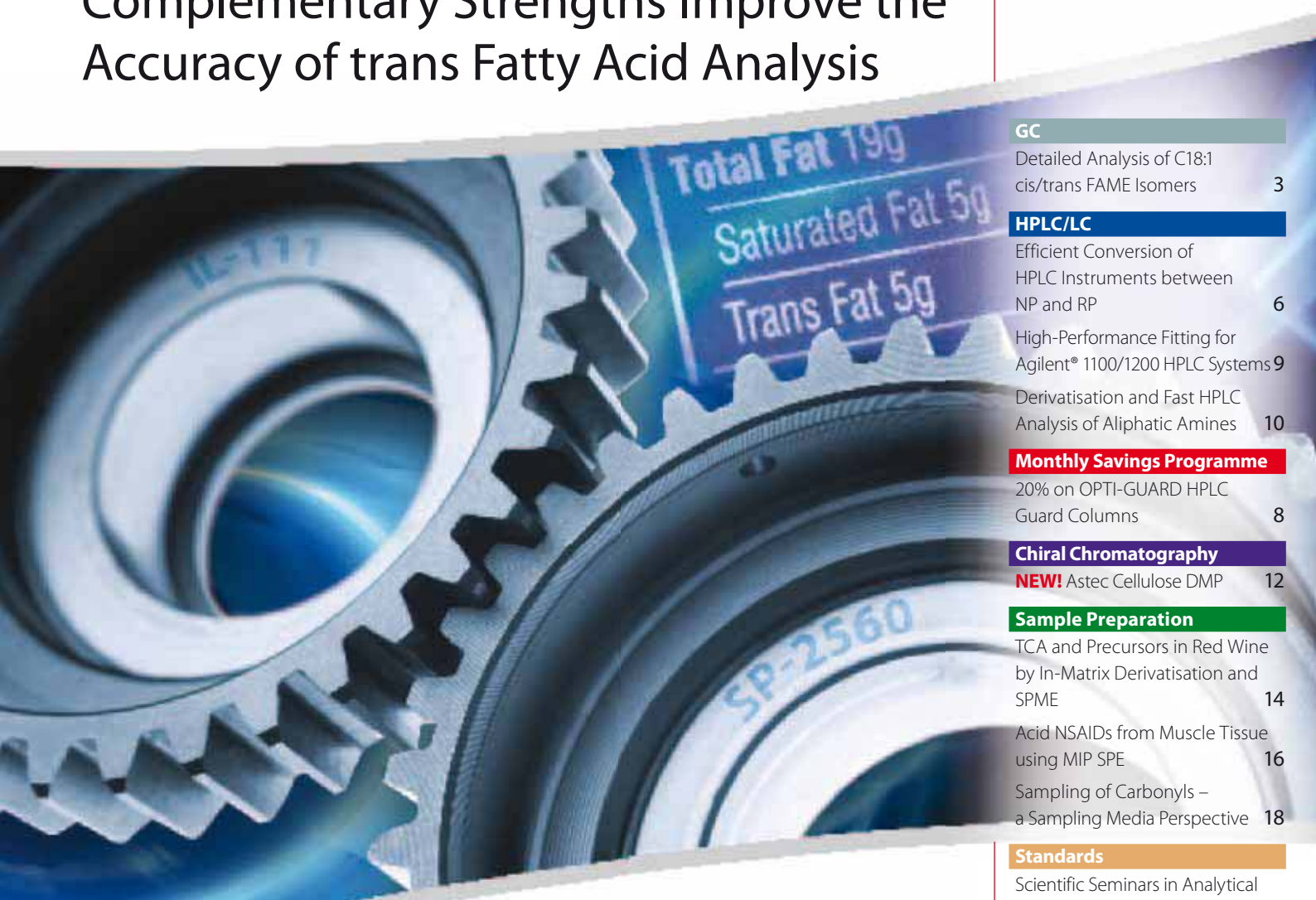


Reporter

Volume 46, May 2011, International



Complementary Strengths Improve the Accuracy of trans Fatty Acid Analysis



When used together, the new Supelco® SLB-IL111 and your current cyanopropyl siloxane GC column provide a more complete and accurate identification of trans fatty acids than currently possible.

GC	
Detailed Analysis of C18:1 cis/trans FAME Isomers	3
HPLC/LC	
Efficient Conversion of HPLC Instruments between NP and RP	6
High-Performance Fitting for Agilent® 1100/1200 HPLC Systems	9
Derivatisation and Fast HPLC Analysis of Aliphatic Amines	10
Monthly Savings Programme	
20% on OPTI-GUARD HPLC Guard Columns	8
Chiral Chromatography	
NEW! Astec Cellulose DMP	12
Sample Preparation	
TCA and Precursors in Red Wine by In-Matrix Derivatisation and SPME	14
Acid NSAIDs from Muscle Tissue using MIP SPE	16
Sampling of Carbonyls – a Sampling Media Perspective	18
Standards	
Scientific Seminars in Analytical and Chromatography	20
LC-MS Mobile Phase Additives	21
Accessories	
NEW! Low Adsorption Vials for Basic Compounds	22

Expanded Quality to Meet Your Analytical Needs – Introducing Cerilliant and Resource Technology Corporation (RTC), the Two Newest Additions to Sigma-Aldrich

Visit us on the web at sigma-aldrich.com/thereporter



Dr. Ingo Haag
Director Marketing

Dear Colleague,

In our efforts to bring you the highest quality and best offering of analytical products, Sigma-Aldrich would like to welcome our two newest family members, Cerilliant Corporation of Round Rock, Texas and RTC of Laramie, Wyoming. The extensive capabilities of these two companies complement Sigma-Aldrich's existing Fluka® and Supelco® analytical brands.

Cerilliant's quality credentials include accreditation to ISO Guide 34 and ISO/IEC 17025, certification to ISO 9001:2008 and incorporation of cGMP and GLP requirements into their everyday activities. They are currently pursuing ISO 13485 and expect to receive their certification in early 2011. Cerilliant offers over 2,800 products and more than 30 years of expertise in the design, development, synthesis, characterisation and packaging of analytical standards, calibrators, controls and certified reference materials for the pharmaceutical, clinical diagnostics, forensic and clinical toxicology, nutraceutical and environmental markets.

RTC is a premium manufacturer of Certified Reference Materials (CRMs), Quality Control (QC) Samples, and Proficiency Testing (PT) Samples with more than 20 years of experience and a strong expertise in the environmental as well as in the pharmaceutical sector. RTC was one of the original Proficiency Test providers recognised by the US EPA. Its quality systems include ISO/IEC 17025, ISO Guide 34 (CRMs), ISO/IEC 17043 (PT) and ISO 9001:2000.

Add the outstanding expertise of Cerilliant and RTC to the over 40 years of experience at Supelco and 60 years at Fluka and you have an unmatched amount of knowledge and products available for your laboratory needs. These companies will combine their talents and capabilities with those of Sigma-Aldrich to create visionary approaches to supply the analytical industry with a continually expanding list of new products that meet the most rigorous quality requirements including cGMP, GLP and ISO requirements.

Currently the products of Cerilliant and RTC are only available through Cerilliant and RTC themselves or one of their distributors. Please contact them directly or visit www.cerilliant.com or www.rt-corp.com to review products, to ask a question or to place an order.

Kind regards,

A handwritten signature in black ink that reads "D. Haag". The signature is fluid and cursive, with a long horizontal stroke extending to the right.

Dr. Ingo Haag

Director Marketing
Ingo.Haag@sial.com

Detailed Analysis of C18:1 cis/trans FAME Isomers Using the New Supelco® SLB™-IL111 and the SP™-2560

Leonard M. Sidisky and Michael D. Buchanan
mike.buchanan@sial.com

Introduction

Fatty acids in the cis configuration are the dominant form in nature. Correspondingly, enzymes have evolved to efficiently digest and metabolise them with a high degree of specificity. Conversely, trans fatty acids are relatively rare in nature. However, they have become predominant synthetic additives to processed foods, especially fried foods and baked goods, because they can increase the shelf life and flavour stability of foods containing them. It is now known that trans fatty acids, formed by partial hydrogenation of vegetable oil, interfere with natural metabolic process. Studies have linked their nutritional contribution to be similar to that of saturated fatty acids, possibly playing a role in the heightened risk of coronary artery disease (1-3).

Many regulatory agencies worldwide now require content labelling to inform buyers of "trans fat" levels of foods and some dietary supplements. Analysis of fatty acids is routinely performed using gas chromatography (GC) following established methodologies that first require derivatisation of the fatty acids to fatty acid methyl esters (FAMEs). Because the differences between cis isomer FAMEs and trans isomer FAMEs of the same carbon length and degree of unsaturation are very small, very efficient capillary GC columns with highly polar phases are required to separate them. This article will determine the suitability of our newest GC column to perform this application through direct comparison to a well-established and widely referenced column.

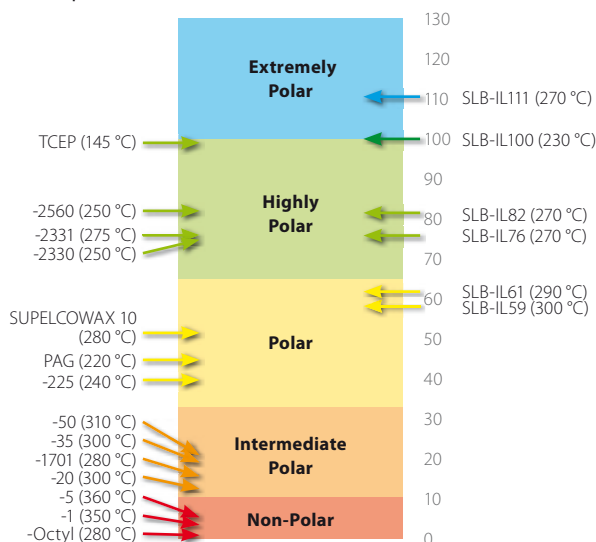
SP-2560 Column

Launched in 1983, the highly polar SP-2560 is specifically designed for the detailed analysis of cis/trans FAME isomers, including those found in hydrogenated vegetable oil. The SP-2560, with 100 m x 0.25 mm I.D., 0.20 µm dimensions, is considered by some as the benchmark column for FAME applications. It is referenced in several methods that employ GC analysis for determining fat content in food (4,5).

Complete column specifications are:

- **Application:** This highly polar biscyanopropyl column was specifically designed for detailed separation of geometric-positional (cis/trans) isomers of fatty acid methyl esters (FAMEs). It is extremely effective for FAME isomer applications.
- **USP Code:** This column meets USP G5 requirements.
- **Phase:** Non-bonded; poly(biscyanopropyl siloxane)
- **Temperature Limits:** Subambient to 250 °C (isothermal or programmed)

Figure 1. Positions of Columns on the GC Column Polarity Scale (Max. Temp)



Supelco Ionic Liquid Capillary GC Columns

Ionic liquids are a class of solvents with low melting points that consist of organic cations associated with (inorganic or organic) anions. In 2005, Prof. Daniel W. Armstrong (University of Texas at Arlington) showed that dicationic and polycationic ionic liquids could successfully be used as viable GC stationary phases. Ionic liquid GC columns have the opportunity to impact current practices along several paths (6).

1. *Identical selectivities as traditional, often flawed, non-ionic liquid columns; but with higher operating temperatures, lower column bleed, and/or less susceptibility to damage from moisture/oxygen*
2. *High thermal stability; decreasing analysis times, and/or allowing additional higher boiling compounds to be analysed*
3. *Completely unique selectivities compared to any/all traditional non-ionic liquid columns; producing good peak shape and resolution for compounds of varying functionality, and/or expanding the polarity range upward*
4. *In multidimensional separations; due to their engineered orthogonality and high thermal stability*

In 2008, Supelco launched the world's first commercially available GC column based on ionic liquid stationary phase technology, the SLB-IL100. Six unique ionic liquid columns are currently available in our evaluation programme. **Figure 1** is a visual depiction of our GC column polarity scale, showing the relationship of ionic liquid

(continued on page 4)

columns to one another and also to non-ionic liquid columns. All polarity number values are relative to both squalane (0 on the scale) and SLB™-IL100 (100 on the scale). This simple but useful scale allows multiple columns to be quickly compared. The positions/maximum temperatures of several of our non-ionic liquid capillary GC columns are shown to the left of the scale. Listed to the right of the scale are the positions/maximum temperatures of our current Supelco® ionic liquid capillary GC columns. Detailed information concerning the scientific basis used to generate this scale can be found at sigma-aldrich.com/il-gc

SLB-IL111 Column

Highly polar columns are well suited for the resolution of polarisable analytes, such as aromatics and unsaturated FAMES. This is due to their ability to undergo various dipole-induced dipole analyte-phase interactions.

The specifications of the SLB-IL111 are:

- **Application:** Launched in late 2010, this extremely polar ionic liquid column was the world's first commercial column to rate over 100 on our GC column polarity scale. As such, it has the most orthogonal selectivity compared to commonly used non-polar and intermediate polar columns, providing increased selectivity for polar and polarisable analytes. It should also be considered for use in GCxGC applications. Its temperature limit of 270 °C is very impressive for such an extremely polar column
- **USP Code:** None
- **Phase:** Non-bonded; proprietary
- **Temperature Limits:** 50 °C to 270 °C (isothermal or programmed)

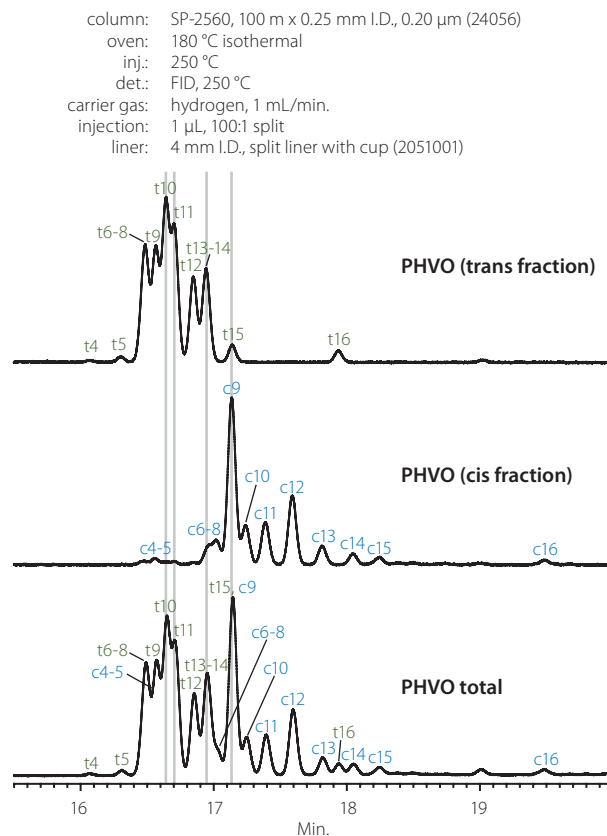
Analysis of PHVO and Fractions

A partially hydrogenated vegetable oil (PHVO) extract was provided by Dr. Pierluigi Delmonte from the United States Food and Drug Administration (US FDA) for our work. A cis fraction extract and a trans fraction extract, both prepared from PHVO using a Ag-ion fractionation procedure, were also supplied by Dr. Delmonte. Complete details and references pertaining to the PHVO extract preparation and Ag-ion fractionation procedures can be found in Reference 7.

The established AOCS method for determining fat content requires analysis on the 100 m SP™-2560 column operated with a 180 °C isothermal oven temperature (5). To determine the optimal oven temperature for these analytes on the 100 m SLB-IL111, the PHVO extract was analysed at several different oven temperatures. In concurrence with work published by Delmonte et al. (7), we determined that an isothermal analysis at 168 °C provided the best resolution for these analytes on this column.

Once optimal conditions were established, the PHVO, cis fraction, and trans fraction extracts were sequentially analysed on each column to determine their ability to separate thirteen C18:1 cis FAME isomers

Figure 2. Partially Hydrogenated Vegetable Oil (PHVO) and Fractions on the SP-2560



(in blue, from C18:1Δ4c to C18:1Δ16c) and thirteen C18:1 trans FAME isomers (in green, from C18:1Δ4t to C18:1Δ16t). **Figure 2** shows the resulting chromatograms on the SP-2560 at 180 °C isothermal, the method-specified oven temperature. **Figure 3** shows the resulting chromatograms on the SLB-IL111 at 168 °C isothermal, the experimentally determined optimal oven temperature.

Results

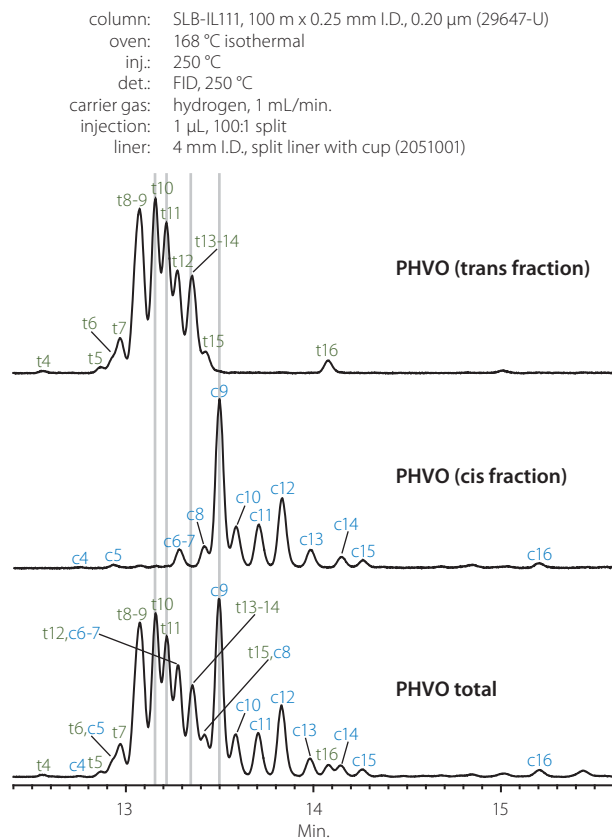
Following the completion of this study and a review of Reference 7, several observations were made.

Elution Temperature. Due to the higher polarity of the SLB-IL111, the FAME isomers eluted in less time compared to the SP-2560, even though the oven temperature necessary for optimal resolution was lower for the SLB-IL111.

Elution Order. The SLB-IL111 resulted in a different elution order than that obtained with the SP-2560. This was predicted due to the different selectivities of the columns, based on the data used to generate our GC column polarity scale.

C18:1 Isomers. The SLB-IL111 was able to provide resolution of C18:1Δ9c from C18:1Δ15t, a separation not possible with the SP-2560. Additionally, the SLB-IL111 offered improved resolution of some isomers that cannot be completely resolved with the SP-2560 either, such as C18:1Δ10t from C18:1Δ11t, and the pair C18:1Δ13t/C18:1Δ14t from other isomers.

Figure 3. Partially Hydrogenated Vegetable Oil (PHVO) and Fractions on the SLB™-IL111



C18:2 Isomers. Although not shown here, Delmonte et al. reported that the SLB-IL111 is able to resolve C18:2Δ9c,11t from C18:2Δ7t,9c. These are the two most abundant conjugated linoleic acid (CLA) isomers found in ruminant fats. This is encouraging as these two important isomers cannot be resolved using any other GC column and thus require time-consuming Ag-ion fractionation (7).

Saturated FAMES. Delmonte et al. also reported that a drawback of the SLB-IL111 was the co-elution of saturated FAMES with minor trans FAME isomers of one carbon less in chain length. Conversely, the SP-2560 is able to resolve many of the saturated FAMES from unsaturated FAMES, the exception being C19:0 and C21:0.

Conclusion

Based on the data presented here and the work reported by Delmonte et al., it appears that the SP™-2560 and SLB-IL111 can be used in a complementary fashion to provide more complete and accurate fatty acid identification and composition information than currently possible. It requires less time and labour to inject one extract on two different columns, than to perform Ag-ion fractionation of an extract prior to injecting multiple fractions on a single column.

References

- Ascherio, A. and Willett, W., "Health Effects of Trans Fatty Acids", *Am. J. Clin. Nutr.*, 1997, 66 (supplement), 1006S.
- Stende S. and Dyerberg, J., "Influence of Trans Fatty Acids on Health", *Annals of Nutrition and Metabolism*, 2004, 48 (2), 61.
- American Heart Association Web Page, <http://www.americanheart.org/presenter.jhtml?identifier=1728> (accessed Jan. 4, 2006).
- AOAC Method 996.06, "Fat (Total, Saturated, and Unsaturated) in Foods" *Official Methods of Analysis, 18th Edition* (on-line) Association of Official Analytical Chemists, Inc.
- AOCS Method Ce 1h-05, "Determination of cis-, trans-, Saturated, Monounsaturated and Polyunsaturated Fatty Acids in Vegetable or Non-ruminant Animal Oils and Fats by Capillary GLC" *AOCS Official Methods* (2005) American Oil Chemists Society.
- Payagala, T., Sidisky, L.M. and Armstrong, D.W., "The Advent and Potential Impact of Ionic Liquid Stationary Phases in GC and GCxGC", *LCGC North America* 27 (2009), 596.
- Delmonte, P., Kia, A-R.F., Kramer, J.K.G., Mossoba, M.M., Sidisky, L. and Rader, J.I., "Separation Characteristics of Fatty Acid Methyl Esters Using SLB-IL111, A New Ionic Liquid Coated Capillary Gas Chromatographic Column", *J. Chromatogr. A* 1218 (2011) 545.

+ Featured Products

Description	Cat. No.
SP-2560, 100 m x 0.25 mm I.D., 0.20 μm	24056
SLB-IL111, 100 m x 0.25 mm I.D., 0.20 μm	29647-U
Inlet liner, 4 mm I.D. split design with cup (unpacked), for Agilent® GC	2051001

+ Related Products

Description	Cat. No.
SLB-IL111, 15 m x 0.10 mm I.D., 0.08 μm	28925-U
SLB-IL111, 30 m x 0.25 mm I.D., 0.20 μm	28927-U
Discovery® Ag-Ion 750 mg/6 mL SPE tube, 30 ea	54225-U
Discovery Ag-Ion 750 mg/1 mL Rezorian™ cartridge, 10 ea	54226-U
Inlet liner, 4 mm I.D. split design with cup (unpacked), for Agilent GC	
5 ea	2051005
25 ea	2051025

+ Related Information

To learn more about Supelco® ionic liquid capillary GC columns, visit our website sigma-aldrich.com/il-gc and to see other techniques, procedures, chromatograms, and products related to the analysis of fatty acids and FAMES visit sigma-aldrich.com/fame

Efficient Conversion of HPLC Instruments between Normal-Phase and Reversed-Phase Solvents

Richard Henry and Carmen T. Santasania
carmen.santasania@sial.com

While reversed-phase (RP) HPLC is still by far the most common mode, normal-phase (NP) HPLC is increasing in popularity with the introduction of new highly polar columns with excellent retention, selectivity and stability. It is easy to inter-convert between RP and HILIC (a type of NP) because both modes employ polar aqueous mobile phases; however, many compounds are not polar enough to be retained under partially aqueous HILIC conditions. When aqueous solvents must be replaced with non-aqueous conditions to study non-polar samples, immiscibility situations can arise during changeover.

Due to many requests to our Technical Service Department this article has been written to explain the best practices for converting between reversed-phase and normal-phase solvents, which are often immiscible.

The first best practice is to dedicate instruments to a specific mode. Significant seal wear-and-tear can be caused by expansion, contraction and extra friction of changing solvents. If possible, columns should also be dedicated to one mode for trouble-free operation. If dedicating the instrument or column is not possible, one should use the following procedure.

Our regular practice is to replace the column with tubing or a union and flush extensively with isopropanol (IPA) before going over to water or hydrocarbon. Before beginning the changeover process, remove the HPLC column, cap and store in the appropriate storage solvent unless the same column is to be used in the new mode.

Columns such as Cyano and Fluorophenyl (F5) can work in either RP or NP mode and may remain installed if desired. In the flushing steps, be sure to include the entire fluid path (pump, autosampler, valves, detector, etc.). Also, include the sample loop and any other fluid paths that are encountered for the normal operation of making injections. This can vary considerably depending on whether the autosampler is an external, or internal loop design. As part of all the washes, make certain the injection needle gets washed as well. It is best to do several full loop injections of a solvent such as IPA that is miscible with both high-aqueous and high-organic mobile phases. The total volume of IPA needed will vary with instrument design, but the waste volume should be monitored (record the volume as a guide for future changeovers) and observed for uniform appearance. UV detectors may remain on (ca. 250 nm) during this step to indicate when the system has returned to a stable baseline.

The second wash step after the IPA should be with methanol (or ethanol). Follow the previous procedure that was used for the IPA wash before going to water. Methanol will help flush the IPA out faster than going directly from IPA to water. If excessive baseline noise or drift is observed with a UV detector, repeat the procedures and allow more time to flush out any poorly swept flow regions.

Table 1. Properties of Organic Solvents Commonly Used in HPLC

Solvent	Polarity	Miscible with Water?	UV Cutoff*	Refractive Index at 20 °C	Solvent Strength e° (silica)	Viscosity at 20 °C, C P	
Hexane	nonpolar	no	200	1.3750	0.00	0.33	
Isooctane	↓	no	200	1.3910	0.01	0.50	
Carbon tetrachloride		no	263	1.4595	0.14	0.97	
Chloroform		no	245	1.4460	0.31	0.57	
Methylene chloride		no	235	1.4240	0.32	0.44	
Tetrahydrofuran		yes	215	1.4070	0.35	0.55	
Diethyl ether		no	215	1.3530	0.29	0.23	
Acetone		yes	330	1.3590	0.43	0.32	
Ethyl acetate		poorly	260	1.3720	0.45	0.45	
Dioxane		yes	215	1.4220	0.49	1.54	
Acetonitrile		yes	190	1.3440	0.50	0.37	
2-Propanol		yes	210	1.3770	0.63	2.30	
Methanol		yes	205	1.3290	0.73	0.60	
Water		polar	yes	-	1.3328	> 0.73	1.00

* typical values

Some Dos and Don'ts for Solvent Changeover:

- Do remove all additives and start with 100% isopropanol in all reservoirs.
- Isopropanol is fully miscible with all common solvents and is the safest changeover solvent for either direction.
- Do use low flow – about half of normal to avoid excessive seal wear and damage due to over-pressuring.
- Don't use acetonitrile routinely as the changeover solvent – it is better than methanol, but is not fully miscible with pure hydrocarbons.
- Don't use methanol routinely as the organic – it is not fully miscible with many normal phase conditions.
- Do check miscibility (use small external vessel) with target mobile phase before starting, especially if IPA is not selected.
- Do use organic (such as IPA) in all lines of a gradient instrument to make certain that water or hydrocarbon is removed from all fluid areas.
- Do operate the injector valve and any other selector valves while doing the IPA flush procedure.
- Do monitor pressure and detector signals during changeover as these are excellent methods to confirm full system equilibration; evaporative detectors such as MS and ELSD cannot be used for this purpose.
- Incomplete mixing shows up as severe detector baseline noise or pressure fluctuations (globules of immiscible solvent can resemble bubbles or particles).
- Do flush detectors and all other components even if baseline is not monitored.
- Total time for changeover can vary but should take about an hour. Do not rush; this may actually slow down the process.
- Do record the volumes of solvent used during changeover for use as a future guide; if changeover is unsuccessful, use more solvent the next time.
- Don't expect fast changeover and baseline equilibration with refractive index detectors – they are extremely slow to equilibrate after changeover.
- Do check gradient blank runs for excessive baseline noise and drift that might indicate pockets of immiscible solvent.
- Good chromatography in the target mode is the most sensitive final test – start with simple binary mobile phases and standard test mixes and work towards real samples with mobile phase additives.

Finally, it is also good practice to contact your LC instrument manufacturer to be sure all details of the changeover are covered. The manufacturer may have additional details and tips for successful changeover to a different chromatographic mode. If columns and instruments are frequently used in different modes, adopt a labelling system to alert a new user about possible solvent compatibility issues.



LC-MS CHROMASOLV®

Pure Quality Solvent

Pure Quality Solvent Solvents and Blends offer:

- Very low level (max. 100 ppb) of inorganic and metal ions – these can form clusters with the analyte, especially when using electrospray ionisation
- No particles (especially for use with Nano-LC inlet filters and sub 2 µm particle columns) and non-volatile compounds
- Low-gradient baseline even with your own optimised protocols

Request your copy of **LC-MS Mobile Phase Additives – Tips & Tricks** (brochure code KCT) by completing this card.

For more information on LC-MS additives in this Reporter edition, please refer to page 21.





Monthly Savings Programme

SAVE 20%



SUPELCO
Analytical



20% Discount on all OPTI-GUARD® HPLC Guard Columns

The finger-tightened OPTI-GUARD 1 mm bed length guard columns have an auto-adjusting design that is no larger than a typical HPLC fitting. They are the perfect choice for effective column protection of any silica-based analytical column without measurable impact on your chromatography. Designed for use with analytical (4.6 mm, 3.0 mm I.D.) and narrow bore (2.1 mm, 1.0 mm) columns, the patented

floating stem design automatically adjusts to any manufacturer's tube stop depth for a zero-dead volume connection every time. In addition, the OPTI-GUARD 3 mm bed length guard system offers greater capacity and more rugged protection. To read more about these useful guard columns, visit us on sigma-aldrich.com and search for LTG as Technical Document.

Part No. Description

OPTI-GUARD 1 mm Guard Column

51177-U	PK5 OPTI-GUARD 1 mm Guard Column C18 (Violet Label)
51183-U	PK5 OPTI-GUARD 1 mm Guard Column C18, Biocompatible
51184-U	PK5 OPTI-GUARD 1 mm Guard Column C8
51185-U	PK5 OPTI-GUARD 1 mm Guard Column Phenyl
51178-U	PK5 OPTI-GUARD 1 mm Guard Column Silica (Orange Label)
51179-U	PK5 OPTI-GUARD 1 mm Guard Column Cn (Blue Label)
51180-U	PK5 OPTI-GUARD 1 mm Guard Column Anion Exchange (Black Label)

Part No. Description

51181-U	PK5 OPTI-GUARD 1 mm Guard Column Cation Exchange (White Label)
51187-U	PK5 OPTI-GUARD 1 mm Guard Column Amino (NH ₂)
OPTI-GUARD 3 mm Guard Column	
51191-U	PK3 OPTI-GUARD 3 mm Cartridge C18
51193-U	PK3 OPTI-GUARD 3 mm Cartridge C8
51194-U	PK3 OPTI-GUARD 3 mm Cartridge Amino (NH ₂)
51196-U	PK3 OPTI-GUARD 3 mm Cartridge Silica
51188-U	OPTI-GUARD 3 mm Peek/SS Holder

To take advantage of this monthly savings offer, please use promotion code **969** when ordering. Offer is valid until **31 July 2011**.

High Performance Fitting for Agilent® 1100/1200 HPLC Systems



Fast HPLC as well as high-resolution HPLC is pushing HPLC systems towards their pressure limits. Whether you are increasing flow rates or column lengths or reducing particle size to gain an advantage in HPLC, these all increase the backpressure in your system. Using PEEK tubing as interconnects is no longer suitable in high-performance HPLC.

PEEK can slip, stretch, or deform, which translates to a decrease in performance. Supelco® High Performance Fittings maintain the integrity of your HPLC systems all the way to 15,000 psi.

Key Benefits

- Eliminates dead volume that contributes to peak broadening and decreased resolution
- Sliding ferrule design allows for use in any port
- Fingertight fittings, no tools required
- Safe to install; will not damage HPLC ports even if over-tightened
- Rated to 15,000 psi
- 316 stainless steel construction

Agilent 1100/1200 Fitting

A high-performance fitting is now available for the Agilent 1100/1200 HPLC system. Semi-rigid 316 stainless steel tubing connects one end to the heater outlet, and allows use of columns by any manufacturer with confidence that the high-performance UHPLC fitting is completely seated in the column inlet port. An important feature of this fitting is the "service loop", which allows the column to be semi-rigidly supported.

+ Featured Product

High Performance Fitting for Agilent 1100/1200

Description	Cat. No.
15 cm x 1/16 inch O.D., 0.005 I.D.	53629-U

+ Related Information

For more information on the complete line of high-performance fittings, request *High Performance HPLC Fittings/Interconnects*, T407140 (KDK), or visit us on the web: sigma-aldrich.com/hplc

Couldn't attend PITTCON 2011?

To get all the Supelco posters and presentations from this year's show, request the *2011 Sigma-Aldrich PITTCON Presentations CD* (NHZ) on the attached postcard, or visit sigma-aldrich.com/pittcon



Presentations (HPLC & Sample Prep)

- Studies on Stationary Phase Selectivity for Solid-Core Particles – Dr. Richard Henry
- Affecting Reversed-Phase/MS Peptide Separations on High Performance Silica Particles – Dr. Richard Henry
- HPLC Enantiomeric Separations of Pharmaceuticals Using Polar Organic Mobile Phases – Jauh-Tzuoh Lee
- Innovative Particles Enable Advances in Chromatographic Separation Devices and Sample Preparation – Dave Bell
- Sample Preparation LC-MS
- High Recovery Method of HybridSPE-Phospholipid for Cleanup of Biological Samples Prior to LC-MS Analysis – Xiaoning Lu

Poster

HPLC

- Optimizing Instruments for Modern HPLC Columns – Tracy Ascah
- Impact of Reversed-Phase Chiral Chromatography on the LC-MS Analysis of Drugs in Biological Fluids – Dave Bell
- Understanding Separations in HILIC Chromatography: We're Not in Water Anymore – Dave Bell

Sample Preparation (SPME/Adsorbents /Air Monitoring)

- SPME – LC Fibres for a Variety of Applications – Robert Shirey
- Characterization of Polymer Carbon Sieves, Graphitized Polymer Carbons and Graphitized Carbon Blacks for Carbon Purification Processes – Len Sidisky
- Benefits of Radial Passive Samplers – Katherine Stenerson

GC

- A Comparison of Ionic Liquid and Polymer Based Capillary Columns for the Analysis of FAME Isomers – Len Sidisky
- The Utility of Headspace Grade Solvents in the Analysis of Organic Volatile Impurities – Katherine Stenerson

Standards

- A New Generation of Certified Reference Materials by the Quantitative 1H-NMR Technique (qNMR) – Len Sidisky

Other

- Selectophore Products – New Ion-Selective Sensor Materials for Food, Environmental, Biomedical and Industrial Applications – Ingrid Hayenga
- New Developments with Ionic Liquid Capillary Columns – Len Sidisky

Derivatisation and Fast HPLC Analysis of Aliphatic Amines

Hugh Cramer and Shyam Verma
shyam.verma@sial.com

The need for the analysis of aliphatic amines in environmental and biological samples has been continuously growing (1). Several methods, such as GC (2, 3), HPLC (4–6) and CE (7, 8), have been reported for detection and separation of aliphatic amines. The aliphatic amines generally do not show ultraviolet absorption or emit fluorescent light which their detection difficult. Huang et al. (1) reported that these amines can be conveniently separated by HPLC following a pre-column derivatisation that improves sensitivity and selectivity. Our study is focused on improving HPLC separation of the aliphatic amines by reducing run time and improving peak shape. This was achieved by using the Ascentis® Express column.

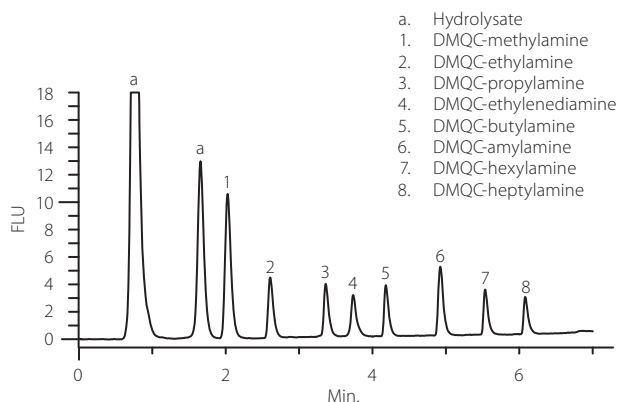
The derivatisation reagent allows quantitative conversion of an analyte to a single detectable derivative, with minimal side reactions under mild conditions. Use of several fluorescent derivatisation reagents has been reported in the literature for this purpose. However, a recent study (1) has demonstrated derivatisation of aliphatic amines with 2,6-dimethyl-4-quinolinecarboxylic acid N-hydroxysuccinimide ester (DMQC-OSu). This reagent is reported to readily react with primary and diamines exhibiting several advantages, such as good selectivity in aqueous solution, fewer byproducts, mild reaction conditions and excellent derivative stability. Huang et al. (1) have reported a method for simultaneous analysis of aliphatic and diamines using reversed-phase HPLC, using a C18 column after pre-column derivatisation with DMQC-OSu. Their results have demonstrated good separation of various primary amines and diamines following this procedure.

In this work, an aqueous solution of 8 different aliphatic amines was used (methyl-, ethyl-, propyl-, ethylenedi-, butyl-, amyl-, hexyl- and heptyl- amines). The solution was derivatised following the optimized method reported by Huang et al (1). The derivatisation conditions for this study, such as concentration of DMQC-OSu, buffer pH, reaction temperature and time, were the same optimised conditions as Huang et al. (1) used for maximising derivatisation yield. A 20 µL sample of amine solution was mixed with 20 µL of derivatisation reagent solution (2 mmole DMQC-OSu in acetonitrile) and 200 µL of 0.2 M boric acid titrate buffered to pH 7.5 with sodium hydroxide at 50 °C for 40 minutes. The mixture was then cooled to room temperature. A 20 µL sample of this derivatised solution was then injected for HPLC analysis.

Ascentis Express column of 10 cm length and 2.7 µm particle size was selected for this study compared to column length of 15 cm and particle size of 5 µm used by Huang et al. (1). The derivatised sample was run by HPLC-FL and experiments were performed by varying gradient time for ACD LC simulator. Simulations were run to develop this test method and simulation conditions were confirmed. The chromatogram is shown in Figure 1.

Figure 1. DMQC-OSu Derivatives of Amines using Ascentis Express C18 Column

column: Ascentis Express C18, 10 cm x 4.6 mm I.D.,
2.7 µm particles (53827-U)
mobile phase A: water
mobile phase B: methanol
gradient: min. %A %B
0 50 50
5 10 90
7 10 90
flow rate: 1.0 mL/min.
temp.: 35 °C
det.: fluorescence, ex=326 nm, em=409 nm
injection: 10 µL



Huang et al. (1) reported that the selectivity of their optimised method was good. They suggested that in an amino acid matrix, DMQC-OSu can react with amino acids, and the retention times of amino acid derivatives were found to be shorter than those of aliphatic amine derivatives under the selected chromatographic conditions. Any interference from amino compounds was ruled out in detection of aliphatic amines. Alcohols were also reported to show no interference with aliphatic amines (1). Consequently, this approach offers high selectivity for analysis of aliphatic amines.

A typical chromatogram from the study of Huang et al. (1) showed that under the defined optimum conditions the eight analytes were separated in 16 minutes. Following their optimised derivatisation method and under similar test conditions, the run time on Ascentis Express column was achieved in 7 minutes with reduced re-equilibration from 30 to 20 minutes at equivalent column volumes. Results from the Ascentis Express column show higher peak resolution, especially early in the chromatogram, and improved peak shapes. These results confirm the suitability of the derivatisation reagent, 2,6-Dimethyl-4-quinolinecarboxylic acid N-hydroxysuccinimide ester and the optimised derivatisation method reported by Huang et al. (1). Also, the Ascentis Express C18 column allowed efficient separation of derivatised primary and secondary aliphatic amines after pre-column derivatisation.

References

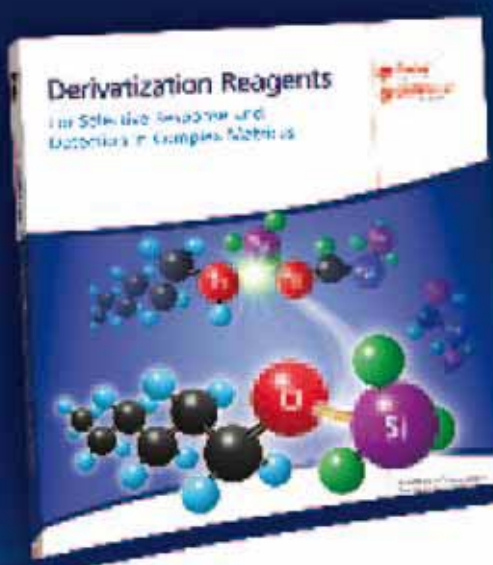
- Huang, K. et al., *Chromatographia*, 2009, 70.
- Wald, G.V., et al., *J. Chromatogr. A*, 2005, 1076:179–182.
- Akyuz, M., *Atmos Environ.*, 2008, 42:3809–19.
- Sahasrabudhey, B. et al. *Analyst*, 1999, 124:1017–1021.
- Lloret, S.M., et al., *J Chromatogr. A*, 2004, 1035:75–82.
- Lamba, S., et al., *Anal Chim Acta*, 2008, 614:190–195.
- Santos, B., et al., *Electrophoresis*, 2004, 25:3231–3236.
- Moliner-Martinez, Y., et al., *J Chromatogr. A*, 2007, 1164:329–333.

+ Featured Products

Description	Cat. No.
Ascentis® Express C18 HPLC Column, 10 cm x 4.6 mm I.D., 2.7 µm particle	53827-U
2,6-Dimethyl-4-quinolinecarboxylic acid N-hydroxysuccinimide ester	49558

+ Related Information

See our guide on “Derivatisation Reagents”. Order a free copy at our website: sigma-aldrich.com/derivatization



- Products sorted by GC, HPLC, Chiral and TLC techniques
- Reagents also listed by “Application”
- Vials, syringes and other useful items for derivatisation reactions
- Up-to-date application information and references

Fluka
Analytical

SUPELCO
Analytical

Derivatisation Reagents Brochure

Listing over 400 Derivatisation Reagents

To order your free copy go to sigma-aldrich.com/derivatization,
or email EurTechServ@sial.com

SIGMA-ALDRICH®

TRADEMARKS: Agilent – Agilent Technologies; Ascentis, Astec, Carbotrap, Center Drain, Cerilliant, CHIRALDEX, CHIROBIOTIC, CLC, CYCLOBOND, DEX, Discovery, P-CAP, Rezorian, “science, smarter”, SLB, Snap-N-Shoot, Snap-N-Spike, SP, Supelco, SupelMIP – Sigma-Aldrich Biotechnology LP; Eternity, Kromasil – Eka Nobel AB; QSertVial – QIS, Inc.; PerkinElmer – PerkinElmer Inc.; PITTCO – The Pittsburgh Conference; Sep-Pak, Xposure – Waters Associates Inc.

New Addition to Astec Chiral HPLC/SFC Line: Astec Cellulose DMP

Tracy Ascah
tracy.ascah@sial.com

Save costs on chiral HPLC columns, without sacrificing performance.

Astec Cellulose DMP is a chiral stationary phase (CSP) comprising spherical, high-purity porous silica coated with DMPC (3, 5-dimethyl-phenyl carbamate)-derivatised cellulose, and packed in analytical to preparative size HPLC columns. It separates a wide range of chiral compounds under normal phase, polar organic, and SFC conditions, with high efficiency, high loading capacity, and excellent column lifetime. Performance is comparable to other DMPC-derivatised cellulose CSPs, but the Astec Cellulose DMP columns are offered at a substantially lower price.

Astec Cellulose DMP is complementary to the other Astec CSPs, including CHIROBIOTIC®, CYCLOBOND™, and the P-CAP™ product lines, and a must-have for every chiral HPLC or SFC screening protocol.

Key Features and Application Areas

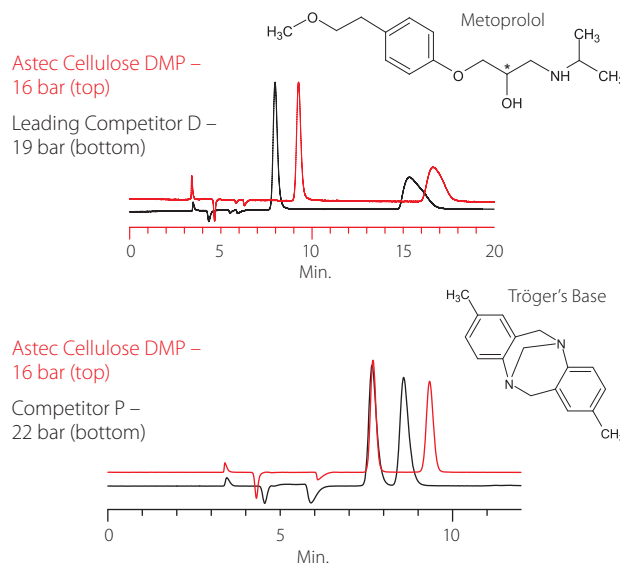
- Classic DMPC-cellulose chiral selectivity
- Efficient, rugged, reproducible and scalable
- Low backpressure
- Ideal for chiral analysis in the pharmaceutical industry and for small analytes in chemical and environmental areas
- Routine chiral column method development screening protocols (HPLC and SFC)
- Approximately one-half the cost of most DMPC-cellulose columns

What Makes Astec Cellulose DMP Unique?

Astec Cellulose DMP is unique in offering classic DMPC-cellulose chiral selectivity with high efficiency, ruggedness, reproducibility and low backpressure, but at approximately one-half the cost of most DMPC-cellulose columns. For the compounds we have tested, Astec Cellulose DMP columns provide similar retention and selectivity, but lower backpressure and higher efficiency compared to competitive columns. Astec Cellulose DMP is not a clone of other DMPC-derivatised cellulose columns on the market, but selectivity and retention is similar enough to use it instead of these columns in your chiral column HPLC and SFC screening protocols. It should also be investigated as a possible replacement for these columns in established methods. The data in **Figure 1** compares Astec Cellulose DMP to two leading competitive columns.

Figure 1. Performance of Astec Cellulose DMP vs. Competitive CSPs

columns: 15 cm x 4.6 mm I.D., 5 µm particles
mobile phase: 10:90:0.1, IPA:heptane:DEA
flow rate: 0.5 mL/min.
temp.: 25 °C
det.: UV, 230 nm
inj.: 2 µL



Ideal for Analytical and Preparative Chiral HPLC

A characteristic of the polysaccharide-based CSPs that has contributed to their popularity is their utility for preparative applications. When we designed the Astec Cellulose DMP, we sought to achieve the high sample loading and throughput that chiral chromatographers have come to expect. The sample loading of Astec Cellulose DMP is comparable to the leading competitor (**Figure 2**). An example of the scale-up of an analytical separation (4.6 mm I.D. column) to a preparative scale (21.2 mm I.D.) is shown in **Figure 3** for the anti-Alzheimer's drug, BAY 73-6691.

Part of the Ever-Expanding Astec Line

The wide choice of CSPs in the Astec line, wider than any other supplier, means they cover many different areas of interest within chiral chromatography. Some of these areas are captured in **Table 1**.

Please visit sigma-aldrich.com/chiral to see our complete line of products and services for chiral chemistry and chiral separations.

Figure 2. Excellent Preparative Loading Capacity

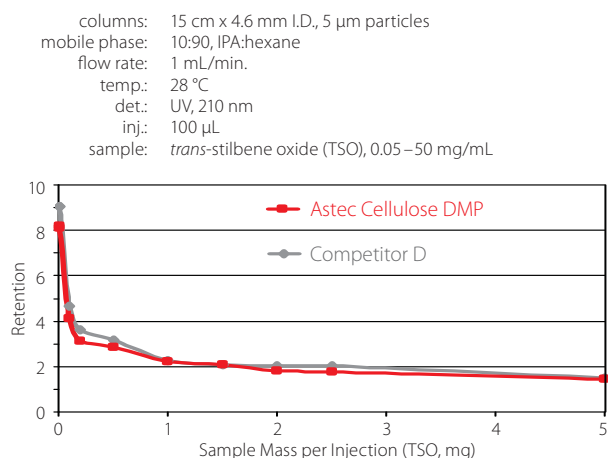


Figure 3. Reliable Scale-Up from Analytical to Prep

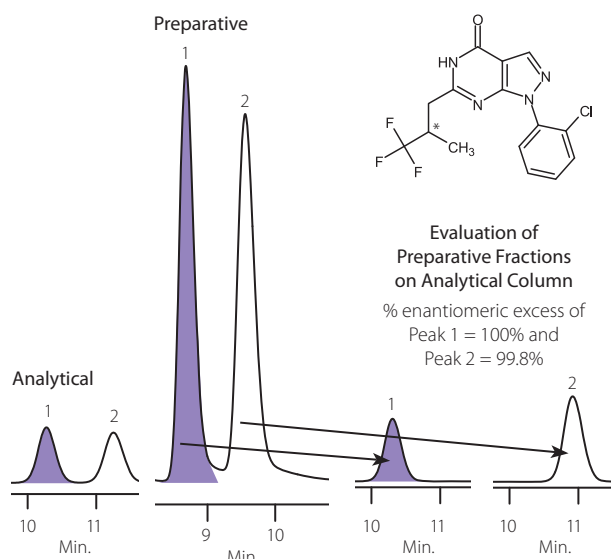
columns: Astec Cellulose DMP, 5 μ m particles
 mobile phase: 80:10:10, heptane:methyl *tert*-butyl ether (MTBE):ethanol
 temp.: 25 $^{\circ}$ C
 det.: UV, 230 nm
 sample: BAY 73-6691 in mobile phase

Analytical:

dimensions: 15 cm x 4.6 mm I.D. (51098AST)
 flow rate: 0.5 mL/min.
 inj.: 10 μ L (2 mg/mL)

Preparative:

dimensions: 25 cm x 21.2 mm I.D. (51103AST)
 flow rate: 13 mL/min.
 inj.: 5000 μ L (3.3 mg/mL)



76 stacked injections were made, processing a total of 10 grams of racemic material

Table 1. Techniques, Applications and Fields of Use for Astec Chiral Phases (HPLC & GC)

	Astec Cellulose DMP	Astec CHIROBIOTIC [®]	Astec CYCLOBOND [™]	Astec P-CAP [™]	Astec CLC [™]	Astec CHIRALDEX [®]	Supelco [®] DEX [™]
Routine Chiral Column Screening	Highly suitable	Highly suitable	Highly suitable	Highly suitable	Highly suitable	Highly suitable	Highly suitable
Normal Phase HPLC	Highly suitable	Highly suitable	Highly suitable	Highly suitable	Highly suitable	Highly suitable	Highly suitable
SFC	Highly suitable	Highly suitable	Highly suitable	Highly suitable	Highly suitable	Highly suitable	Highly suitable
Reversed-Phase HPLC	Highly suitable	Highly suitable	Highly suitable	Highly suitable	Highly suitable	Highly suitable	Highly suitable
Hydrophilic Interaction HPLC (HILIC)	Highly suitable	Highly suitable	Highly suitable	Highly suitable	Highly suitable	Highly suitable	Highly suitable
Polar Organic Mode (HPLC)	Highly suitable	Highly suitable	Highly suitable	Highly suitable	Highly suitable	Highly suitable	Highly suitable
Polar Ionic Mode (HPLC)	Highly suitable	Highly suitable	Highly suitable	Highly suitable	Highly suitable	Highly suitable	Highly suitable
Ligand Exchange Mode HPLC	Highly suitable	Highly suitable	Highly suitable	Highly suitable	Highly suitable	Highly suitable	Highly suitable
Gas Chromatography (GC)	Highly suitable	Highly suitable	Highly suitable	Highly suitable	Highly suitable	Highly suitable	Highly suitable
Prep (LC and/or SFC)	Highly suitable	Highly suitable	Highly suitable	Highly suitable	Highly suitable	Highly suitable	Highly suitable
Polar/Ionic Analytes	Highly suitable	Highly suitable	Highly suitable	Highly suitable	Highly suitable	Highly suitable	Highly suitable
Amino Acids, Peptides	Highly suitable	Highly suitable	Highly suitable	Highly suitable	Highly suitable	Highly suitable	Highly suitable
Non-Aromatic Organic Acids	Highly suitable	Highly suitable	Highly suitable	Highly suitable	Highly suitable	Highly suitable	Highly suitable
Mass Spec (LC/ESI)	Highly suitable	Highly suitable	Highly suitable	Highly suitable	Highly suitable	Highly suitable	Highly suitable
Mass Spec (LC/APCI)	Highly suitable	Highly suitable	Highly suitable	Highly suitable	Highly suitable	Highly suitable	Highly suitable
Mass Spec (GC/MS)	Highly suitable	Highly suitable	Highly suitable	Highly suitable	Highly suitable	Highly suitable	Highly suitable
Bioanalysis (drugs in biological fluids)	Highly suitable	Highly suitable	Highly suitable	Highly suitable	Highly suitable	Highly suitable	Highly suitable

+ Featured Products

Description	Length (cm)	I.D. (mm)	Cat. No.
Astec Cellulose DMP Columns – 5 μm particles			
	15	2.1	51100AST
	25	2.1	51101AST
	10	4.6	51097AST
	15	4.6	51098AST
	25	4.6	51099AST
	25	10.0	51102AST
	25	21.2	51103AST
Astec Cellulose DMP Guard Cartridge Kits*			
For 2.1 mm I.D. columns	2	2.1	51105AST
For 4.6 mm I.D. columns	2	4.0	51107AST
Astec Cellulose DMP Replacement Guard Cartridges			
For 2.1 mm I.D. columns	2	2.1	51104AST
For 4.6 mm I.D. columns	2	4.0	51106AST
For 10 mm I.D. columns	1	10.0	51108AST
For 21.2 mm I.D.	1	21.2	51109AST

* Kits contain holder (59660-U), one cartridge, 2" x 1/16" tubing, two nuts, and two ferrules.

Holders for Guard Cartridges	
For 2 cm x 2.1 mm and 4 mm I.D. Guard Cartridges	59660-U
For 1 cm x 10 mm I.D. Guard Cartridges	567499-U
For 1 cm x 21.2 mm I.D. Guard Cartridges	581392-U

TCA and Precursors in Red Wine Using In-Matrix Derivatisation Followed by SPME on the SLB™-5ms

Katherine K. Stenerson
katherine.stenerson@sial.com

Introduction

Cork taint refers to a musty odour in wine caused by the presence of 2,4,6-trichloroanisole (TCA). The source of TCA is thought to be the fungal methylation of chlorophenols present in the wine. Chlorophenols can originate from the cork or other sources, such as biocides, fungicides, and exposure of processing equipment to antiseptic cleaning products that contain chlorophenols (1). This article demonstrates the determination of TCA and several chlorophenol precursors in a red wine.

Experimental

Calibration standards were prepared in 12% ethanol in water using a mixture of TCA and chlorophenols. Two samples were based on a California (USA) shiraz, packaged in a wax-lined carton-type container with a plastic closure. The first sample was the wine as is, and the second sample was the wine after being spiked with each analyte at 100 ng/L.

The chlorophenols were derivatised in-matrix using acetic anhydride, and the acylated derivatives were then extracted from the headspace using solid phase microextraction (SPME). TCA, which is not derivatised, was simultaneously extracted with the chlorophenols. An SPME fibre coated with 100 µm polydimethylsiloxane (PDMS) was chosen based on past work we performed with TCA, and also on published findings for derivatised chlorophenols (1). The extraction conditions were based on published findings as well, with adjustments made to sample volume and corresponding amounts of reagents. Final analysis was performed by GC-ECD (gas chromatography with an electron capture detector) on the SLB-5ms capillary column, selected due to its low bleed characteristic.

Results

Derivatisation and Extraction. The chlorophenols were acylated with acetic anhydride prior to extraction. Acetic anhydride will hydrolyse in the presence of water. However, the phenolic groups present on the analytes are more reactive, making it possible to conduct derivatisation in an aqueous matrix (2). The reaction is shown in **Figure 1**. The addition of potassium carbonate (K_2CO_3) drives the reaction by removing the acetic acid that is formed. The resulting derivatives demonstrated good peak shape and response by ECD, allowing for easy and consistent integration. The use of headspace extraction and selective detection (ECD is selective for halogens) resulted in minimal background interference.

Linearity. Five calibration standards from 10-300 ng/L prepared in 12% ethanol in water were extracted, then analysed. **Figure 2** shows the chromatogram resulting from the mid-level standard. Linearity results

Figure 1. Derivatisation Reaction

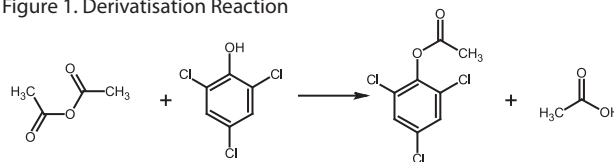


Figure 2. Calibration Standard

sample/matrix: 1.5 mL of each analyte at 100 ng/L in a 12% ethanol in water solution + 600 µL 5% potassium carbonate (K_2CO_3) + 240 mg sodium chloride (NaCl) + 60 µL acetic anhydride
 SPME fibre: metal fibre coated with 100 µm PDMS (57928-U)
 extraction: headspace, 50 °C for 30 min., with stirring
 desorption process: 250 °C for 3 min.
 column: SLB-5ms, 30 m x 0.25 mm I.D., 0.25 µm (28471-U)
 oven: 50 °C (1 min.), 25 °C/min. to 280 °C
 det.: ECD, 290 °C
 carrier gas: helium, 1.5 mL/min., constant
 liner: 0.75 mm I.D. SPME (2637501)

1. 2,4,6-Trichloroanisole
2. 2,4,6-Trichlorophenol (acylated)
3. 2,3,4,6-Tetrachlorophenol (acylated)
4. Pentachlorophenol (acylated)

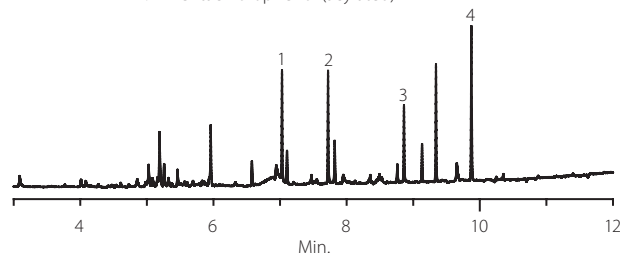


Table 1. Calibration Linearity for Five Standards from 10-300 ng/L

Analyte	Correlation Coefficient
2,4,6-Trichloroanisole	0.9908
2,4,6-Trichlorophenol	0.9978
2,3,4,6-Tetrachlorophenol	0.9932
Pentachlorophenol	0.9873

are summarised in **Table 1**. A linear response was observed over this concentration range, with correlation coefficients of >0.990 for all analytes except pentachlorophenol. For this compound, excluding the 10 ng/L standard results in a correlation coefficient of 0.998.

Recovery. Immediately after calibration, unspiked and spiked (100 ng/L) wine samples were prepared, extracted, and analysed. Resulting chromatograms are presented in **Figures 3 and 4**. Using the calibration curves, the levels of TCA and chlorophenols were calculated, along with a percent recovery for the spiked wine sample. These results are summarised in **Table 2**. It appears that the wine matrix may have interfered, as indicated by the low % recovery values.

Table 2. Recovery from Red Wine Spiked at 100 ng/L

Analyte	Unspiked (ng/L)	Spiked (ng/L)	Recovery (%)
2,4,6-Trichloroanisole	not detected	60.7	61
2,4,6-Trichlorophenol	22.7	96.3	74
2,3,4,6-Tetrachlorophenol	not detected	55.7	56
Pentachlorophenol	3.3	33.5	30

Table 3. Average Detector Response and Reproducibility for 100 ng/L Spikes

Analyte	Spiked Method Blank Samples (12% ethanol in water) (n=3)			Spiked Red Wine Samples (n=3)		
	Avg.	Std. Dev.	%RSD	Avg.	Std. Dev.	%RSD
2,4,6-Trichloroanisole	4190	112	3	3004	286	10
2,4,6-Trichlorophenol	4389	99	2	3496	1013	29
2,3,4,6-Tetrachlorophenol	3006	205	7	1811	184	10
Pentachlorophenol	5611	539	10	2610	238	9

Reproducibility. A check of reproducibility was performed by processing three spiked samples in 12% ethanol in water and another three spiked samples in wine. This data is summarised in **Table 3**. Reproducibility for the three spiked samples in 12% ethanol in water was good, with <10% RSD values. The lower average area counts and overall increased variability for the three spikes samples in wine indicates the effect of the wine matrix.

Conclusions

SPME can be used for the extraction of 2,4,6-trichloroanisole and its chlorophenol precursors from wine. In the case of the latter, derivatisation makes the analytes easier to extract and analyse by GC.

Headspace extraction in combination with ECD can be used to reduce background interference. The method appears to be quantitative, although further work would be necessary to optimise extraction efficiency from wine matrix.

References

- Insa, S., Salvado, V., Antico, E., "Development of Solid-Phase Extraction and Solid-Phase Microextraction Methods for the Determination of Chlorophenols in Cork Macerate and Wine Samples", *J. Chromatogr. A* 1047, 2004, 15.
- Blau, K. and Halket, J., "Handbook of Derivatives for Chromatography, Second Edition", John Wiley & Sons, New York, 1993, 38.

Figure 3. Unspiked Red Wine

sample/matrix: 1.5 mL red wine + 600 µL 5% potassium carbonate (K₂CO₃) + 240 mg sodium chloride (NaCl) + 60 µL acetic anhydride

Conditions and Peak IDs same as Figure 2

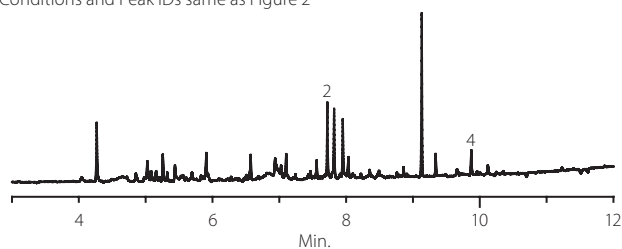
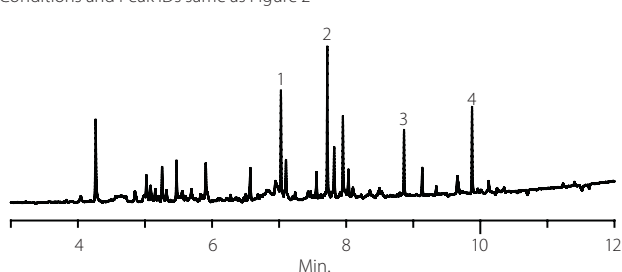


Figure 4. Spiked Red Wine

sample/matrix: 1.5 mL red wine spiked with each analyte at 100 ng/L + 600 µL 5% potassium carbonate (K₂CO₃) + 240 mg sodium chloride (NaCl) + 60 µL acetic anhydride

Conditions and Peak IDs same as Figure 2



+ Featured Products

Description	Cat. No.
Metal SPME fibre assembly, 100 µm PDMS coating, 23 gauge, autosampler design	57928-U
SLB™-5ms capillary GC column, 30 m x 0.25 mm I.D., 0.25 µm	28471-U
Inlet liner, 0.75 mm I.D. SPME design, for Agilent® 5890 and 6890 GCs	2637501
Acetic anhydride, 10 x 2 mL	33085
Potassium carbonate (K ₂ CO ₃), ACS reagent, ≥99.0%	209619
Sodium chloride (NaCl), BioXtra, ≥99.5%	S7653
Ethanol, 200 proof	459828
Standards	
2,4,6-Trichloroanisole, 100 µg/mL in methanol, 1 mL	47526-U
2,4,6-Trichlorophenol, 5000 µg/mL in methanol, 1 mL	40019
2,3,4,6-Tetrachlorophenol, 5000 µg/mL in methanol, 1 mL	48264
Pentachlorophenol, 5000 µg/mL in methanol, 1 mL	40062

+ Related Information

To discover the features and benefits of SPME, visit sigma-aldrich.com/spme

To learn more about derivatisation reagents and procedures, visit sigma-aldrich.com/derivatization and/or order publication KDI.

To view our full-line of GC columns and accessories, visit sigma-aldrich.com/gc

The Extraction of Acid NSAIDs from Muscle Tissue Using Molecularly Imprinted Polymer SPE

Contributed Article

The following was generated with the assistance of an outside source using Sigma-Aldrich products. Technical content was generated and provided by:

Dr. Anja Siewing

LAVES, Veterinary Institute Oldenburg, Germany

Introduction

The determination of acidic pharmaceuticals, such as non-steroidal anti-inflammatory drugs (NSAIDs), at low levels in muscle tissue requires highly selective and sensitive analytical procedures. Final analysis by LC-MS-MS requires clean samples with minimal matrix effects; therefore, the removal of matrix components during sample preparation is critical for analysis. Here we describe a very simple method of concentrating and cleaning up NSAIDs from tissue using SupelMIP® (molecularly imprinted polymer) phases.

MIPs are highly cross-linked polymer-based molecular recognition elements, which are capable of binding one target, one compound, or a class of structurally related target compounds with high selectivity. The 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 analytes.

Most of NSAID compounds have acidic functionality. The following subgroups can be recognised among NSAIDs and they include aryl-propionic acid derivatives (Carprofen, Vedaprofen), oxicam derivatives (Meloxicam), nicotinic acid derivatives (Flunixin) and anthranilic acid derivatives (Diclofenac, Tolfenamic acid). Acidic NSAIDs are characterised by their ability to suppress the inflammatory process and associated symptoms such as pain, swelling, redness and loss of function.

NSAIDs are widely used in veterinary medicine in the treatment of food-producing animals. Although they have short half-lives, these veterinary drugs and their metabolites may leave residues in edible tissue, which could have direct toxic effects on the consumers. In order to avoid risks, a strict legislative framework controls the use of these drugs, with the aim of minimising the risk to human health. The European Union (EU) has constituted maximum residue limits (MRLs) for numerous substances, which are administered for food-producing animals (Council Regulation No. 37/2010), to ensure human food safety (1). The acidic NSAIDs is a group of substances, for which maximum residue limits (MRL) have been set. The tolerance value in poultry muscle tissue (the national agreed value for this specific matrixes) for all NSAIDs analytes in the present work is 50 µg/kg.

The method presented here was sensitive and selective enough to detect these compounds at below the tolerance level. The described method was fully validated according to the European Commission Decision 2002/657/EC (2-4) for the analysis of veterinary drug residues.

Materials and Methods

The extraction procedure was adopted from (5). Five (5) g of muscle tissue was weighed into 25 mL centrifuge tubes; internal standards and analytes were added to the samples. Four (4) mL ammonium acetate buffer (10 mM, pH 5) and 6 mL acetonitrile were added. The mixture was shaken well by hand for 30 seconds, followed by a pH adjustment between pH value 4 and 5 with formic acid. The sample was shaken for 20 min. and then centrifuged for 5 min. (3000 g at 20 °C) to separate the phases.

10 mL of the supernatant was decanted into a clean 50 mL centrifuge tube and diluted with 5 mL of 10 mM ammonium acetate buffer. The pH value of the samples (pH 3) was checked before proceeding to the solid phase extraction stage (6).

The next step was the solid phase extraction (SPE) with SupelMIP. All of the wash and elution steps were done according to the SupelMIP extraction protocol for NSAIDs. The SPE method is presented in **Table 1**.

Table 1. SPE Extraction Conditions

condition:	1 mL acetonitrile, 1 mL methanol 1 mL pH 3 ammonium formate buffer
load:	5 mL sample
wash:	1. 1 mL water – vacuum was applied for 5 min. 2. 1 mL acetonitrile:water (40:60) – vacuum was applied for 2 min. 3. 1 mL toluene:heptane (40:60) vacuum was applied for 4 min.
elution:	1 mL 1% acetic acid in 20:80 acetone:methanol

The eluate was evaporated to dryness under nitrogen at 50 °C and reconstituted in 150 µL of a mixture of acetonitrile:methanol (9:1 v/v) with 0.1% formic acid. The extracts were filtered through a 0.2 µm filter and transferred into HPLC vials. The LC-MS conditions are presented in **Figure 1**.

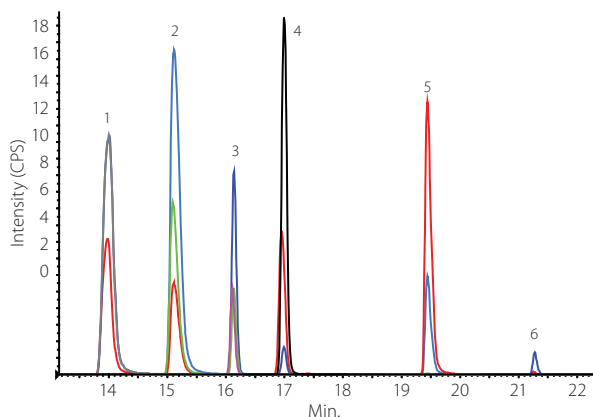
Results and Discussion

We looked for a sample preparation method that would allow low NSAIDs detection limits and provide easy cleanup from poultry muscle tissue. The first liquid-liquid extraction step using acetonitrile:water efficiently precipitated the proteins. If that extraction step is not performed, the samples can form a gel-like consistency in the presence of water and are hard to handle. Experiments were also carried out to compare the efficiency of protein separation by liquid-liquid extraction with that by hydrolysis using protease. The use of protease resulted in a loss in analyte which was probably due to less efficient protein removal.

Figure 1. Extracted Ion Chromatogram of a Muscle Matrix Sample Spike with Six Standard Analytes (48 µg/mL) and the Internal Standards (80 µg/mL) Mix of NSAIDs

column: Ascentis® Express C18, 15 cm x 3.9 mm I.D., 2.7 µm
 det.: MS-MS, negative ESI with two MRM transitions, curtain gas 40.00 psi; ion spray voltage 4500 V; temperature 400 °C; gas 1 40 psi; gas 2 70 psi
 flow rate: 400 µL/min.
 injection: 10 µL
 mobile phase: gradient (A) water: acetonitrile (90:10) with 1 mM acetic acid; (B) acetonitrile; 90% A to 10% A in 23 min., re-equilibration at 90% A for 12 min.
 temp.: 40 °C

1. Meloxicam (red, blue, grey – IS)
2. Flunixin (green, red, blue – IS)
3. Carprofen (red, green, blue – IS)
4. Diclofenac (red, blue, black – IS)
5. Tolfenamic acid (blue, green, red – IS)
6. Vedaprofen (red, green, blue – IS)



The extracted ion chromatogram after sample preparation showed that the peaks for NSAID analytes were baseline-resolved in **Figure 1**. Two MRM transitions for each compound are shown and one MRM transition for the internal standard is also shown.

During method validation the sample preparation procedures were applied to 16 poultry muscle samples. The samples were spiked at the following concentration levels:

Meloxicam, Flunixin, Carprofen, Diclofenac, and Tolfenamic acid:

Level 1: 16 µg/kg Level 3: 48 µg/kg
 Level 2: 32 µg/kg Level 4: 64 µg/kg

Vedaprofen:

Level 1: 30 µg/kg Level 3: 90 µg/kg
 Level 2: 60 µg/kg Level 4: 120 µg/kg

Quantification was done by using standard matrix-matched calibration curves. On the basis of these results we calculated decision limit CC_{α} , detection capability CC_{β} , recovery as well as the within-laboratory reproducibility. **Table 2** shows the calculated validation results for poultry muscle.

The recovery values for each compound at the level of interest are above 90%, thus meeting the requirements of Commission Decision 2002/657/EC. The ion suppression due to matrix effects was low. Our calculated decision limits CC_{α} for the level of interest are below the required value.

Table 2. Summary of the Calculated Validation Results for Poultry Muscle Samples

Substance	CC_{α} [µg/kg]	CC_{β} [µg/kg]	RSD [%]	Recovery [%]
Carprofen	19.7	22.4	9.3	96.1
Vedaprofen	40.1	47.3	13.9	92.3
Meloxicam	19.9	22.8	9.5	96.8
Flunixin	20.3	23.4	10.2	97.4
Diclofenac	20.8	24.1	11.0	103.1
Tolfenamic acid	20.6	23.8	11.3	94.1

Conclusions

In this article we described a new successfully developed and validated LC-MS-MS method allowing the extraction of NSAIDs from poultry muscle tissue using MIP SPE. SupelMIP® offered the selectivity necessary to achieve sensitivity, detection limits and quantitation in tissue at the low concentration level that was below the maximum residue limits. Furthermore, cleaner extracts were obtained with this rapid extraction technique when analysing difficult and dirty sample matrices. Running cleaner samples resulted in less contamination to LC-MS instrument and less maintenance was required. The ruggedness of the SupelMIP SPE was proven by a systematic variation of the validation factors on two levels. Thus, the extraction method is fit for purpose and can be applied in routine analysis.

References

1. European Commission, Council regulation 37/2010/EEC, Off. J. Eur. Commun., L 15, 2010, 1.
2. Commission Decision (2002/657/EC) of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and interpretation of results, Off. J. Eur. Commun., L221, 2002, 8.
3. Document SANCO SANCO/2004/2726 rev 4 (2008), Guidelines for the implementation of decision 2002/657/EC.
4. Council Directive 96/23/EC of April 1996 on measures to monitor certain substances and residues thereof in live animals and animal products, 1996, Off. J. Eur. Commun., L125:10-3.
5. Peters, R.J.B. et al., *J. Chromatogr. A* 1216, 2009, 8206–8216.
6. Zorita, S. et al. *Analytica Chimica Acta*, Volume 626, Issue 2, 26 September 2008, 147–154.

+ Featured Products

Description	Qty.	Cat. No.
SupelMIP SPE-NSAIDs, 3 mL/25 mg	50	52769-U
Ascentis Express C18, 15 cm x 3.0 mm, 2.7 µm	1	53816-U

Did you know?

For more information on SupelMIPs, the brochure (JOZ) and other phases, please visit sigma-aldrich.com/supelmip or request a copy on the enclosed reply card.

Sampling of Carbonyls – a Sampling Media Perspective

Kristen Schultz
kristen.schultz@sial.com

Carbonyls such as formaldehyde, acetaldehyde, glutaraldehyde and acetone are common indoor air pollutants most often collected on devices which contain 2,4-dinitrophenylhydrazine (DNPH) treated sampling media which derivatises the carbonyls to the more stable hydrazone derivatives. The derivatives are measured by reversed-phase HPLC with UV detection.

There are a number of analytical methods available to collect and measure carbonyls in indoor and ambient environments which range from the use of glass impingers to filter methods to solid phase extraction (SPE) cartridges – active and passive sampling. In the past decade, passive sampling has become an accepted method for sampling and measurement of carbonyls.

Current Trends in Carbonyl Measurement

In 2005, OSHA released Method 1007 for measurement of formaldehyde using diffusive samplers. Several different samplers were investigated in the method, including the Supelco® DSD-DNPH (28221-U) sampler. At the time, the Radiello Aldehyde (RAD165) sampler was not available for inclusion in the method. Both the DSD-DNPH and Radiello devices are suitable for the method.



Radiello Aldehyde Sampler can be used for sampling a wide range of aldehydes such as formaldehyde, acetaldehyde, benzaldehyde, butanal, hexanal, glutaraldehyde and more. Sampling rates are faster than other available passive samplers (example: RAD165 formaldehyde sampling rate 99 mL/min.).



NEW! BPE-DNPH is an innovative dual-bed cartridge device designed for sampling of carbonyls in ambient, indoor and industrial atmospheres. It contains also a bed of 2,4-DNPH but offers the advantage of a 1,2-bis(2-pyridyl) ethylene (BPE) silica gel bed in front, functioning as a built-in ozone scrubber that is not affected by high humidity or rainy conditions.

Traditional Sampling Methods

Solid sorbent-based media such as high-purity silica gel coated with 2,4-DNPH is most widely used for sampling carbonyls. The following regulatory methods specify a single bed of 350 mg silica gel coated with 1 mg of 2,4-DNPH in a solid phase extraction (SPE) style cartridge: NIOSH 2016, ASTM D5197, EPA TO-11A, and EPA IP-6A. Carbonyl capacity: <75 µg (formaldehyde equivalent).

Supelco offers a variety of cartridge configurations suitable for your application containing low background and low pressure drop (Lp) DNPH packings:



LpDNPH S10 cartridge is a 3 mL SPE style cartridge with a slip luer design and a built-in reservoir for easy extraction and elution. Reusable adapters are available.

The S10 Starter Kit contains all needed adapters (tubing and cartridge adapter).



LpDNPH S10x cartridge is shorter than the S10 cartridge and designed to fit into automated systems.



LpDNPH S10L cartridge is a 3 mL reversible cartridge design for EPA Method TO-11A which is designed for the analyst who prefers shorter dimensions and does not require an adapter for sampling. The cartridge is eluted by connecting to an empty SPE cartridge that acts as a reservoir for gravity-fed elution solvent. The S10L is equivalent to Waters Sep-Pak™ or XPOsure™ cartridges.



DNPH Rezorian™ is a 3 mL cartridge which features luer-lock end-fittings that can be used to connect a pump tubing to two cartridges in a series for “piggybacked” sampling to monitor breakthrough or increase capacity.

Request your copy of the newest Air Monitoring catalogue. Visit us at sigma-aldrich.com/air_monitoring

Sampling Carbonyls in Higher Concentration Environments

In some instances, a high-capacity carbonyl sampling device may be required for high concentration environment. The need for such a device is commonly determined when the capacity of a traditional device would be exceeded. Supelco® offers cartridges to meet these special conditions, known as our LpDNPH H Series cartridges. These cartridges contain silica gel with a higher loading of 2,4-DNPH plus, for the H30 and H300 type cartridges, a larger bed weight.



LpDNPH H10 cartridge is a 3 mL S10 style cartridge with a 350 mg bed of higher loading capacity DNPH-silica.
Carbonyl capacity: <225 µg



LpDNPH H30 cartridge is a 6 mL SPE style cartridge containing a 1 gram bed of higher loading capacity DNPH-silica.
Carbonyl capacity: <643 µg



LpDNPH H300 cartridge is a 20 mL SPE style cartridge containing 10 grams of higher loading capacity DNPH-silica.
Carbonyl capacity: <6.4 mg

Sampling Carbonyls in the Presence of High Ozone Levels

Ozone is known to interfere with carbonyl sampling and therefore must be removed or “scrubbed” from the sampling environment. The most common ozone scrubber is 1.5 g of potassium iodide (KI) which prevents negative ozone interference in DNPH coated devices. KI scrubbers are available in both a reversible SPE tube design, like the S10L (bottom), and the Rezorian (top) design. Our scrubbers have an ozone capacity of 100,000 ppb/hr when tested at 200 ppb ozone, 50% RH, 25 °C. Potassium iodide scrubbers are not recommended in high-humidity environments. For sampling carbonyls high-humidity environments, we recommend the new BPE-DNPH cartridge.



Other Sampling Methods for Carbonyls



For sampling select carbonyls, filter methods are still used for compounds such as glutaraldehyde (OSHA 64), crotonaldehyde (OSHA 81), and valeraldehyde (OSHA 85). ORBO-827 glass fibre filters are coated with 2,4-DNPH, actively sampled and desorbed in the same manner as traditional DNPH devices.

Summary

We have a wide range of products available for sampling carbonyls in most applications, from routine sampling situations to the most unique. In addition to the sampling media, we offer HPLC columns and aldehyde standards to complement your method.

References

1. Uchiyama, S., Aoyagi, S. and Ando, M., “Evaluation of a Diffusive Sampler for Measurement of Carbonyl Compounds in Air”, *Atmospheric Environment*, 2004, 38, 6319–6326.
2. Uchiyama, S. and Hasegawa, S., “A Reactive Sensitive Diffusion Sampler for the Determination of Aldehydes and Ketones in Ambient Air”, *Atmospheric Environment*, 1999, 33, 1999–2005.

+ Featured Products

Description	Qty.	Cat. No.
Passive/Diffusive Sampling		
Radiell® Aldehyde Adsorbent Cartridge	20	RAD165
Radiello Blue Diffusive Body	20	RAD1201
Radiello Triangular Support Plate	20	RAD121
Radiello Vertical Adapter (optional)	1	RAD122
Active Sampling		
LpDNPH S10, 3 mL/350 mg	10	21026-U
	50	21014
Packaged in nylon bags, 1 per bag	50	54072-U
LpDNPH S10 Starter Kit	10	21024-U
LpDNPH S10x	10	505293
LpDNPH S10L	10	505358
	50	505361-U
LpDNPH Rezorian™ 3 mL/350 mg	10	54074-U
	50	54075-U
BPE-DNPH, 3 mL,130/270 mg	10	54278-U
	50	54279-U
LpDNPH H10, 3 mL/350 mg	10	505315
	50	505320-U
LpDNPH H30, 6 mL/1 g	10	505323
LpDNPH H300, 20 mL/10 g	10	505331
ORBO-827 DNPH coated filter, 37 mm	25	20069
Ozone Scrubbers		
Reversible Cartridge, 1.5 g KI	10	505285
Rezorian Cartridge, 1.5 g KI	10	54078-U

+ Related Products

Description	Qty.	Cat. No.
Ascentis® Express Columns, 15 cm x 4.6 mm I.D., 2.7 µm		
C18	1	53829-U
RP-Amide	1	53929-U



Supelco® Thermal Desorption Tubes in TDS³™

For DANI, Markes (MI™), Shimadzu®, OI Analytical® and PerkinElmer® Instruments

Available in both Stainless Steel and Glass (fritted) Styles. ¼ in. (6.35 mm) O.D x 3.5 in. (89 mm) Long

- All Carbotrap™ products supplied with our adsorbent technology inside
- Better adsorbent bed integrity
- More consistent back pressure from tube to tube
- Stainless steel tube markings easy to read
- Fritted glass tubes supplied with frit at optimised location and barcode
- Sealed with TDS³ storage container



Scientific Seminars on Analytical and Chromatography

As a service-driven supplier for Analytical and Chromatography products, Sigma-Aldrich has a long history in providing technically advanced presentations for end users. Attendees value these seminars because they are given both by experienced scientists from R&D or Product Management and by acknowledged external guest speakers, providing an overview on recent developments and useful guidance, tips and tricks for the lab.

On the one hand, the presentations cover the fundamentals of analytical science such as theory, method development, trouble-shooting

and most recent innovative approaches; on the other hand, they cover dedicated application areas such as pharmaceutical, environmental or food analysis.

Feedback from former participants:

- "Presentations from experienced scientists were highly instructive"
- "Competent team of speakers"
- "Very interesting, informative and professionally presented"

Overview on forthcoming Supelco and Fluka® seminars in Europe:

Austria			26.05.2011	Munich:	Food Analysis
31.05.2011	Vienna:	Food Analysis	07.06.2011	Berlin:	Karl Fischer Titration
Czech Republic			Italy		
01.06.2011	Prague:	Food Analysis	06.07.2011	Milan:	SPME User Meeting
Denmark			Norway		
10.05.2011	Copenhagen:	Recent developments in Sample Preparation	10.05.2011	Oslo:	Clinical Analysis
France			12.05.2011	Bergen:	Clinical Analysis
07.07.2011	Nice:	SPME User Meeting	Sweden		
Germany			19.05.2011	Stockholm:	Recent Developments in Sample Preparation
03.05.2011	Munich:	Karl Fischer Titration	Switzerland		
18.05.2011	Cologne:	Food Analysis	24.05.2011	Basle:	Food Analysis
19.05.2011	Hanover:	Food Analysis	UK		
			08.07.2011	London:	SPME User Meeting

Please find details on these and further seminars at sigma-aldrich.com/events

LC-MS Mobile Phase Additives

Shyam Verma
shyam.verma@sial.com

LC-MS is commonly used as an analytical tool in research and industrial laboratories. There is growing emphasis on test sensitivity, specificity and speed of analysis. Continuously improving LC systems have lowered the limits of detection. Consequently, it requires use of high-purity chemicals for sample preparation, mobile phase and post-column additives. The purity and composition of the chemicals used in LC-MS can affect its performance in many ways, such as:



- Inorganic salts form adducts with polymers, including biopolymers like proteins, and complicate the mass spectra, causing a broad distribution of charged sodium, potassium and chloride adducts.
- Salts can suppress ionisation in ESI sources even with small molecules.
- Risk of contaminating the analysis.

Sigma-Aldrich offers many solvents and additive reagents that are specifically designed to meet the requirements of high purity and consistency. High-purity solvents and additives offer many advantages for both small and large molecule analysis. Our offering includes the most commonly used acids, bases, volatile salts and a sodium source. These products are of high purity, usually puriss p.a., and are tested for LC-MS application.

Acid additives: Volatile, low molecular weight organic acids like formic and acetic acid are commonly used as additives in LC-MS mobile phase. Primarily, these acids improve ionisation and resolution of a wide range of molecules (1).

TFA suppression effect: The ionisation-suppressing effect of trifluoroacetic acid (TFA) can be partly overcome by addition of other LC-MS compatible organic acid, like formic or propionic acids (2). Mobile phases for HPLC of proteins and peptides usually contain TFA to control the pH and improve peak shape and resolution. TFA enhances retention by ion pairing with the peptide and improves peak shape by reducing silanol interactions (3). However, TFA also has adverse effects on MS detection. Its high surface tension prevents efficient spray formation and TFA ions in the gas phase ion-pair with the peptide basic group, thus suppressing their ionisation and reducing the MS signal (4, 5).

Neutral salts: Neutral volatile salts, such as, ammonium acetate and ammonium formate, offer a much broader influence on analyte separation and ionisation than do acids (6). Their use, of course, is dictated by the particular LC-MS separation objective.

It may be necessary under certain circumstances to use more neutral conditions, either because the analytes are sensitive to acids or do not exhibit optimal resolution at low pH. When acids are not suitable, volatile salts like ammonium formate or acetate may be the additives of choice. However, limited solubility of the salt in organic solvents, changing pH value during a gradient and the mildly acidic pH provided by the salt that permits both positive and negative ion mode detection are issues of concern (6).

Sodium adduct formation: Alkali adduct diminishes the instrument sensitivity. When adduct formation tendency is strong, addition of small and defined amounts of sodium ions (mostly pre-column) can help to obtain uniform and stable molecular ions for detection in LC-MS (7). In addition to sensitivity, stability and perhaps specificity of the molecular ion are also important. The ability to form alkali adducts is useful for quantifying certain classes of molecules and for selectively enhancing the LC-MS signals.

References

1. Emmert, J., Reporter, 2006, No. 2, 8.
2. Emmert, J. and Rueck, A., Reporter, 2006, No. 3, 16.
3. Supelco® Application Note 168 (T302168).
4. Apffel, A., Fisher, S., Goldberg, G., Goodley, P.C., Kuhlmann, F.E., *J. Chromatogr. A.*, 1995, 712, 17780–190.
5. Wang, G., Cole, R.B., *J. Am. Soc. Mass Spectrom.*, 1996, 7(10), 1050–1058.
6. Emmert, J. and Leitner, A., Reporter, 2006, No. 4, 9.
7. Emmert, J. and Waelti, T., Reporter, 2006, No. 5, 6.

+ Featured Products

Description	Qty.	Cat. No.
Puriss p.a.* Eluent Additives for LC-MS		
Trifluoroacetic acid, puriss p.a.	10 x 1 mL	40967
Formic acid, puriss p.a.	50 mL	56302
Acetic acid, puriss p.a.	50 mL	49199
Propionic acid, puriss p.a.	50 mL	49916
Ammonium formate, puriss p.a.	50 g	55674
Ammonium bicarbonate, puriss p.a.	50 g	40867
Ammonium hydroxide solution 25%, puriss p.a.	100 mL	44273
Triethylamine, puriss p.a.	50 mL	65897

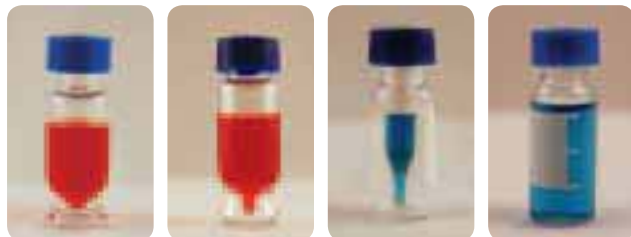
* "puriss" quality grade is defined as >98.5% assay, <0.1% ash, and specification $n_D^{20} \pm 0.001$, $d_4^{20} \pm 0.001$ with no extraneous colour and a homogeneous appearance. "p.a." or pro analysis denotes a product with guaranteed trace impurity levels and/or suitability for the indicated analytical application.

+ Related Information

For more information on LC-MS Chromasolv Solvents, Blends and Additives, request Literature Code KCT on the attached postcard or visit sigma-aldrich.com/chromasolv

NEW! Low Adsorption Vials for Basic Compounds

Four Styles of LA Vials: Center Drain™, MRQ30, QSertVial™ and Standard Autosampler Vial



Features and Benefits

- Maintains sample integrity during storage
- Minimises pH shifts in the sample
- Reduce metal contamination in the sample
- Compatible with most autosamplers

Supelco®'s new Low Adsorption (LA) vials are manufactured using a process that decreases the number of hydroxyl groups on the vial's glass surface, significantly reducing surface activity while improving analytical quantitation and minimising pH shifts in the sample. This same process also removes unwanted surface metals such as sodium and boron that can contaminate samples and interfere with trace analysis. Unlike other methods used to decrease vial surface activity, the elimination of surface activity in LA vials is integral to the manufacturing process and is not a chemical surface treatment.

Our study of samples containing trace levels of basic, acidic and neutral compounds stored in an LA vial product versus three other national brands found the LA vial to be of particular benefit when working with small, polar amine compounds (See [Table 1](#)).

Table 1. % Loss of Compounds After 4 Hours

	LA-2 mL	LA-MRQ	Brand A	Brand B	Brand C
Chlorhexidine*	1.4	0	26.4	49.3	29.3
Chlorpheniramine	2.1	1	14.1	18	12
Ibuprofen	< 5	< 5	< 5	< 5	< 5
Acenaphthene	< 2	< 2	< 2	< 2	< 2

* Analyte concentration @ 5 µg/mL, except acenaphthene which was < 100 ppb

Supelco's LA vials are manufactured from Type 1 borosilicate glass and are offered in four styles: Center Drain (CD), MRQ30, QSertVial and a standard 12 x 32 mm autosampler vial. These new low adsorption CD, MRQ30 and QSertVial products now offer the benefit of maximum sample extraction without the worry of trace analytes being adsorbed by the vial surface.

MSQ Polypropylene Cap/PTFE White Silicone Septa

We recommend using LA vials with our new MSQ polypropylene cap/PTFE white silicone septa. This new cap and septa combination was purposely designed for use with mass spectrometry. It shows little to no background contamination when compared to other cap and septa products in the marketplace.



For more information on how our LA vials and MSQ cap/septa products can improve your analyses, please email our Technical Service chemists at EurTechServ@sial.com

+ Featured Products

Description	Cat. No.
CD (Center Draining) Vial Kits	
<i>Kits include 100 each of vial, cap, and septa</i>	
Clear glass vial, 1.5 mL, PTFE/silicone septa	29655-U
Clear glass vial, 1.5 mL, PTFE/silicone septa with slit	29656-U
MRQ30 Vial Kits	
<i>Kits include 100 each of vial, cap, and septa</i>	
Clear glass vial, 1.2 mL, PTFE/silicone septa	29658-U
Clear glass vial, 1.2 mL, PTFE/silicone septa with slit	29659-U
QSertVial (0.3 mL) Vial Kits	
<i>Kits include 100 each of vial, cap, and septa</i>	
Clear glass, natural PTFE/silicone septa	29661-U
Clear glass, natural PTFE/silicone septa with slit	29662-U
Amber glass vial, natural PTFE/silicone septa	29663-U
Amber glass vial, natural PTFE/silicone septa with slit	29664-U
Standard 2 mL (12 x 32 mm) Vial Kits	
<i>Kits include 100 each of vial, cap, and septa</i>	
Clear glass vial with marking spot, natural PTFE/silicone septa	29651-U
Clear glass vial with marking spot, natural PTFE/silicone septa with slit	29652-U
Amber glass vial with marking spot, natural PTFE/silicone septa	29653-U
Amber glass vial with marking spot, natural PTFE/silicone septa with slit	29654-U
Mass Spec Quality (MSQ) Caps with Septa	
Caps with septa, 9 mm, natural PTFE/silicone	29665-U
Caps with septa, 9 mm, natural PTFE/silicone with slit	29666-U

Supel™-Select HLB SPE

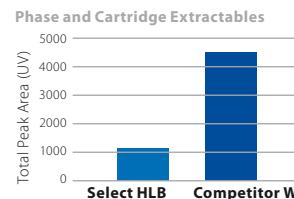
Sample Prep Performance at an attractive price



Supel-Select HLB SPE is a hydrophilic modified styrene-based polymer developed for solid phase extraction. (HLB: Hydrophilic Lipophilic Balance)

- Generic Methodology
- Extracts and Recovers a Broad Range of Analytes
- Low UV & MS Extractables

To learn more about Supel-Select HLB SPE, or to request a FREE product sample, please visit sigma-aldrich.com/supel-select



Sigma-Aldrich Offices

Austria

Tel: (+43) 1 605 81 10
Fax: (+43) 1 605 81 20

Belgium

Free Tel: 0800 14747
Free Fax: 0800 14745
Tel: (+32) 3 899 13 01
Fax: (+32) 3 899 13 11

Czech Republic

Tel: (+420) 246 003 200
Fax: (+420) 246 003 291

Denmark

Tel: (+45) 43 56 59 00
Fax: (+45) 43 56 59 05

Finland

Tel: (+358) 9 350 9250
Fax: (+358) 9 350 92555

France

Free Tel: 0800 211 408
Free Fax: 0800 031 052
Tel: (+33) 474 82 28 88
Fax: (+33) 474 95 68 08

Germany

Free Tel: 0800 51 55 000
Free Fax: 0800 64 90 000
Tel: (+49) 89 6513 0
Fax: (+49) 89 6513 1160

Hungary

Ingyenes telefonszám: 06 80 355 355
Ingyenes fax szám: 06 80 344 344
Tel: (+36) 1 235 9063
Fax: (+36) 1 269 6470

Ireland

Free Tel: 1800 200 888
Free Fax: 1800 600 222
Tel: (+353) 402 20370
Fax: (+353) 402 20375

Italy

Tel: (+39) 02 3341 7310
Fax: (+39) 02 3801 0737

The Netherlands

Free Tel: 0800 022 9088
Free Fax: 0800 022 9089
Tel: (+31) 78 620 5411
Fax: (+31) 78 620 5421

Norway

Tel: (+47) 23 17 60 00
Fax: (+47) 23 17 60 10

Poland

Tel: (+48) 61 829 01 00
Fax: (+48) 61 829 01 20

Portugal

Free Tel: 800 202 180
Free Fax: 800 202 178
Tel: (+351) 21 924 2555
Fax: (+351) 21 924 2610

Russia

Tel: (+7) 495 621 5828
Fax: (+7) 495 621 5923

Slovakia

Tel: (+421) 255 571 562
Fax: (+421) 255 571 564

South Africa

Free Tel: 0800 1100 75
Free Fax: 0800 1100 79
Tel: (+27) 11 979 1188
Fax: (+27) 11 979 1119

Spain

Free Tel: 900 101 376
Free Fax: 900 102 028
Tel: (+34) 91 661 99 77
Fax: (+34) 91 661 96 42

Sweden

Tel: (+46) 8 742 4200
Fax: (+46) 8 742 4243

Switzerland

Free Tel: 0800 80 00 80
Free Fax: 0800 80 00 81
Tel: (+41) 81 755 2828
Fax: (+41) 81 755 2815

United Kingdom

Free Tel: 0800 717 181
Free Fax: 0800 378 785
Tel: (+44) 1747 833 000
Fax: (+44) 1747 833 313

Internet

sigma-aldrich.com



Accelerating Customers'
Success through Innovation and
Leadership in Life Science,
High Technology and Service

Order/Customer Service (800) 325-3010 • Fax (800) 325-5052
Technical Service EurTechServ@sial.com • sigma-aldrich.com/techservice
Safety-related Information sigma-aldrich.com/safetycenter

World Headquarters
3050 Spruce St.
St. Louis, MO 63103
(314) 771-5765
sigma-aldrich.com



Faster HPLC on **ANY** System

Go to sigma-aldrich.com/express

1. Learn about the Fused-Core® Advantage
2. Find the phases you need (7 choices) →
3. Request application support



- C18
- RP-Amide
- C8
- Phenyl
- F5 (pentafluorophenyl) **NEW!**
- HILIC (Si)
- Peptide ES-C18

Ascentis® is a registered trademark of Sigma-Aldrich Biotechnology LP.
Fused-Core is a registered trademark of Advanced Materials Technology, Inc.

Date: 05/2011;
SAMS Code: NFX