphenicol with 2 x 1 mL methanol:acetic acid:MS-grade water (89:1:10, v/v/v) 5. Evaporate combined eluate to dryness at 50 °C under nitrogen. Reconstitute 150 µL LC mobile phase prior to LC-MS analysis. 	<p>SPE Procedure: Conventional Hydrophilic Polymer SPE, 500 mg/12 mL</p> <ol style="list-style-type: none"> 1. Condition and equilibrate SPE phase with 3 mL methanol, 4 mL DI water, and 4 mL 10 mM HCl 2. Apply the pre-treated milk extract to the cartridge. 3. Elute interferences using the following wash scheme: 4 mL MS-grade water 2 mL 5% methanol 2 mL 50% methanol 4. Elute chloramphenicol with 2 mL methanol 5. Evaporate combined eluate to dryness at 50°C under nitrogen. Reconstitute 0.4 mL DI water <p>Liquid-liquid Extraction:</p> <ol style="list-style-type: none"> 1. Liquid-liquid extract of reconstituted eluate with 0.6 mL acetonitrile:dichloromethane (4:1, v/v). 2. Centrifuge at 7k rpm for 5 min. Transfer upper organic layer to a fresh tube. 3. Repeat steps 1 & 2 of the LLE procedure two additional times on the lower aqueous layer. 4. Combine all organic layers, evaporate to dryness at 60 °C under nitrogen. Reconstitute with 0.2 mL LC mobile phase and filter through a 0.2 µm nylon filter.

Improved Selectivity and Recovery Using SupelMIP SPE

Upon sample extraction using the two procedures described in Table 1, resulting extracts were analyzed via LC-MS. Recovery was determined for each protocol against a calibration curve (data not shown) using external standards. An average chloramphenicol recovery of 84% (n=4) was obtained using the SupelMIP method and 79% (n=2) for the hydrophilic polymer SPE method. However, a pronounced difference in selectivity was determined between the two extraction methods. In Figure 2, we see that signal/noise ratio for the hydrophilic polymer SPE method was double that of the SupelMIP ion-chromatograms (320-323 m/z range); and blank milk samples processed using the SupelMIP were free of interfering responses in the elution area of chloramphenicol. In Figure 3, a significantly cleaner mass spectra is observed for the SupelMIP SPE extract relative to the conventional hydrophilic polymer extract. Also, unlike the conventional hydrophilic polymer method that required an extensive sample pre-treatment involving a protein precipitation step, an SPE cleanup procedure, and three LLE steps, the SupelMIP method only required a simple sample pre-treatment followed by a single SPE cleanup step.

Conclusion

In this report, we discussed the utility of molecular imprinted polymer SPE technology for the extraction of chloramphenicol from milk. Because selectivity is introduced during the development of the MIP phase itself, it allows for a binding site that is sterically and chemically complementary to the target analyte(s). The multiple interactions that take place between the imprint binding site and analyte(s) of interest offer strong interactions enabling the use of harsher wash conditions during the SPE process. For chloramphenicol, the SupelMIP SPE approach provided simpler methodology and significant increases in selectivity relative to the described conventional hydrophilic polymer SPE method. Both points are particularly advantageous where trace detection limits and routine analysis are required.

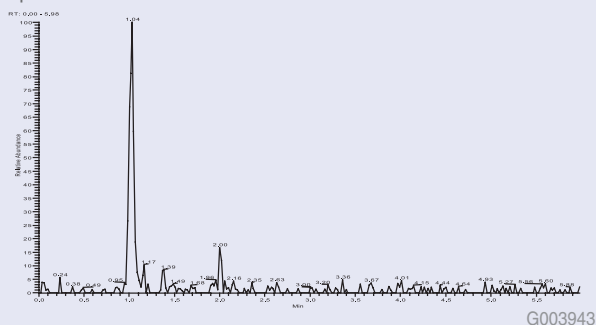
Reference

1. P.A. Guy et al. in J. Chromatogr. A 1054 (2004) 365-371

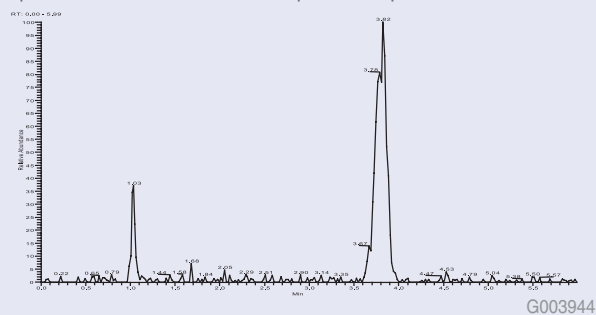
Figure 2. Chloramphenicol Spiked Milk Samples Extracted on SupelMIP SPE vs. Conventional Hydrophilic Polymer SPE

column: Ascentis C18, 2.1 mm x 10 cm I.D., 3 μ m particles (581301-U)
 instrument: Jasco HPLC interfaced with a ThermoFinnigan Advantage ion trap mass spectrometer via an electrospray ionization source
 mobile phase: 100 mM ammonium acetate (pH unadjusted):
 MS-grade water:acetonitrile (10:60:30)
 temp.: 35 °C
 flow rate: 0.2 mL/min., split to MS
 det.: MS, ESI(-) (320-323 m/z range)
 injection: 5 μ L

SupelMIP SPE Extract of Blank Milk



SupelMIP SPE Extract of Chloramphenicol Spiked Milk



Conventional SPE Extract of Chloramphenicol Spiked Milk

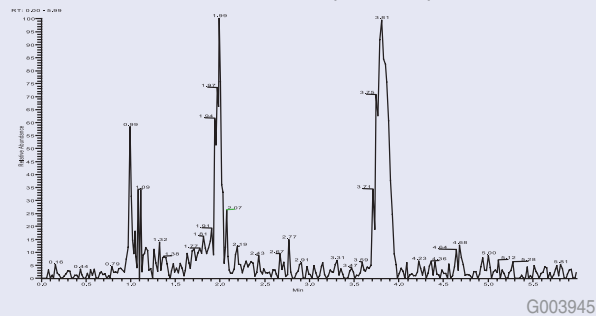
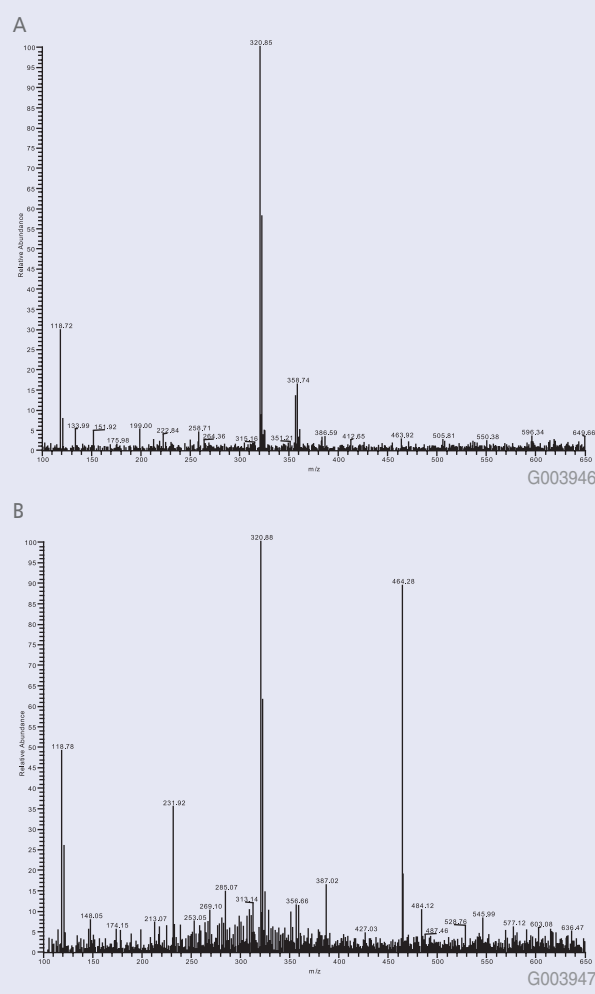


Figure 3. Mass Spectrum of Full Ion-chromatograms (3.65-4.00 min.) of the SupelMIP SPE Extract (A) and the Hydrophilic Polymer SPE Extract (B)



+ Related Products

SupelMIP SPE Cartridges	Sorbent Mass (mg)	Cartridge Volume (mL)	Cartridges per Box	Cat. No.
Clenbuterol	25	10	50	53201-U
Beta-agonists (class selective)	25	10	50	53202-U
Beta-blockers (class selective)	25	10	50	53218-U
Full Beta Receptor (Beta-agonists and Beta-blockers)	25	10	50	53223-U
Chloramphenicol	25	10	50	53210-U
NNAL (4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol)	25	10	50	53206-U
TSNAs (4 different Tobacco specific Nitrosamines: NNK, NNN, NAB, NAT)	25	10	50	53221-U
Riboflavin (vitamin B2)	25	10	50	53207-U
Triazines (class selective)	25	10	50	53208-U

radiello® - Diffusive Sampler with Sampling Rates Close to Active Sampling

Does diffusive air monitoring always require long exposure times? **Not any more!**

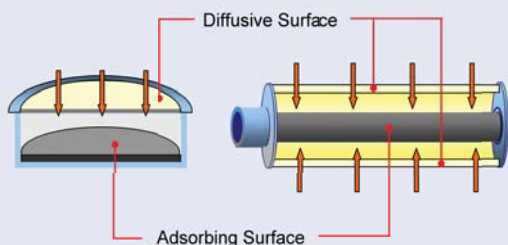
A common misconception is that diffusive or passive air samplers always require long sampling/exposure times due to the low sampling rates of conventional devices. Long sampling times is the norm for axial samplers because the parallel positioned diffusion and adsorbing surfaces are essentially the same size.

However, the **radiello®** sampler replaces the inefficient axial configuration with a radial design. Here, the diffusive surface is "wrapped" around a cylindrical adsorbent bed. The diffusion surface is larger than the adsorbing surface and therefore more analyte molecules can approach the adsorbent bed. Since the surface surrounds the adsorbent bed, the analytes can access the packing material through an entire 360° (Figure 1).

The result is a significantly higher amount of adsorbed compound in the same time period compared to an axial sampler. Sampling rates are 3- to 8-times higher for Radiello samplers than for comparable axial samplers. This enables analysts to achieve sampling times approaching those of active sampling, providing a higher sensitivity and reproducibility. In addition Radiello samplers due to their design and the used materials show a better stability against wind and weather conditions.

NIOSH method 2549 for analysis of volatile organic compounds (VOC) via active sampling (thermal desorption tubes) recommends flow rates of 10-50 mL/min. Table 1 shows the **radiello®** sampling rates for VOC/BTEX using

Figure 1. Axial and radial sampler design



activated charcoal filled adsorbent cartridges for solvent desorption, which are in the range from 8-125mL/min. There is also a version for thermal desorption available that covers a more selective analyte spectrum and provides sampling rates in the range of 20-30 mL/min.

For more information on passive diffusive air sampling and the complete line of Radiello products, please request your free copy of the Radiello brochure (IXV), the Radiello CD (IXW) or visit our Web site

www.sigma-aldrich.com/radiello.

radiello® diffusive samplers are available for:

- Aldehydes
- VOCs and BTEX
- NO₂ and SO₂
- HF
- HCl
- O₃
- Phenols
- H₂S
- NH₃
- Anesthetic gases and vapors



Table 1. Sampling rates Q (at 25°C) for Radiello sampler VOC/BTEX RAD130 (chemical desorption)

	Q ₂₉₈ mL·min ⁻¹	Q ₂₉₈ mL·min ⁻¹	
acetone	77	isobutanol	77
acetonitrile	73	isobutylacetate	63
acrylonitrile	75	isooctane	55
benzylalcohol	37	isopropanol	52
amylacetate	52	isopropylacetate	66
benzene	80	isopropylbenzene	58
bromochloromethane	70	limonene	43
butanol	74	methanol	125
sec-butanol	64	methylacetate	80
tert-butanol	62	methyl-ter-butylether(MTBE)	65
butylacetate	60	methylcyclohexane	66
2-butoxyethanol	56	methylcyclopentane	70
2-butoxyethylacetate	41	methylethylketone	79
carbontetrachloride	67	methylisobutylketone	67
cyclohexane	54	methylmetacrylate	68
cyclohexanone	68	2-methylpentane	70
cyclohexanol	54	3-methylpentane	70
chlorobenzene	68	2-methoxyethanol	35
chloroform	75	2-methoxyethylacetate	56
n-decane	43	1-methoxy-2-propanol	55
diacetonolcohol	43	1-methoxy-2-propylacetate	60
1,4-dichlorobenzene	51	naphthalene	25
1,2-dichloroethane	77	n-nonane	48
1,2-dichloropropane	66	n-octane	53
dichloromethane	90	pentane	74
N,N-dimethylformamide	82	_pinene	53
1,4-dioxane	68	propylacetate	65
n-dodecane	8	propylbenzene	57
n-heptane	58	styrene	61
n-hexane	66	tetrachloroethylene	59
1-hexanol	52	tetrahydrofuran b	74
ethanol	102	toluene	74
diethylether	78	1,1,1-trichloroethane	62
ethylacetate	78	trichloroethylene	69
ethylbenzene	68	1,2,4-trimethylbenzene	50
2-ethyl-1-hexanol	43	n-undecane	24
2-ethoxyethanol	55	m-xylene	70
2-ethoxyethylacetate	54	o-xylene	65
ethyl-tert-butylether(ETBE)	61	p-xylene	70

Separation of Alcohols and Esters in Spirits Using Serially Connected Gas Chromatography Columns

Dr. Maurizio Baccarini¹ and Michael D. Buchanan²

1. Dister SPA, Faenza, Italy, e-mail: maurizio.baccarini@dister.it

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

Introduction

In addition to containing ethyl alcohol, alcoholic spirits are complex mixtures of many compounds that may each influence the flavor of the product. It is the ratios of these other compounds that give each spirit its uniqueness of flavor. Therefore, it is critical for the alcoholic spirit industry (distilleries) to be able to identify and quantify these compounds to maintain a consistent tasting product for their consumers. Because these types of samples are complex, containing a multitude of similar compounds, it may be difficult to find the proper column to resolve all analytes. The following application, submitted by Dr. Maurizio Baccarini with Dister SPA in Faenza, Italy, provides a unique solution to this analytical problem.

Application Problem / Solution

An alcoholic mix injected at low concentration normally does not create any separation problem. However, problems become clearly visible when there is a need to inject raw products, such as when it is important to identify impurity compounds present at trace levels. It is a well-known problem that methanol/ethyl acetate co-elute on polar columns and that methanol/acetaldehyde co-elute on non-polar columns. Therefore, the need to identify each of these compounds in spirits typically requires two analyses on separate columns, each of different phase polarity.

Through many years of experience working in distilleries performing tests using different column phases, Dr. Baccarini has determined that using a combination of two columns serially-connected can solve these well-known separation problems. Table 1 describes the column sets used for the work presented in this article.

Table 1. Descriptions of Column Sets

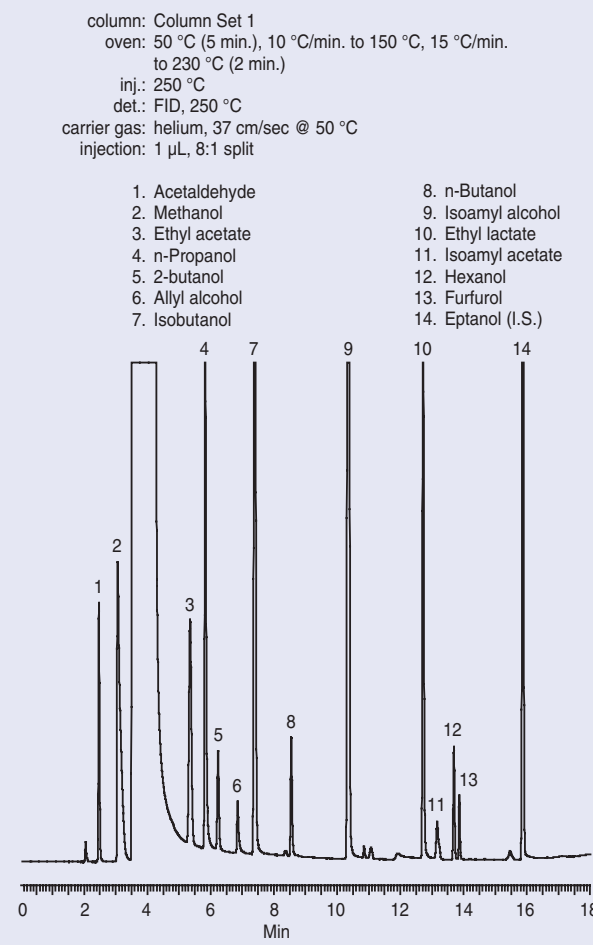
	Front Column	Back Column
Set 1	Equity-1	SUPELCOWAX 10
	8.7 m x 0.32 mm I.D., 5.0 µm ¹	30 m x 0.32 mm I.D., 0.25 µm (24080-U)
Set 2	Equity-1	SUPELCOWAX 10
	30 m x 0.32 mm I.D., 5.0 µm (28062-U)	10 m x 0.32 mm I.D., 0.25 µm ²

1. Made by cutting down a 30 m (28062-U) column.

2. Made by cutting down a 15 m (24078) or a 30 m (24080-U) column.

Column set 1 allows the optimum separation between the acetaldehyde, methanol, and ethanol peaks that historically are found in alcoholic spirits. However, isopropanol co-elutes with ethanol. Therefore, this column set is a viable option only when the analysis of isopropanol is not required. Chromatography and analytical conditions are shown in Figure 1.

Figure 1. Analysis of an Alcoholic Spirit Using Column Set 1

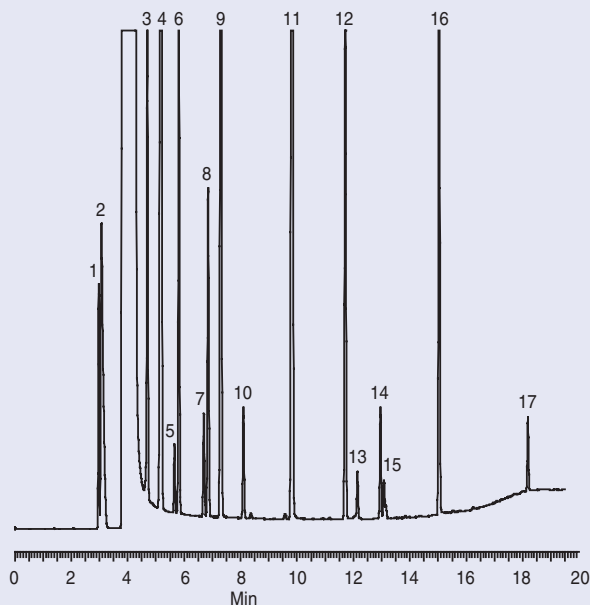


As shown in Figure 2, column set 2 solves the ethanol/isopropanol separation problem while maintaining acceptable separation between the acetaldehyde, methanol, and ethanol peaks, as well as all other compounds. Therefore, column set 2 presents a better choice for the separation of all compounds of interest in a single analytical test.

Figure 2. Analysis of an Alcoholic Spirit Using Column Set 2

column: Column Set 2
 oven: 45 °C (1 min.), 10 °C/min. to 150 °C, 15 °C/min.
 to 240 °C (2 min.)
 inj.: 250 °C
 det.: FID, 250 °C
 carrier gas: helium, 36 cm/sec @ 45 °C
 injection: 1 µL, 11:1 split

- | | | |
|------------------|---------------------|-----------------------|
| 1. Acetaldehyde | 7. 2-butanol | 13. Furfurol |
| 2. Methanol | 8. Ethyl acetate | 14. Hexanol |
| 3. Isopropanol | 9. Isobutanol | 15. Isoamyl acetate |
| 4. 3-butanol | 10. n-Butanol | 16. Eptanol (I.S.) |
| 5. Allyl alcohol | 11. Isoamyl alcohol | 17. Diethyl succinate |
| 6. n-Propanol | 12. Ethyl lactate | |



Practical Considerations

When serially connecting columns, care must be taken to insure the ends are connected with no, or minimal, dead-space. This can best be accomplished if the column ends are butted together, end-to-end, using a device such as the Capillary Column Butt Connector. The use of these devices is describe in detail in the article on page 19.

Another popular choice for connecting columns is the use of a press fitting, such as a GlasSeal connector. These result in a perfect seal forming between the two columns. Using a small drop of a polyimide resin on each column makes the connection extremely durable. Care must be

taken to ensure none of the resin enters either column or plugs the connector.

Whichever method is selected, it is recommended that the connection be evaluated with an electronic leak detector. Avoid liquid leak detector solutions with capillary columns due to the risk of pulling some of the solution into the system.

Conclusion

As shown, the use of serially connected columns of different phase polarity allows the analysis of the various components in spirits in a single run. This novel, time-saving approach is an improvement over the need for two analyses on separate columns, and should be considered for solving separation problems associated with alcohol beverage analysis.

+ Featured Products

Description	Cat. No.
Equity-1 30 m x 0.32 mm I.D., 5.0 µm	28062-U
SUPELCO WAX 10 30 m x 0.32 mm I.D., 0.25 µm	24080-U

+ Related Products

Description	Cat. No.
SUPELCO WAX 10 15 m x 0.32 mm I.D., 0.25 µm	24078
Capillary Column Butt Connector Body only, ferrule not included	23804
Supectex M-2B Ferrules for Butt Connector, pack of 2 To connect 0.32 to 0.32 mm I.D.	22454
GlasSeal Connectors	
Fused silica, pack of 5	23627
Fused silica, pack of 25	23628
Borosilicate glass, pack of 12	20479
Polyimide Sealing Resin, 5 g	23817
GOW-MAC Miniature Leak Detector	
115 V, 60 Hz	22807
230 V, 50 Hz	22808

The Use of Derivatization Reagents for GC

Jay Jones and Katherine Stenerson
jjones2@sial.com

Introduction

Analyte range is somewhat limited when it comes to GC analysis when compared to other techniques, such as HPLC. A couple of the causes for this can be attributed to compounds having low or no volatility, or those having poor thermal stability. An approach to overcoming these issues is to modify the analyte into a more "GC-amenable" form, using the process of derivatization.

BSTFA + TMCS as a Derivatization Reagent for GC

Derivatization is a technique that usually involves a reaction of the analyte(s) of interest with a particular derivatization reagent. There are a multitude of reagents that can be used depending on the character and functionality of the analytes. One of the more commonly used in GC analyses involves a reaction that adds a trimethylsilyl (TMS) functional group to the compound. This is also known as trimethylsilylation. The reagent N,O-bis(trimethylsilyl)trifluoroacetamide (also called BSTFA) is regularly used for this reaction.

The ease of derivatization of various functional groups for a given silylating reagent follows this order: alcohol > phenol > carboxylic acid > amine > amide. Within this sequence, reactivity towards a particular silylating reagent will also be influenced by steric hinderance. Therefore, the ease of reactivity for alcohols follows the order: primary > secondary > tertiary, and for amines: primary > secondary.

For moderately hindered or slowly reacting compounds, a catalyst may be added to the BSTFA. Some of the more common catalysts are trimethylchlorosilane (also called TMCS), trifluoroacetic acid, hydrogen chloride, potassium acetate, piperidine, O-methylhydroxylamine hydrochloride, and pyridine.

Derivatization Procedure Considerations

There are a few parameters that must be considered when performing a derivatization reaction. For instance, an alcohol may fully derivatize in just a matter of minutes at room temperature. However, it may take hours at an elevated temperature to complete the reaction for an amide or a sterically hindered carboxylic acid.

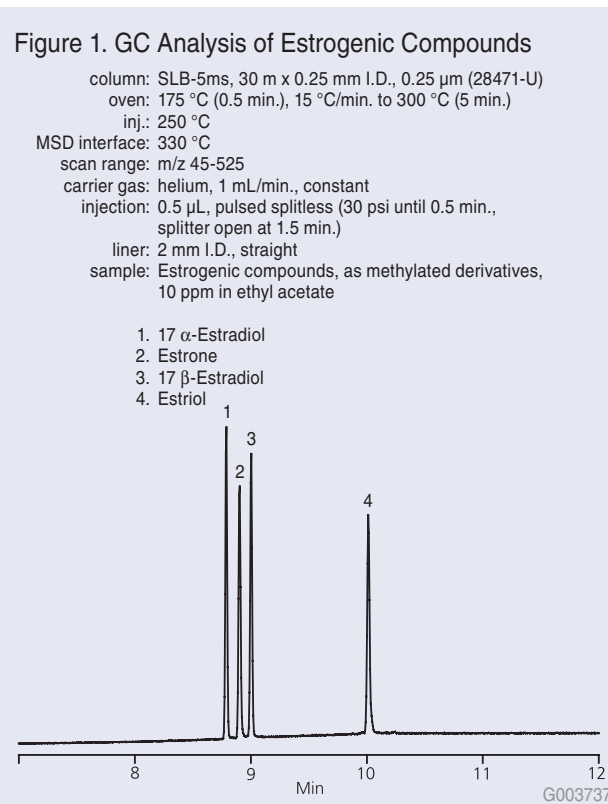
Along with time and temperature, the concentration of the reagent is important. It is recommended to add the silylating reagent in excess. As a general rule, add at least a 2:1 molar ratio of BSTFA to active hydrogens.

Most derivatization reactions are sensitive to water. The presence of water may slow or completely stop the reaction. Moisture may also decompose the TMS reagent or the derivatives that are formed. Therefore, it is recommended that derivatization reagents are stored in a secondary container that contains desiccant. Additionally, moisture should be removed from the extract that is to be derivatized.

Derivatization Examples

Without derivatization, estrogenic compounds and lysergic acid amide (LSD) show little or no response by GC analysis. With the addition of the TMS group, these analytes show great peak shape and response. The following paragraphs detail the steps taken to ensure the derivatization reactions go to completion by manipulating the above mentioned procedure considerations.

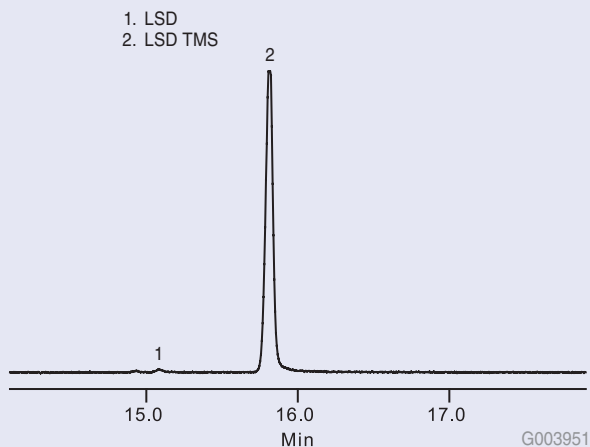
Of the four estrogenic compounds, three were fully derivatized within 30 minutes at 75 °C. However, estriol appeared to only have two of its active hydrogens silylated in 30 minutes (according to spectral data obtained from GC-MS analysis). The extract was derivatized a second time at 75 °C. Additionally, the reaction time was increased to 45 minutes. After being allowed to sit overnight at room temperature, GC-MS analysis then confirmed that all three active hydrogens had been replaced with TMS groups. Figure 1 shows the final chromatogram.



Lysergic acid diethylamide (LSD), was derivatized under similar conditions as the estrogenic compounds but at 68 °C. This reaction was sampled at intervals of 30 minutes. At this temperature, this reaction never went beyond 60 % completion, even after 5 hours. The temperature was then increased to 75 °C. The elevated temperature pushed the reaction to approximately 95 % completion. Even after almost three hours, both the LSD and the LSD (TMS) peak are still detected by GC analysis, which means that the reaction has not gone to 100 % completion. This chromatogram is shown in Figure 2.

Figure 2. GC Analysis of LSD-TMS

column: SLB-5ms, 30 m x 0.25 mm I.D., 0.25 µm (28471-U)
 oven: 150 °C (1.5 min.), 20 °C/min. to 300 °C (20 min.)
 inj.: 250 °C
 MSD interface: 330 °C
 scan range: m/z 45-525
 carrier gas: helium, 0.7 mL/min. constant
 injection: 0.5 µL, pulsed splitless, 30 psi. (0.20 min.), purge on (1.5 min.), purge flow (50 mL/min.)
 liner: 2 mm I.D., straight
 sample: derivatized LSD using BSTFA



Conclusion

Derivatization reactions allow an expanded range of analytes that are capable of being analyzed by GC. However, there are a few parameters that may require some trial and error to optimize the reaction. In the examples shown, both temperature and time were crucial to having the reactions go to completion. These two examples illustrate that each derivatization reaction must be optimized to achieve a high derivatization completion percentage, resulting in good peak shape and detector response.

Reference

1. D. R. Knapp, Handbook of Analytical Derivatization Reactions, John Wiley & Sons, New York, 1979.

Featured Products

Description	Cat. No.
BSTFA, Derivatization Grade, 25 mL	33027
TMCS, Derivatization Grade, 100 mL	33014
SLB-5ms, 30 m x 0.25 mm I.D., 0.25 µm	28471-U

Related Products

Description	Cat. No.
BSTFA, Derivatization Grade, 144 x 0.1 mL	33084
BSTFA, Derivatization Grade, 20 x 1 mL	33024
Sylon BFT (BSTFA + TMCS, 99:1), 144 x 0.1 mL	33154-U
Sylon BFT (BSTFA + TMCS, 99:1), 20 x 1 mL	33148
Sylon BFT (BSTFA + TMCS, 99:1), 25 mL	33155-U
Sylon BFT (BSTFA + TMCS, 99:1), 50 mL	33149-U

Related Information

For Supelco Bulletin 909 (*Guide to Derivatization Reagents for GC*), request T196909 (BGL). For more information on Supelco Low Bleed SLB-5ms capillary columns, visit our website sigma-aldrich.com/slb

Did you know...?

Supelco Bulletin 909 (T196909 BGL) contains detailed information regarding a large number of reagents used to prepare derivatives (using acylation, alkylation, or silylation) for gas chromatography. This bulletin describes each category, and presents information on how to choose the proper reagent based on the functional group(s) on the compound to be derivatized. Additionally, Supelco Technical Service Chemists (e-mail EurTechServ@sial.com) are a valuable resource for providing guidance with the selection and use of derivatization reagents.

FREE Technical Literature

Reagent	T#	Code
BCl ₃ , Methanol	T496123	BAX
BF ₃ , Methanol	T496125	BAZ
BSA + TMCS, 5:1 (Sylon BT)	T496018	AWH
BSA + TMCS + TMSI, 3:2:3 (Sylon BTZ)	T496019	AWI
BSTFA + TMCS, 99:1 (Sylon BFT)	T496021	AWK
5% DMDCS in Toluene (Sylon CT)	T496023	AWM
HMDS + TMCS, 3:1 (Sylon HT)	T496025	AWO
HMDS + TMCS + Pyridine, 3:1:9 (Sylon HTP)	T496026	AWP
Methanolic Base	T497007	BEG
Methanolic HCl	T497099	BIV
Perfluoro Acid Anhydrides	T497104	BIY
TFA	T496027	BFD
BSTFA	T496020	AWJ
TMCS	T496028	BFE

To receive electronic copies of the literature above, email EurTechServ@sial.com

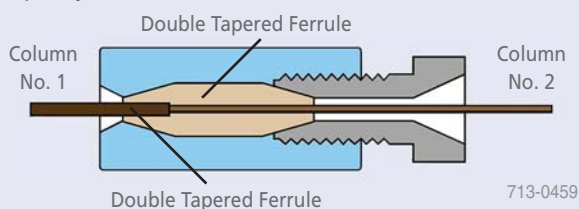
Extend the Lifetime of Your Capillary Columns With Guard Columns and Butt Connectors

Robert F. Wallace
 rwallace@sial.com

Introduction

A decrease in peak shape quality in a capillary gas chromatographic system can typically be traced to the inlet end of the column. Over time, the inlet end of the column becomes contaminated from an accumulation of non-volatile material. The phase can also be damaged from the continuous condensation and vaporization of solvent and analytes. Inevitably, active analytes will adsorb to this contaminated / damaged section (the analytes "drag" when passing through the inlet end of the column). Poor peak shape (peak tailing), loss in resolution, and reduced response may be observed. When the chromatographic system degrades to an unacceptable level, performance is restored by clipping the contaminated / damaged section off the inlet end of the column. A decrease in retention times and resolution results each time the column is clipped, as theoretical plates are lost. Eventually, the column will be rendered useless.

Capillary Column Butt Connector



Capillary Guard Columns

To extend the lifetime of capillary columns, Supelco recommends attaching a 5 m long capillary guard column with a capillary column butt connector. A guard column is a short piece of uncoated deactivated fused silica tubing which is placed in-line between the GC injection port and the analytical column. The guard column is used to take the brunt of the contamination / damage from the solvent and sample. By clipping the guard column periodically to restore performance instead of the analytical column, the analytical column remains unaltered. Therefore, chromatography (retention times and resolution) is not affected. It is important to match the polarity of the deactivation treatment of the guard column to the polarity of the solvent (see Table 1). It is also recommended to match the I.D. of the guard column to the I.D. of the analytical column.

Table 1. Choices of Tubing Deactivation Treatments

Treatment	Application	Max. Temp.
Non-Polar	Low polarity solvents (alkanes, carbon disulfide, ethers)	360 °C
Intermediate Polar	Intermediate polarity solvents (acetone, methylene chloride, toluene)	360 °C
Polar	Polar solvents (acetonitrile, methanol, water)	260 °C
Untreated	General purpose, where high inertness is not necessary	360 °C

Making the Connection

The Supelco Capillary Column Butt Connector consists of a double-tapered ferrule and a stainless steel compression housing with a threaded cap. Small and light (2.3 cm x 0.6 cm, 4.4 g with ferrule), it provides a gas tight seal without a change in column efficiency or inertness. The columns to be connected can have the same or different internal and external diameters. The butt connection is made inside the special double-tapered ferrule. The ferrule is then compressed within the housing. When the column ends are butted squarely and tightly together, the butt connector will not alter the chromatographic performance of your capillary columns. There is little or no dead volume and little chance of gas flow disruption by following these steps.

1. Make sure the bore of the ferrule is clean. Blow out any ferrule fragments with nitrogen. Using a magnifier, examine the column ends to be connected. Make sure each cut is clean and square. The two ends must butt squarely, without any gaps.
2. With white typewriter correction fluid, place a reference mark 1/4 inch from the end of the column with the larger bore. This mark will help you to confirm visually that the end of the column is centered within the 1/2 inch ferrule.
3. Replace the ferrule inside the housing and loosely tighten the nut. Feed the unmarked column completely through the ferrule and out the opposite end. Cut off ~1 inch (25 mm) of the column to ensure no ferrule fragments are in the column. Draw the column back far enough to insert the marked column into the ferrule to the indicating mark. Tighten the nut about 1/8 turn past finger tight.
4. Press the ends of the columns together, observing the reference mark to make certain they connect at the center of the ferrule. Tighten the ferrule to about 1/4-1/2 turn past finger tight. Gently pull on both columns to ensure they are secure. If they are loose, additional tightening is necessary.

5. Any undetected leaking connection, including this butt connection, can allow oxygen and water vapor to enter the system. Leak check the butt connector in the same manner as any capillary column connection. DO NOT USE LIQUID LEAK INDICATORS. Liquids can contaminate the capillary system. We recommend using a GOW-MAC® Leak Detector. This thermal conductivity detector is highly sensitive to trace amounts of hydrogen or helium, and will not contaminate the system.

Conclusion

The butt connector enables you to protect your analytical column without compromising its integrity or performance. It is more economical to discard the guard column than to break off the contaminated inlet of an otherwise good analytical column.

+ Featured Products

Description	Cat. No.
Capillary Column Butt Connector Includes 0.4 mm I.D. Supeltex M-2 ferrule, to connect 0.10/0.25 to 0.10/0.25 mm I.D.	23796

+ Related Products

Description	Cat. No.
Capillary Column Butt Connector	
Body only, ferrule not included	23804
Supeltex™ M-2B ferrules, pack of 2	
To connect 0.10/0.25 to 0.10/0.25 mm I.D.	22453
To connect 0.32 to 0.32 mm I.D.	22454
To connect 0.53 to 0.53 mm I.D.	22591
To connect 0.10/0.25 to 0.53 mm I.D.	22455-U
To connect 0.32 to 0.53 mm I.D.	22586
Capillary Guard Columns	
Non-Polar, 5 m x 0.25 mm I.D.	25742
Non-Polar, 5 m x 0.32 mm I.D.	25743
Non-Polar, 5 m x 0.53 mm I.D.	25744
Intermediate Polar, 5 m x 0.25 mm I.D.	25747
Intermediate Polar, 5 m x 0.32 mm I.D.	25748-U
Intermediate Polar, 5 m x 0.53 mm I.D.	25339
Polar, 5 m x 0.32 mm I.D.	25752-U
Polar, 5 m x 0.53 mm I.D.	25753
GOW-MAC Miniature Leak Detector	
115 V, 60 Hz	22807
230 V, 50 Hz	22808

Supeltex M-2 composition is DuPont VESPEL® SP-1 (100% polyimide), maximum temperature 350° C.

Supeltex M-2B composition is DuPont VESPEL SP-211 (75% polyimide, 15% graphite, 10% PTFE fluorocarbon resin), maximum temperature 350° C.



Analytical Standards Catalogue

Indispensable reference guide for analytical chemists!

- Comprehensive offering of over 8000 standards
- 608 pages packed with standards from all areas
- Over 200 new products, including *TraceCERT*™ standards
- Large collection of Certified Reference Materials
- Service on Custom Standards

To receive your free copy of the catalog please call or register at www.sigma-aldrich.com/standardcatalog



SIGMA-ALDRICH

Explosive Calibration Standards for Site Assessment and Remediation

Vicki Yearick

vyearick@sial.com

Supelco offers explosive/energetic calibration standards in support of the analytical monitoring of US Department of Defense base closures and remediation (sites and procedures). These same standards may also be used as part of an on-going preventative and/or remediation site assessment program to monitor soil and groundwater for possible contamination at energetic production facilities, firing ranges, mines, and long-term storage facilities.

Our calibration standards were designed to meet the requirements of analysts following US EPA Method 8330, a method designed for monitoring remediation of contaminated soils and water by HPLC using UV

detection. They are also suitable for use with EPA Method 8095 for analysis of explosives using capillary column gas chromatography by GC/ECD.

Fourteen single component solutions are available, both individually and in a kit. Two multi-component solutions which, when combined, provide a composite analytical calibration set for Method 8330 are also offered. The standards are gravimetrically prepared in acetonitrile at 1000 µg/mL. They are flame sealed in an amber ampul under nitrogen to prevent oxidation and photo degradation. A certificate of analysis accompanies each product.

For additional information about these products, please contact our Technical Service Department at EurTechServ@sial.com

Description	Concentration	Cat. No.
Single component solutions	1000 µg/mL in acetonitrile	
1,3-Dinitrobenzene solution		47746-U
2,4-Dinitrotoluene solution		47747
2,6-Dinitrotoluene solution		47748-U
Nitrobenzene solution		47239
2-Nitrotoluene solution		47240
3-Nitrotoluene solution		47241
4-Nitrotoluene solution		47242
Tetryl solution		47238
1,3,5-Trinitrobenzene solution		47243
2,4,6-Trinitrotoluene		47244
2-Amino-4,6-dinitrotoluene solution		47749-U
4-Amino-2,6-dinitrotoluene solution		47750
HMX solution		47236
RDX solution		47237
EPA 8330 Mix A	100 µg/mL each component	47283
1,3,5-Trinitrobenzene	2-Amino-4,6-dinitrotoluene	
1,3-Dinitrobenzene	HMX	
2,4,6-Trinitrotoluene	Nitrobenzene	
2,4-Dinitrotoluene	RDX	
EPA 8330 Mix B	100 µg/mL each component	47284
2,6-Dinitrotoluene	3-Nitrotoluene	
4-Amino-2,6-dinitrotoluene	4-Nitrotoluene	
2-Nitrotoluene	Tetryl	
EPA 8330 Energetic Materials Kit - Individually packaged, 1 mL solutions @ 1000 µg/mL in acetonitrile		47245
2-Amino-4,6-dinitrotoluene	HMX RDX	
4-Amino-2,6-dinitrotoluene	Nitrobenzene Tetryl	
1,3-Dinitrobenzene	2-Nitrotoluene 1,3,5-Trinitrotoluene	
2,4-Dinitrotoluene	3-Nitrotoluene 2,4,6-Trinitrotoluene	
2,6-Dinitrotoluene	4-Nitrotoluene	



Looking for an analytical standard? Our Standards Explorer makes sourcing products fast and easy. To learn more, visit sigma-aldrich.com/standards and link to the Standards Explorer.

New! Ginsenoside Certified Reference Materials

Vicki Yearick
vyearick@sial.com

Ginseng is a root crop that is highly regarded for its medicinal properties in many cultures. These properties are due to the presence of a group of active chemicals known as triterpene saponins or, more commonly, ginsenosides.

Scientists have identified thirty-one ginsenosides to date. These compounds are named ginsenoside Rx according to their mobility on a TLC plate with polarity decreasing from index a to h. The seven ginsenosides having the most pharmacological interest are Rg1, Rb1, Rb2, Rc, Rd, Re, and Rf. All have the same general structure, but vary in the degree of monosaccharide substitution. The ginsenoside composition of various ginsengs is species-dependent and varies whether the root is grown above ground or below ground. For example, the highest amount of Rb1, Rg1, and Rc are found in the underground roots.

Ginseng is an expensive root crop to produce. Consequently, adulteration and substitution with cheaper products does occur. Companies producing medicines, foods, and beverages with ginseng must quality control the ginseng they source before use in manufacturing. High Performance Liquid Chromatography (HPLC) using a C18 column is the preferred method for quality control and requires the use of a certified reference material.

Sourcing quality reference materials poses a problem, as there are few suppliers and costs are often prohibitive. Sigma Aldrich has addressed this need by establishing an agreement with KT & G Central Research Institute, Taejon,

Korea to offer ginsenoside Rb1 and ginsenoside Rg1 certified reference materials (CRMs). These materials are appropriate for quality control and the investigation of pharmaceutical efficacy. They may also be used as instrument calibration standards.

The CRMs are prepared, tested, and certified by KT & G Central Research Institute. The certified value of each of these standards is traceable to the International System of Units (SI), which the Korea Research Institute of Standards and Science (KRISS) has established. Traceability, homogeneity, stability, certification method and uncertainty are determined according to the standards of KS A ISO Guide 34 and ISO Guide 35.

Each CRM is packaged as 1.2 mL per ampul and supplied with a certificate of analysis, which provides both the certified value and the uncertainty value. Ginsenoside Rb1 and ginsenoside Rg1 are offered at 1000 µg/mL in methanol.

! Related Information

For more information, please email our Technical Service department at EurTechServ@sial.com

+ Featured Products

Description	Cat. No.
Ginsenoside CRMs	
Ginsenoside Rb1 Solution	93537-1.2 mL
Ginsenoside Rg1 Solution	18826-1.2 mL

New! Supelco Syringe Brochure

Our new 40-page Supelco Syringe brochure features the most frequently requested autosampler, manual, and specialty syringes offered by Hamilton®, SGE, and VICI Precision Sampling. An overview of each manufacturer's product line will assist in the selection of syringe models that will work best for your application. Our syringe selection guide will help you in choosing a syringe on the basis of sample type, volume range, and application. Tips on how to care for your syringe are also presented.

For a free copy of the Supelco Syringe brochure, or Vials brochure use the attached request form to request JCS, IXH, or visit us at sigma-aldrich.com/vials



Versa Vial™ Autosampler Vial

Our extensive line of autosampler vials now includes the unique Versa Vials.

These vials are a combination of a shell vial and a 12 x 32 mm autosampler vial. Versa Vials can be used in autosamplers that currently use other 12 x 32 mm vials. The neck has been modified to allow the vial to be picked up by an autosampler. An elongated pick-up area in the neck accommodates variations in robotic arm settings. Wide, 9 mm, neck openings allow samples to be added using pipettors.

Versa Vials are available in clear or amber borosilicate glass and polypropylene. The larger volume of the polypropylene vials is an advantage for ion chromatography applications.



The soft plug caps of the Versa Vial are designed to press-fit securely into the neck opening of the vial and are available in several materials. The siliconized chlorobutyl and PTFE/Silicone Versa Vial plugs are autoclavable. A variety of limited volume inserts are available.

Description	Cat. No.
Vials, 12 x 32 mm, pk of 100	
2 mL, Clear Glass	29083-U
2 mL, Clear Glass with marking spot	29085-U
2 mL, Amber Glass	29084-U
2 mL, Amber Glass with marking spot	29086-U
1.5 mL, Natural Polypropylene	29087-U
Closures, 12 mm diameter	
Green Polypropylene Plug	29088-U
Siliconized Gray Chlorobutyl Plug	29089-U
White PTFE/Silicone Plug	29091-U
Polypropylene, flat bottom, 350 µL	29096-U

45% off your next order:

Please quote promotion code Y75 when ordering.
Offer expires on July 15th 2007.

Syringes and Vials

Vials to fit Agilent® 1090 & 1100 Series LC Autosamplers

Sigma-Aldrich offers a wide range of high-quality vials for use in Agilent LC autosamplers. Our large inventory of these products is available for immediate shipment. The vials are offered in three styles: crimp neck, 9 mm short thread, and snap ring. They are available in clear glass and amber glass. Amber glass is suggested to protect sensitive samples from exposure to UV light. The marking spot available on many vials provides a convenient place for sample identification information.

Description	Cat. No.
Crimp Neck Vials, 11.6 x 32 mm, Large Opening, pk of 100	
1.5 mL Clear Glass with marking spot	SU860064
1.5 mL Amber Glass with marking spot	854998
1.5 mL Clear Glass, high recovery	27274
0.3 mL Clear Glass with glass insert, limited volume	24714
Screw Top Vials, 9 mm (Short Thread), 11.6 x 32 mm, Large Opening, pk of 100	
1.5 mL Clear Glass	854105
1.5 mL Clear Glass with marking spot	854165
1.5 mL Amber Glass with marking spot	SU860033
Snap Ring Vials, 11 mm, 11.6 x 32 mm, Large Opening, pk of 100	
1.5 mL Clear Glass with marking spot	SU860081
1.5 mL Amber Glass with marking spot	SU860082



sigma-aldrich.com/supelco

Hamilton Syringes to use with Agilent 1090 and 1100 Series Autosamplers

Sigma-Aldrich offers a wide range of syringes for a variety of analytical and chromatographic applications. We recommend the 1700 Series removable needle syringes from Hamilton Company for Agilent LC autosamplers. They are available in 25 µL or 250 µL volumes. Needles are not required when these syringes are used with Agilent 1090 and 1100 Series autosamplers.

Description	Cat. No.
1702RN, Removable needle, 25 µL	20781
1725RN, Removable needle, 250 µL	20784



! Related Information

If you have additional questions or require help in choosing the correct product, please contact Supelco Technical Service at EurTechServ@sial.com or visit sigma-aldrich.com/supelco

*31st International Symposium on High Performance
Liquid Phase Separations and Related Techniques*

HPLC 2007

*International Convention Centre
Ghent, Belgium
June 17-21, 2007*

Info: www.hplc2007.org

Call for Papers

sigma-aldrich.com


Contact your local sales office. Website sigma-aldrich.com/supelco



EKE

*The
SIGMA-ALDRICH
Family*

 **SIGMA**
Biochemicals and
Reagents for Life
Science Research

 **ALDRICH**
Organics and
Inorganics for
Chemical Synthesis

 **Fluka**
Speciality Chemicals
and Analytical Reagents
for Research

 **Riedel-de Haën**
Laboratory Chemicals
and Reagents for
Research and Analysis

 **SUPELCO**
Chromatography
Products for Analysis
and Purification

 **ISOTEC**
Promoting Research
and Discovery

©2007 Sigma-Aldrich Co. Printed in Germany Sigma brand products are sold through Sigma-Aldrich, Inc. Sigma-Aldrich, Inc. warrants that its products conform to the information contained in this and other Sigma-Aldrich publications. Purchaser must determine the suitability of the product(s) for their particular use. Additional terms and conditions may apply. Please see reverse side of the invoice or packing slip. SIGMA and  are registered trademarks of Sigma-Aldrich Co. and its division Sigma-Aldrich Biotechnology LP. Riedel-de Haën®: trademark under license from Riedel-de Haën GmbH.