

# Reporter

Volume 27.4

## Food & Beverage Analysis



*During the last decade, analysis of food and beverage products for both harmful and beneficial compounds has become a topic of major interest.*

- Liquid Chromatography
- Sample Handling
- Gas Chromatography
- Standards
- Accessories
- Chiral Chromatography



An Trinh

Product Manager, Sample Prep

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### Dear Colleague:

As LC-MS becomes increasingly commonplace, analysts are expected to develop assays that are faster, provide greater throughput, and achieve sensitivity levels that were near impossible just a decade prior.

I highlight below some of the recent trends observed in sample prep technology:

**Multi-Residue Methods.** Most solid phase extraction procedures target a specific analyte or class of analytes; however, many researchers, especially in the field of food safety/quality, must monitor for dozens of classes of analytes. In such applications, finding a single SPE condition that can adequately retain such a broad range of compounds would be impossible. To negotiate this issue, researchers have developed SPE techniques that target the retention and removal of specific interferences for a given sample matrix. These researchers have recognized that if the sample prep method can remove one to three key interferences, targeted LLODS can often be achieved. A classic example is the recently popular QuEChERS technique for multi-residue pesticide analysis.

**Simple and Generic Methods.** In order to maximize the throughput benefits of LC-MS technology, most researchers desire faster and easier sample prep. As a result, simple and generic techniques such as protein precipitation have become the preferred method when analyzing biological plasma. When improved selectivity is desired, such analysts look towards SPE phases that are more amenable to generic methodology such as hydrophilic polymer SPE phases and mixed-mode SPE technology.

**Highly Selective Techniques.** As mentioned previously, more and more assays are targeting lower limits of detection. As trace analysis becomes mainstream, highly selective sample prep techniques are becoming more prevalent. For example, molecularly imprinted polymers have seen a surge in publications in recent years; and many of the more recent and exciting developments in sample prep technology are application specific. In the last 5 years, Supelco has commercialized an array of application specific products including: HybridSPE™-Precipitation technology for protein and phospholipid removal, SupelMIP™ molecularly imprinted polymers, silver-ion SPE for FAMES analysis, Supelclean™ sulfoxide SPE for PCB fractionation... to name a few.

To learn more about these trends and our solutions for them, visit our web site at [sigma-aldrich.com/sampleprep](http://sigma-aldrich.com/sampleprep) or call our Technical Service Group at 800-359-3041 (US and Canada only) and 814-359-3041.

Best regards,

An Trinh

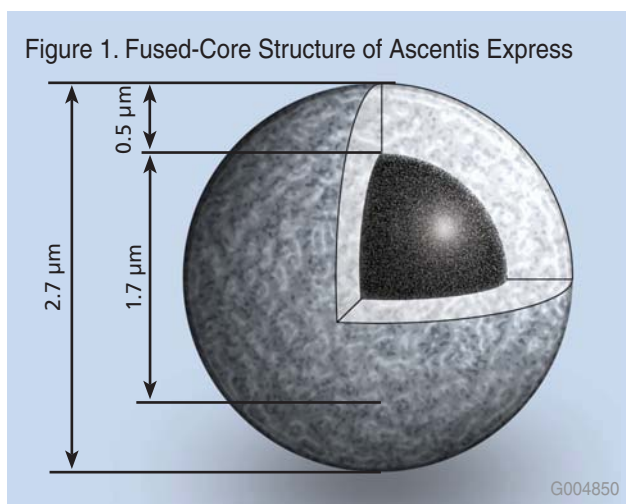
Product Manager, Sample Prep

# Ultra-High Performance Liquid Chromatography (UHPLC) with the new Ascentis Express Phenyl-Hexyl Column

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Peak resolution is a requirement for sensitive, accurate chromatographic analysis. Scientific meetings and publications have been dominated recently by reports on HPLC columns with Fused-Core™ particles and porous particles in the sub-2 μm range, which can both significantly enhance resolution and speed by producing either higher efficiency (N) for the same column length or equivalent efficiency with shorter column length.

Ultra-high performance liquid chromatography (UHPLC) comes at a price of much higher pressure when sub-2 μm particles are employed. The revolutionary Ascentis Express 2.7 μm Fused-Core silica particle (Figure 1) has quickly become accepted as an attractive alternative because it is equivalent in performance to particles in the sub-2 μm range. With a very narrow particle size distribution, Ascentis Express columns employ conventional 2 μm frits and operate ruggedly at much lower pressures that are within the comfort zone of conventional HPLC instruments.



## Why Do We Need Different HPLC Phases?

Even though new column technologies have more than doubled the plates per meter possible with traditional 5 μm columns, resolution still cannot be routinely achieved in every case without the ability to adjust retention and selectivity by proper selection of column stationary and mobile phases. This article features Ascentis Express Phenyl-Hexyl phase, a new addition to the Fused-Core

column family, and describes how column selectivity and higher efficiency can be coupled to achieve much faster separations than have previously been possible.

The vast majority of UHPLC separations have been carried out with C18 columns in the classic reversed-phase (RP) mode; however, suppliers now offer many different phases. Although no one would dispute the fact that UHPLC columns with different phases are needed, very little has been published yet on the performance that can be expected from UHPLC columns having different, complementary selectivity to C18 and C8. Two of the most popular polar-RP phases are RP-Amide, which is often categorized as an embedded polar-group phase, and Phenyl, which can interact with solutes by  $\pi$ - $\pi$  mechanisms. A brief retention and selectivity comparison for the Ascentis Express column family is given in Table 1.

Table 1. Brief Overview of Ascentis Express Column Retention and Selectivity

Ascentis Express Fused-Core Phase	Principle Retention Mode	Principle Solute Interaction
C18	Reversed-Phase (RP)	Hydrophobic (dispersive)
C8	Reversed-Phase (RP)	Hydrophobic (dispersive)
RP-Amide	RP with embedded polarity	Hydrophobic and H-bonding
Phenyl-Hexyl	RP with pendant aromaticity	Hydrophobic and $\pi$ - $\pi$
HILIC (Silica)	HILIC (or normal phase)	Hydrophilic (dipole, H-bonding, ion exchange)

C18 and C8 phases are highly popular because they are stable, reproducible, and easy-to-use. Retention correlates closely with log P values, which have been established for many solutes. Solute ionization causes retention to decrease in a predictable manner and is relatively easy to control by adding dilute acids, bases, and buffers to the mobile phase. Changing the organic component of the mobile phase between acetonitrile and methanol (or other solvents) allows the user to tweak resolution because solvation affects phase structure and selectivity. Temperature is also a useful variable for optimizing phase selectivity. Columns with C18 and C8 phases will frequently give optimum resolution when solutes are nonpolar or slightly polar; however, columns with polar-RP phases such as RP-Amide or Phenyl-Hexyl will often show improved

(continued on page 4)

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(continued from page 3)

retention and selectivity for more polar solutes. It should be emphasized that even polar-RP phases have a significant alkyl phase character in addition to their polar character. The same mobile phase solvents and techniques may be employed with polar-RP phases, with comparable phase stability to C18.

The RP-Amide phase is complementary to C18 because the amide group has several unique features: 1) strong interaction by H-bonding when solutes can donate or accept protons, 2) effective shielding of silanols by internal H-bonding between amide group and silica surface, and 3) the ability to wet and operate well, even in 100% aqueous solvents. H-bonding allows solutes with carboxyl and phenol groups to be retained much longer and separate much better on RP-Amide than on C18 or C8. Shielding prevents solutes with amino groups from interacting with silanols and can result in shorter retention and sharper peaks on amide phases. Another interesting feature of amide phases is that methanol and other alcohols become much stronger solvents when H-bonding between phase and solute occurs. Except for the special situations listed above, an RP-Amide phase often performs similar to C18 due to the long alkyl chain extending away from the surface.

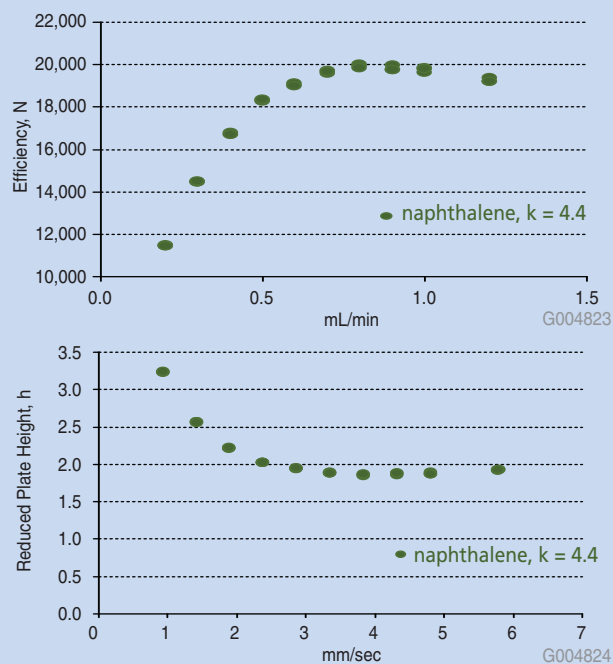
The Phenyl phase has unique selectivity arising from solute interaction with the aromatic ring and its delocalized electrons. It is complementary (orthogonal) to both C18 and RP-Amide phases because of this unique aromaticity. An unsubstituted phenyl ring is a  $\pi$ -donor or Lewis base, which interacts strongly with  $\pi$ -acceptors and any electron-deficient Lewis acid. Phenyl phases also tend to exhibit good shape selectivity, which may originate from solute multipoint interaction with the planar ring system. More retention and selectivity will often be observed for solutes with aromatic electron-withdrawing groups (fluorine, nitro, etc.) or with a delocalized heterocyclic ring system such as the benzodiazepine compounds studied.

#### UHPLC Results with Ascentis Express Phenyl-Hexyl

Low-pressure drop with high efficiency and a flat van Deemter curve have been confirmed for Phenyl-Hexyl, as shown in Figure 2. In general, more than twice the column efficiency of 5  $\mu$ m particles can be expected for all Ascentis Express Fused-Core columns at pressures that are easily managed with all HPLC instruments. Note that 20,000 plates have been achieved for a 10 cm x 3 mm I.D. column operating at optimum flow. A Jasco X-LC HPLC instrument was used for the study. As shown in Figure 3,

Figure 2. Flow Performance of Ascentis Express Phenyl-Hexyl Column with Neutral Probes

10 cm x 3 mm I.D. column, water:acetonitrile, 50:50, 35 °C, 250 nm, 2  $\mu$ L



the selectivity of Ascentis Express Phenyl-Hexyl is very similar to that of other commercial Phenyl columns, so methods can be readily transferred between columns. The difference in efficiency and pressure drop for the two porous 3  $\mu$ m columns can be explained by different particle size distributions.

Figures 4-6 show comparisons of five benzodiazepines separated on the four Ascentis Express RP phases in water:acetonitrile and water:methanol mobile phases. No additives were employed in order to observe the interaction between these polar solutes and the different phases; however, a dilute buffer will normally be used for development of a validated method. The addition of 10-20 mM buffer at neutral pH typically has little or no effect upon the separation with these highly deactivated column phases.

Note that overall retention in acetonitrile is similar for the four bonded phases, but elution order is different. The two less polar compounds, diazepam and desmethyldiazepam, elute late and show the same order for all columns due to predominance of hydrophobic interactions. The more polar solutes, however, elute earlier and interact differently with Phenyl-Hexyl and the other phases. With this test sample and operating conditions, three of the four Ascentis Express RP columns provide good resolution with different selectivity; however, Phenyl-Hexyl shows the best retention and selectivity.

Figure 3. Comparison of Phenyl Column Selectivity for Benzodiazepines

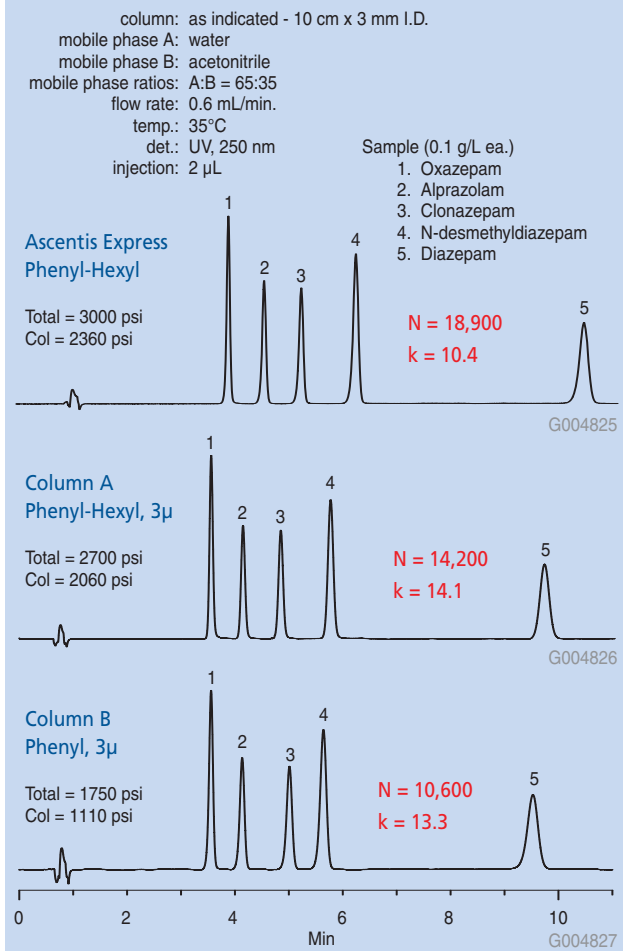


Figure 4. Benzodiazepine Structures

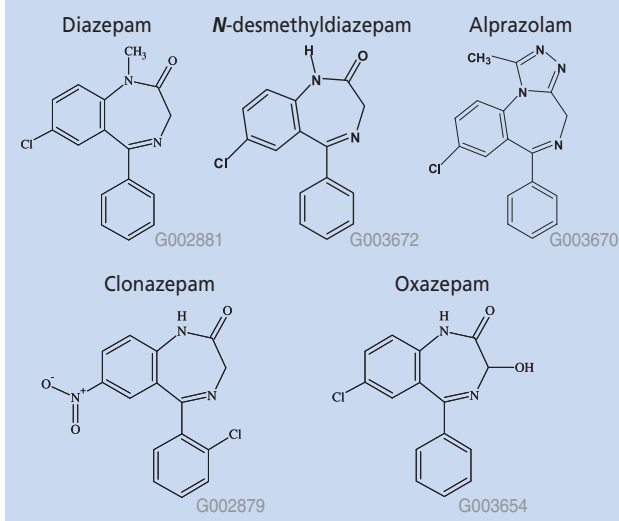
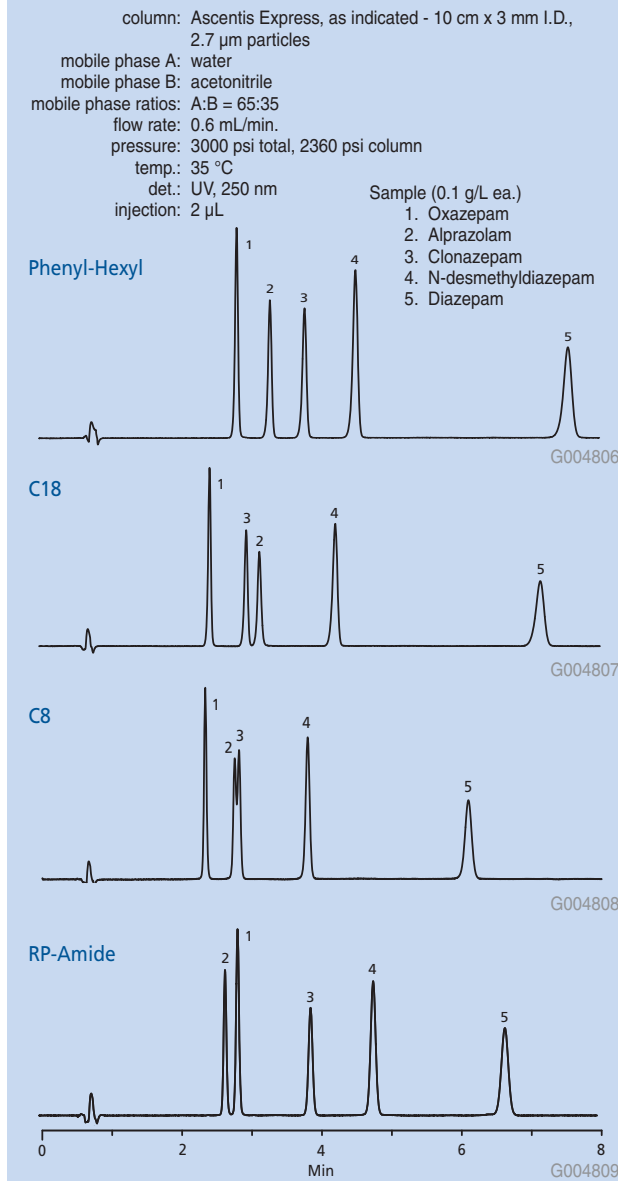


Figure 5. Benzodiazepines in 35% Acetonitrile Mobile Phase with no Additive



A switch to water:methanol in Figure 6 (page 6) shows a dramatic change in retention for Ascentis Express Phenyl-Hexyl. In water:methanol mobile phase, the phenyl group interacts much more strongly than the other phases with the solute heterocyclic ring system, presumably by a  $\pi$ - $\pi$  mechanism. Kazakevitch (1) has published evidence that methanol forms only monolayer coverage on aromatic phases (and also thinly solvates other phases), which allows the aromatic selectivity to shine through more strongly. Elution order for the polar compounds also changes from that of water:acetonitrile conditions. For this test sample, Ascentis Express Phenyl-Hexyl selectivity is clearly superior in water:methanol to the other phases.

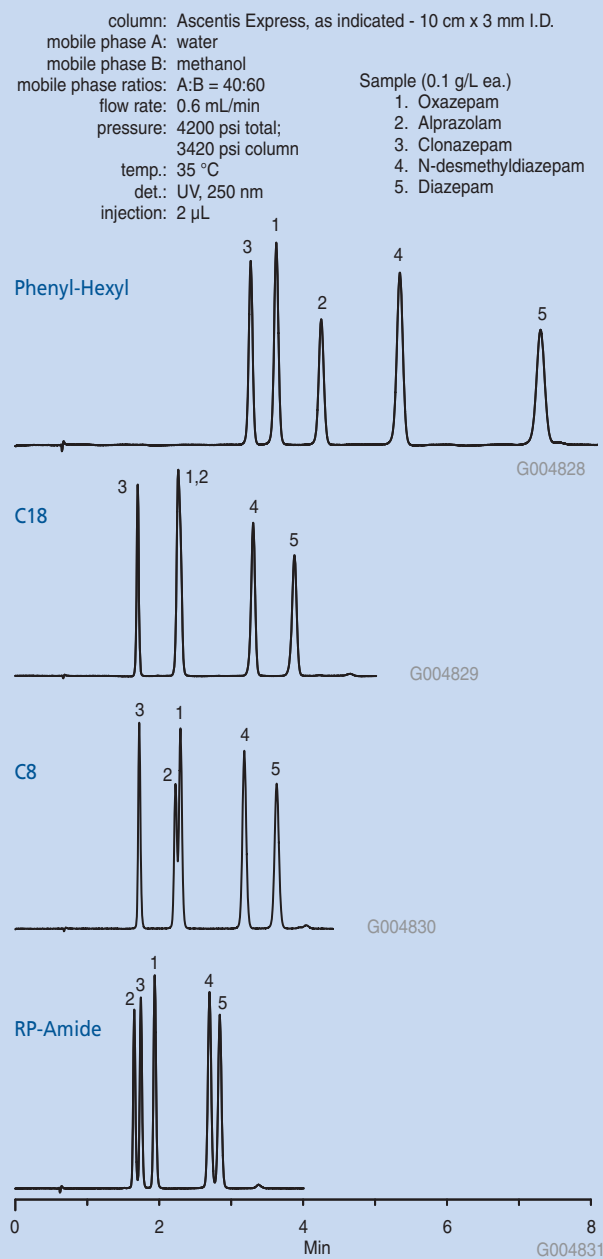
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Liquid Chromatography

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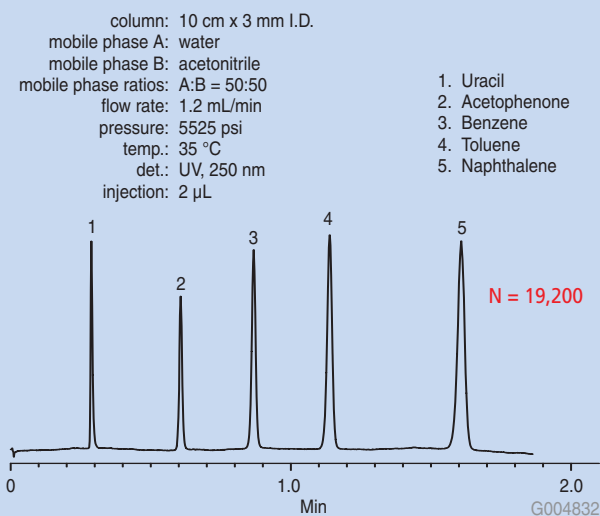
Figure 6. Benzodiazepines in 60% Methanol Mobile Phase with no Additive



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A major objective for development of UHPLC columns is to gain separation speed and sample throughput by using shorter columns at higher flow rates. These columns also improve sensitivity and uses less solvent. The flat van Deemter curve shown in Figure 2 allows an increase in flow rate and mobile phase velocity without a significant loss in resolution. This result is illustrated in Figure 7 for a neutral polar test mix in water:acetonitrile mobile phase.

Figure 7. Ascentis Express Phenyl-Hexyl High-Speed Separation



### Conclusions

A new Phenyl-Hexyl phase has been paired with Fused-Core particles to complete the primary Ascentis Express column family. High performance with lower pressure drop than other UHPLC columns has been confirmed for all Fused-Core particle phases. Ascentis Express Phenyl-Hexyl correlates well to other Phenyl phases for easy method development or method transfer. Selectivity for benzodiazepine compounds has been compared to the other Ascentis Express RP phases in water:acetonitrile and water:methanol. The extra retention possible with Phenyl phases in water:methanol has been demonstrated for these heterocyclic aromatic compounds. The potential for faster, more sensitive assays using Ascentis Express Phenyl-Hexyl and all Ascentis Express phases has been shown.

### References

1. Kazakevitch, Y. V., et al. *J. Chromatogr., A.* 2005, 1082, 158–165.
2. Consultant to Supelco/Sigma-Aldrich.

## Featured Products

### Ascentis Express Phenyl-Hexyl HPLC Columns

Length (cm)	I.D. (mm)	Cat. No.
3	2.1	53332-U
5	2.1	53334-U
7.5	2.1	53335-U
10	2.1	53336-U
15	2.1	53338-U
3	3.0	53341-U
5	3.0	53342-U
7.5	3.0	53343-U
10	3.0	53345-U
15	3.0	53346-U
3	4.6	53347-U
5	4.6	53348-U
7.5	4.6	53351-U
10	4.6	53352-U
15	4.6	53353-U

# NEW! Ultra-High Performance Liquid Chromatography Accessories

Ultra-high performance liquid chromatography (UHPLC) columns containing particles that are smaller than 3 micron have created very narrow peak widths that can no longer be routinely measured by all HPLC systems. The efficiency of separations performed with low-volume columns is highly dependent on the system having components designed to minimize band-width. With UHPLC systems it is good laboratory practice to install the proper fittings, ferrules, and other accessories to ensure the analytical results show no extra column effects created by improperly assembled accessories. Typical, more popular PEEK and stainless steel fittings, pre-column filters, guard columns, and tubing, must be reliable enough to hold at the pressures needed (200 bar) with today's UHPLC instrumentation.

Upgrading the UHPLC system can bring on many challenges due to the vast array of commercially available products. To assist in achieving the system and pressure requirements, Supelco has introduced a line of UHPLC accessories from the most trusted names in the industry into this rapidly growing area of chromatography making the product selection process easier. This newly expanded line of accessories for low volume and high sensitivity analytical applications helps to maximize the efficiency of your analysis and protect your column investment. These new products compliment the vast array of HPLC accessory products we currently offer.

For assistance with new product selection, please contact our Technical Service group at [techservice@sial.com](mailto:techservice@sial.com) or visit us on the web at [sigma-aldrich.com/hplc](http://sigma-aldrich.com/hplc)

## Featured Products

Description	Pkg. Size	Cat. No.
<b>Pre-Column Filters</b>		
2 µm Solvent Filter Assembly with frit (1.4 µL swept volume)	1	51231-U
0.5 µm Solvent Filter Assembly with frit (1.3 µL swept volume)	1	51232-U
0.5 µm Solvent Filter Assembly with frit ( 0.84 µL swept volume)	1	51233-U
<b>Mini Microfilter Assembly</b>		
1 µm, SS Frit	1	51242-U
1 µm, Titanium Frit	1	51243-U
<b>Ultra-High Performance Fittings for 1/32 in. Tubing</b>		
PEEK LiteTouch® Nut (Black) 10-32	10	51256-U
PEEK LiteTouch® Micro Ferrule (Black) 1/32 in.	10	51257-U
<b>Ultra-High Performance Fittings for 1/16 in. Tubing</b>		
PEEK LiteTouch® Ferrule Assy 1/16 in. (Black)	10	51258-U
PEEK Fingertight I Nut (Black) 10-32	10	51262-U
PEEK SealTight™ Short Fitting (Black) with Ferrule 10-32	10	51263-U
<b>Ultra-High Pressure Stainless Steel Fittings</b>		
for 1/16 in. Tubing, (with ferrule)	10	51264-U
for 1/32 in. Tubing, (with ferrule)	10	51265-U
<b>Ultra-High Pressure Stainless Steel Adapter</b>		
for adapting 1/16 in. OD to 1/32 in. OD Tubing	1	51267-U
<b>Ultra-High Performance MicroTight Unions</b>		
for 1/32 in. Tubing PEEK and SS (Fittings & Capsule Union Included)	1	51274-U
for 360 µm Tubing PEEK and SS	1	51277-U
for 1/32 in. Tubing PEEK and SS (Fittings Included)	1	51279-U
Adapter for 1/16 in. O.D. and 1/32 in. Tubing PEEK and SS	1	51281-U
<b>MicroTee</b>		
MicroTee PEEK for 1/32 in. for O.D. Tubing	1	51283-U
MicroTee PEEK for 360 µm O.D. Tubing	1	51285-U
<b>MicroTight® PEEK Tubing Sleeves</b>		
MicroTight Sleeve Natural 0.009 I.D. (230 µm)	10	51296-U
MicroTight Sleeve Blue 0.011 I.D. (280 µm)	10	51297-U
MicroTight Sleeve Natural 0.021 I.D. (535 µm)	10	51298-U
MicroTight Sleeve Purple 0.006 I.D. (152 µm)	10	51303-U
<b>PEEKsil™ Tubing</b>		
1/32 O.D. x 25 µm I.D. x 50 cm	2	51316-U
1/32 O.D. x 50 µm I.D. x 50 cm	2	51321-U
1/32 O.D. x 75 µm I.D. x 50 cm	2	51328-U
1/32 O.D. x 150 µm I.D. x 50 cm	2	51329-U
1/16 O.D. x 50 µm I.D. x 50 cm	2	51332-U
1/16 O.D. x 25 µm I.D. x 50 cm	2	51335-U
1/16 O.D. x 100 µm I.D. x 50 cm	2	51337-U
<b>OPTI-SOLV® Mini Filters</b>		
OPTI-SOLV Mini Filter, 0.5 µm	5	51168-U
OPTI-SOLV Mini Filter, 2 µm	5	51170-U
<b>OPTI-SOLV Micro Filters</b>		
OPTI-SOLV Micro Filter, 1.0 µm	5	51172-U
OPTI-SOLV Micro Filter, 2.0 µm	5	51173-U
OPTI-SOLV Biocompatible Micro Filter, 0.5 µm	5	51174-U
<b>OPTI-SOLV Nano Filter</b>		
OPTI-SOLV Biocompatible Nano Filter, 0.5 µm	5	51176-U
<b>OPTI-GUARD®</b>		
OPTI-GUARD 1 mm Guard Column, C18	5	51177-U
OPTI-GUARD 1 mm Guard Column, Silica	5	51178-U
OPTI-GUARD Biocompatible 1 mm Guard Column, C18	5	51183-U
OPTI-GUARD 1 mm Guard Column, C8	5	51184-U
OPTI-GUARD 1 mm Guard Column, Amino NH <sub>2</sub>	5	51187-U
OPTI-GUARD 3 mm PEEK/SS Holder	1	51188-U
OPTI-GUARD 3 mm Cartridge, C18	3	51191-U
OPTI-GUARD 3 mm Cartridge, C8	3	51193-U
OPTI-GUARD 3 mm Cartridge, Amino, NH <sub>2</sub>	3	51194-U
<b>OPTI-SOLV EXP® Pre-Column Filter Holder</b>		
Holder w / EXP Titanium Hybrid Ferrule, (2 Ferrules, 1 Nut in pack)▼	1	51163-U
▼Note: Cartridges not included with holder.		
<b>OPTI-SOLV EXP® Pre-Column Replacement Filter Cartridges</b>		
OPTI-SOLV EXP Pre-Column Filter Cartridge, 0.5 µm	5	51164-U
OPTI-SOLV EXP Pre-Column Filter Cartridge, 0.5 µm	10	51165-U
OPTI-SOLV EXP Pre-Column Filter Cartridge, 0.2 µm	5	51166-U
OPTI-SOLV EXP Pre-Column Filter Cartridge, 0.2 µm	10	51167-U

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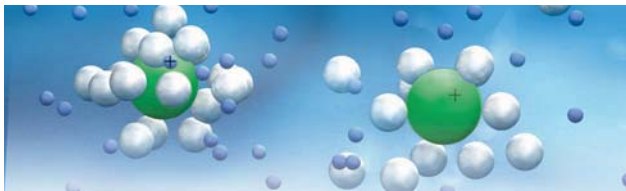
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# Reversed-Phase HPLC Buffers

## High quality buffers (solutions, solids or concentrates)

Shyam Verma

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Preparation of aqueous mobile phase is the most critical step in reversed-phase chromatography (RPC) method development for ionic analytes. This includes consideration of the effects of pH on analyte retention, type of buffer to use, and its concentration, solubility in the organic modifier and its effect on detection, among other considerations. The improper choice of buffer, in terms of buffering species, ionic strength and pH, can result in poor or irreproducible retention and tailing in reversed-phase separation of polar and ionizable compounds.

Problems like partial ionization of the analyte and strong interaction between analytes and residual silanols or other active sites on the stationary phases can be overcome by proper mobile phase buffering (maintaining the pH within a narrow range) and choosing the right ionic species and its concentration (ionic strength) in the mobile phase (1-2). In sensitive LC-MS separations that depend heavily on the correct choice of acid, base, buffering species and other additives (3), a buffer must be chosen based on its ability to maintain, and not suppress analyte ionization in the MS interface.

### Mobile Phase pH and Reversed-Phase Retention

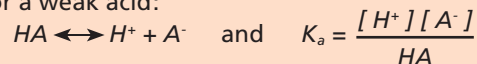
Retention of ionic analytes in RPC is fundamentally affected by mobile phase pH. The dissociation properties of the ionic functional groups also impact analyte retention. Retention of non-ionic analytes is minimally affected by mobile phase pH.

For acidic moieties (usually carboxylates), a pH below the  $pK_a$  (within limits) and for basic moieties (usually amines), a pH above the  $pK_a$  (within limits) of the compound enhances retention. Dramatic effects are observed on retention of these analytes in pH range near the  $pK_a$  of a given functional group. This becomes apparent in consideration of chemical dissociation as illustrated in Figure 1.

The last two equations are commonly known as the Henderson-Hasselbach equation. These equations suggest

Figure 1. Chemical Dissociation of an Ionic Analyte

For a weak acid:



By definition:

$$pH = -\log [H^+], \quad \text{or} \quad pH = pK_a + \log \frac{[A^-]}{[HA]}$$

The corresponding equation for a weak base is:

$$\text{or } pH = pK_a + \log \frac{[B]}{[BH^+]}$$

that for pH far removed from the  $pK_a$ , a small change in the pH has a minimal effect on the ratio of unprotonated-to-protonated species. Therefore, moderate change in pH will not affect retention significantly. However, at pH near the  $pK_a$ , a small change in pH will produce a significant change in the ratio of the two species. Therefore, changing the pH within a range of values sufficiently close to the  $pK_a$  will dramatically affect retention. When pH is used to increase reversed-phase retention, the pH should be changed in the direction that decreases analyte ionization.

### Buffer Selection

Buffers are solutions of a weak acid and its conjugate base, or a weak base and its conjugate acid. They mitigate the influence of hydrogen/hydronium and hydroxide ions and subsequently reduce the pH fluctuations, even upon dilution. The typical pH range for reversed-phase on a silica-based packing is pH 2 to 8. Choice of buffer is typically governed by the desired pH. It is important that the buffer has a  $pK_a$  close to the desired pH since buffers control pH best at their  $pK_a$ . A rule of thumb is to choose a buffer with a  $pK_a$  value <2 units of the desired mobile phase pH (see Table 1).

Phosphoric acid and its sodium or potassium salts are the most common buffer systems for reversed-phase HPLC. Phosphate's two  $pK_a$  values, 2.1 and 7.1, and UV transparency make it ideal for most HPLC separations. Its  $pK_a$  of 12.3 is suitable for buffering in 11.3-13.3 pH range. Phosphonate buffers can be replaced with sulfonate buffers when analyzing organophosphate compounds. With the growth in popularity of LC-MS, volatile buffer systems, such as TFA, acetate, formate, and ammonia, are frequently used.

**Buffer Concentration:** A higher buffer concentration that enhance buffer capacity will give more reproducible separation of compounds partially ionized at the pH of the

Table 1. HPLC Buffers, pK<sub>a</sub> Values and Useful pH Range

Buffer	pK <sub>a</sub> (25°C)	Useful pH Range
TFA	0.5	<1.5
Sulfonate	1.8	<1-2.8
Phosphate	2.1	1.1-3.1
Chloroacetate	2.9	1.9-3.9
Formate	3.8	2.8-4.8
Acetate	4.8	3.8-5.8
Sulfonate	6.9	5.9-7.9
Phosphate	7.2	6.2-8.2
Ammonia	9.2	8.2-10.2
Phosphate	12.3	11.3-13.3

mobile phase, by reducing local perturbations of the pH of the migrating analyte peak. Generally, a buffer concentration of 10-50 mM is adequate for small molecules.

**Buffer Solubility:** It is especially important when performing gradient separations. Solubility can be empirically determined by mixing given volume fractions of buffer and the organic solvent. Appearance of precipitates or opaque solution indicates solubility issues. A general rule is no more than 50% organic should be used with a buffer. This will depend on the specific buffer as well as its concentration.

**Effects on Detection:** The choice of buffer is also dependent upon means of detection. For traditional UV detection, the buffer needs to be effectively transparent in this region, especially critical for gradient separations. Buffers listed in Table 1 have low enough absorption below 220 nm.

More common issues today are related to compatibility with mass spectral (MS) detection. Preferred buffers addressing the issue of volatility are formate, acetate, and ammonia. In regard to the issue of suppression of ionization, formate and acetate are ideal choices for positive-ion

mode detection. TFA, however, can negatively impact detector response even in positive-ion mode (4,5), while it strongly suppresses ionization with negative ion mode. Acetic acid is good for negative-ion mode. LC-MS applications further limit buffer selection and buffer concentration.

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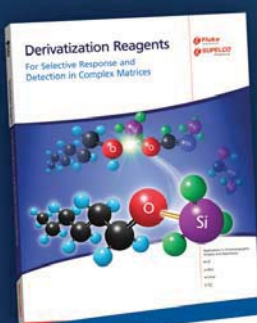
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### HPLC-Grade (puris p.a.) Buffers and Additives

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Ammonium formate, >99.0% (NT) (dried material)	50 g, 250 g	17843
Ammonium hydroxide solution, ~10% (T) in water	100 mL, 1 L	17837
Ammonium phosphate monobasic, >99.0% (T)	250 g	17842
Ammonium trifluoroacetate, >99.0% (NT)	10 g, 50 g	17839
Potassium phosphate dibasic anhydrous, >99.0% (T)	250 g	17835
Sodium formate, >99.0% (NT)	250 g	17841
Sodium phosphate dibasic dihydrate, ~99% (T)	50 g, 250 g	71633
Sodium phosphate monobasic anhydrous, >99.0% (T)	50 g, 250 g	17844
Sodium trifluoroacetate, >99.0% (T)	10 g	17840
Trifluoroacetic acid:Triethylamine 2M:1M	500 mL	9746
Trifluoroacetic acid:Triethylamine 2M:2M	100 mL	9747



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# Discovery BIO GFC 3 $\mu\text{m}$ Packings

Hillel Brandes and Hugh Cramer

*hillel.brandes@sial.com*

Gel filtration chromatography (GFC) (or size exclusion chromatography) is a common technique for resolution of biological macromolecules based on size and/or shape.

Analytes that are large relative to the pore size of the stationary phase are excluded from the pores and therefore elute relatively early. Conversely, analytes that are small relative to the pore size, are well-retained and therefore elute later. Shape also affects retention: Molecules with a more elongated structure are not as retained as much as those that are more spherical or globular.

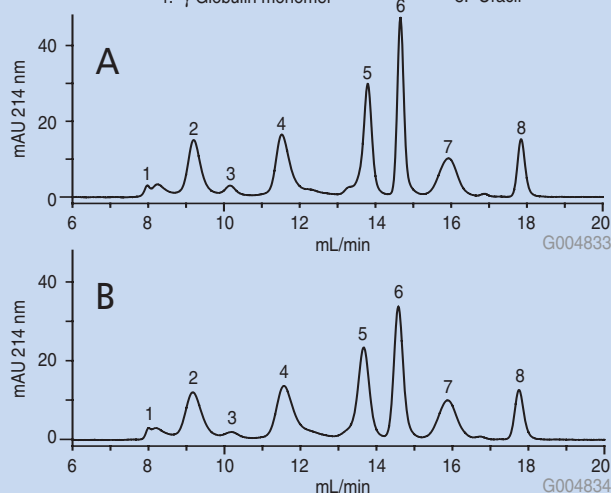
The Discovery BIO GFC product line for gel filtration of macromolecules is based on uniform, spherical, high-purity silica with a hydrophilic surface. To date, Discovery BIO GFC has been available as a 5  $\mu\text{m}$  product line; now, the 3  $\mu\text{m}$  particles have been added. While the 3  $\mu\text{m}$  will generate higher backpressures, the relatively low flow rates that are generally optimal for gel filtration chromatography only generate modest back pressures. However, the tremendous advantage of the 3  $\mu\text{m}$  columns is their higher efficiency. This will directly translate to higher resolution and peak capacity. Request Application Note 187 (T309187) for further discussion of peak capacity.

Figure 1 compares the elution profiles of a protein mixture on Discovery BIO GFC, 3  $\mu\text{m}$  versus 5  $\mu\text{m}$ , with the same y-axis scaling. The improvement in the 3  $\mu\text{m}$  packing is evident in the narrower and taller peaks. Also evident is improvements in fine detail of the chromatogram, which reflects the higher resolution. A comparison of the calculated peak efficiencies, however, is particularly striking. This is shown in Table 1 with a sampling of analytes. The greatest gains are evident with moderately retained analytes, with peak efficiencies improving on the order of 100%. Even for uracil, representative of a fully retained analyte, efficiency has improved 30%, which itself is significant.

Figure 1. Elution Profiles of 3  $\mu\text{m}$  Discovery BIO GFC 300 (A) and 5  $\mu\text{m}$  Discovery BIO GFC 300 (B)

column: Discovery BIO GFC 300, 30 cm x 7.8 mm I.D.,  
3  $\mu\text{m}$  particles (567337-U)  
mobile phase: 150 mM potassium phosphate monobasic, pH 7.0  
(adjusted with potassium hydroxide)  
flow rate: 0.7 mL/min.  
pressure: 1100 psi  
temp.: ambient  
det.: UV, 214 nm  
injection: 1  $\mu\text{L}$   
sample: 1 g/L ea. (except uracil, 0.1 g/L) in mobile phase

1. Thyroglobulin aggregate
2. Thyroglobulin
3.  $\gamma$ -Globulin dimer
4.  $\gamma$ -Globulin monomer
5. Ovalbumin
6. Myoglobin
7. Poly-DL-alanine
8. Uracil



## Featured Products

Description	Cat. No.
<b>Discovery BIO GFC 100: (100 – 100,000 mw range)</b>	
5 cm x 4.6 mm I.D., 3 $\mu\text{m}$ particles	567322-U
30 cm x 4.6 mm I.D., 3 $\mu\text{m}$ particles	567323-U
5 cm x 7.8 mm I.D., 3 $\mu\text{m}$ particles	567324-U
30 cm x 7.8 mm I.D., 3 $\mu\text{m}$ particles	567325-U
<b>Discovery BIO GFC 150: (500 – 150,000 mw range)</b>	
5 cm x 4.6 mm I.D., 3 $\mu\text{m}$ particles	567328-U
30 cm x 4.6 mm I.D., 3 $\mu\text{m}$ particles	567329-U
5 cm x 7.8 mm I.D., 3 $\mu\text{m}$ particles	567330-U
30 cm x 7.8 mm I.D., 3 $\mu\text{m}$ particles	567331-U
<b>Discovery BIO GFC 300: (5,000 – 1,250,000 mw range)</b>	
5 cm x 4.6 mm I.D., 3 $\mu\text{m}$ particles	567334-U
30 cm x 4.6 mm I.D., 3 $\mu\text{m}$ particles	567335-U
5 cm x 7.8 mm I.D., 3 $\mu\text{m}$ particles	567336-U
30 cm x 7.8 mm I.D., 3 $\mu\text{m}$ particles	567337-U

Table 1. Peak Efficiencies (N) of Discovery BIO GFC, 3  $\mu\text{m}$  versus 5  $\mu\text{m}$

Protein/Analyte	GFC 100			GFC 150			GFC 300		
	5 $\mu\text{m}$	3 $\mu\text{m}$	% Improvement	5 $\mu\text{m}$	3 $\mu\text{m}$	% Improvement	5 $\mu\text{m}$	3 $\mu\text{m}$	% Improvement
BSA monomer	--	--	--	5200	9200	77	10000	19300	93
Ovalbumin	--	--	--	5600	11500	105	9700	18300	89
Myoglobin	13500	24500	81	12600	28800	129	16600	32900	98
Ribonuclease A	14200	25100	77	12900	23400	81	16100	31500	96
Uracil	36400	47900	32	26600	37300	40	26300	36200	38

# Comparing the Enantioselectivity of Cyclodextrin-Based GC Chiral Stationary Phases

Len Sidisky, Kathy Stenerson, Greg Baney  
tracy.ascah@sial.com

Supelco currently offers the widest selection of cyclodextrin-based chiral stationary phases. The most versatile and popular commercial stationary phases are prepared using alpha-, beta- or gamma-cyclodextrins that have been functionalized with various derivative groups at the 2, 3 and 6 positions on the sugar molecules. Functional groups that have been used to derivatize the cyclodextrins include trifluoroacetyl, permethyl, dimethyl, diacetyl and others. Each of the functional groups imparts a unique enantioselectivity on the resulting chiral stationary phase.

We recently compared the selectivity of four popular chiral GC stationary phases using a series of test probes. The phases we studied include the Supelco  $\beta$ -DEX™ 110,  $\beta$ -DEX 120 (both permethylated beta-cyclodextrins imbibed in an intermediate polarity stationary phase), Astec CHIRALDEX™ B-DA (a dipentylated beta cyclodextrin) and Astec CHIRALDEX G-TA (a gamma-cyclodextrin trifluoroacetylated at the 3-hydroxy position and pentylated at the 2- and 6-hydroxy positions). The Supelco  $\beta$ -DEX 110 and 120 and the Astec CHIRALDEX B-DA phases are thought to interact with chiral compounds primarily using an inclusion mechanism to affect the separation of the chiral moieties. The Astec CHIRALDEX G-TA interacts primarily using a surface interaction with the chiral compounds.

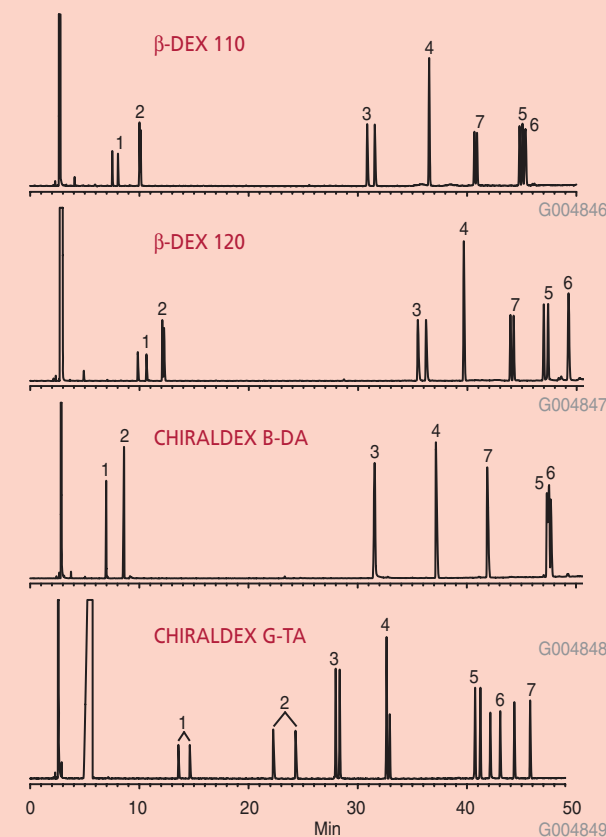
The temperature-programmed analysis of seven chiral probes shows the Supelco  $\beta$ -DEX 110 and 120 phases provide similar polarity and chiral selectivity, though there are differences in their selectivity (Figure 1). These phases provide chiral resolution primarily via inclusion complexation. The Astec CHIRALDEX B-DA column did not provide the chiral selectivity for the compounds evaluated compared to the permethylated and Astec CHIRALDEX G-TA phase. This phase also uses inclusion complexation as its primary separation mechanism. The Astec CHIRALDEX G-TA column had the best chiral selectivity for the compounds used in this study. The G-TA uses surface interactions as its primary mechanism to resolve chiral compounds, and has broad applicability for a variety of chiral compounds.

The Supelco DEX and Astec CHIRALDEX lines comprise many more phase chemistries than described here. The columns are also available in convenient and economical kits containing the most popular phase chemistries. A partial listing is shown. To view the complete list, please visit [sigma-aldrich.com/chiral-chromatography](http://sigma-aldrich.com/chiral-chromatography).

Figure 1. Selectivity of 4 Chiral GC Stationary Phases

columns: Supelco  $\beta$ -Dex 110, 30 m x 0.25 mm I.D., 0.25  $\mu$ m (24301)  
Supelco  $\beta$ -Dex 120, 30 m x 0.25 mm I.D., 0.25  $\mu$ m (24304)  
Astec CHIRALDEX B-DA, 30 m x 0.25 mm I.D., 0.12  $\mu$ m (72023AST)  
Astec CHIRALDEX G-TA, 30 m x 0.25 mm I.D., 0.12  $\mu$ m (73033AST)  
oven: 60 °C (0 min.), 2 °C/min. to 160 °C (hold 20 min.)  
inj.: 250 °C  
det.: FID, 250 °C  
carrier gas: helium, 25 to 30 cm/sec. set @ 75 °C  
injection: 1  $\mu$ L, 100:1 split

1. Methyl lactate
2.  $\beta$ -Butyrolactone
3. 1-Phenylethanol
4.  $\delta$ (+), l(-)-Carvone
5.  $\alpha$ -Ionone
6. S(+), R(-) Methyl mandelate
7. 1-Octanolactone



## + Featured Products

Description	Cat. No.
Supelco $\beta$ -DEX 110, 30 m x 0.25 mm I.D., 0.25 $\mu$ m	24301
Supelco $\beta$ -DEX 120, 30 m x 0.25 mm I.D., 0.25 $\mu$ m	24304
Astec CHIRALDEX B-DA, 30 m x 0.25 mm I.D., 0.12 $\mu$ m	72023AST
Astec CHIRALDEX G-TA, 30 m x 0.25 mm I.D., 0.12 $\mu$ m	73033AST
Supelco DEX Kit I Kit I contents: One 30 m x 0.25 mm I.D., 0.25 $\mu$ m column of each type: $\alpha$ -DEX 120, $\beta$ -DEX 120 and $\gamma$ -DEX 120	24340
Supelco DEX Kit II Kit II contents: One 30 m x 0.25 mm I.D., 0.25 $\mu$ m column of each type: $\beta$ -DEX 120, $\beta$ -DEX 225, $\gamma$ -DEX 225 and $\beta$ -DEX 325	24328-U
Astec CHIRALDEX Kit Kit contents: One 30 m x 0.25 mm I.D., 0.12 $\mu$ m column of each type: Astec CHIRALDEX G-TA, B-DM and B-DA	71030AST

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Chiral Chromatography

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# Determination of Triglycerides and Waxes in Food Products Using Cool On-Column Injection and the MET-Biodiesel Capillary Column

**Michael D. Buchanan**  
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## Introduction

Food nutrition (composition, fat content, labeling requirements, etc.) and food chemistry (ingredients, purity, flavors & fragrances, etc.) are two areas of study in a food & beverage laboratory. Examples of each are:

- Desired nutritional data includes the triglyceride content of various food products, providing information that complements that of the fatty acid composition
- Food chemistry analysis includes the characterization of wax content in olive oil, used to help ensure the product is unadulterated

Both of these analyses can be accomplished with the use of capillary gas chromatography (GC). The suitability of the MET-Biodiesel capillary column to perform both of these applications was investigated.

## MET-Biodiesel Column

The Supelco MET-Biodiesel capillary GC column was designed specifically for the determination of free and total glycerin in B100 biodiesel samples. Table 1 lists the column specifications. Several features and benefits of this column indicate that it is also well-suited for the analysis of triglycerides and waxes in food products. These features and benefits include:

- Metal column holds up better than fused silica, virtually eliminating column breakage
- Integrated guard protects the analytical column, extending column life with a leak-free connection
- Integrated guard also acts as a retention gap, minimizing peak broadening
- Provides good peak shape and resolution for glycerides
- Able to separate mono-, di-, and triglycerides (the mono- and diglycerides analyzed as TMS derivatives)
- Short column length, allowing for fast analysis times
- Operates at a maximum temperature of 380 °C (isothermal) and 430 °C (programmed)

Our goal was to obtain the acceptable resolution in as fast of a run time as possible. Several analyses, each using a different mix, were performed to illustrate the chromatographic results that can be obtained with this column. All separations were performed using cool on-column injection, which improves the recovery of high molecular weight substances.

**Table 1. MET-Biodiesel Specifications**

Application:	This rugged metal column was designed specifically for the determination of free and total glycerin in B100 biodiesel samples. A guard is integrated, thereby providing protection with a leak-free connection (the guard and analytical column are one continuous piece of tubing; there is no union between the guard and analytical column).
USP Code:	None
Phase:	Bonded; proprietary
Temperature Limits:	-60 °C to 380/430 °C

## Triglycerides

Triglycerides (also called triacylglycerol, triacylglyceride, or TAG) are the main constituent of vegetable oil and animal fat, and make up most of the fats digested by humans. They are important in that they allow the uptake and transport of fat-soluble vitamins. Plus, they play a role in metabolism (unused saturated or monounsaturated fatty acids are stored by the body as triglycerides). However, triglyceride intake should be monitored because high levels of triglycerides have been linked to an increased risk of heart disease and stroke.

Most natural fats contain a complex mixture of individual triglycerides. Therefore, efficient capillary columns with the ability to provide ample resolution are required for proper identification. Triglycerides are large compounds requiring a relatively high final oven temperature for elution in a reasonable time.

## Triglycerides in Butter and Lard

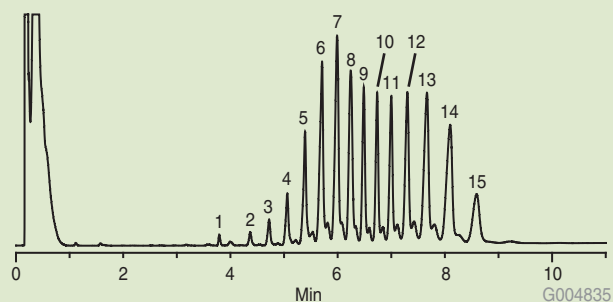
The extraction and analysis of triglycerides in butter and lard samples were performed, with the resulting chromatograms shown in Figure 1 and Figure 2, respectively. Peak IDs follow the nomenclature that T## (Triglyceride Number or Triacylglycerol Number) signifies the total number of carbons on the fatty acid chains, regardless of degree of saturation or position on the glycerol backbone. For example, T54 could be SSS, a triglyceride containing three stearic acids (C18:0). It could also be SOS or SSO, a triglyceride containing two stearic acids (C18:0) and one oleic acid (C18:1).

### Figure 1. Butter Triglycerides

Chromatogram courtesy of Dr. M. Povolò and Dr. G. Contarini (CRA-FLC, Lodi, Italy)

column: MET-Biodiesel, 14 m x 0.53 mm I.D., 0.16 µm with integrated 2 m x 0.53 mm I.D. guard (28668-U)  
 oven: 150 °C, 30 °C/min. to 350 °C (15 min.)  
 det.: FID, 400 °C  
 carrier gas: helium, 15 mL/min.  
 injection: 1 µL, cool on-column  
 sample: butter extract

- |        |        |        |         |         |
|--------|--------|--------|---------|---------|
| 1. T26 | 4. T32 | 7. T38 | 10. T44 | 13. T50 |
| 2. T28 | 5. T34 | 8. T40 | 11. T46 | 14. T52 |
| 3. T30 | 6. T36 | 9. T42 | 12. T48 | 15. T54 |

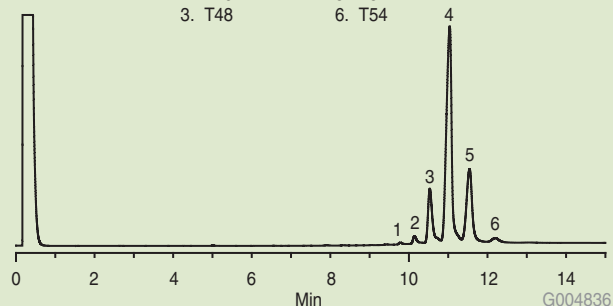


### Figure 2. Lard Triglycerides

Chromatogram courtesy of Dr. M. Povolò and Dr. G. Contarini (CRA-FLC, Lodi, Italy)

column: MET-Biodiesel, 14 m x 0.53 mm I.D., 0.16 µm with integrated 2 m x 0.53 mm I.D. guard (28668-U)  
 oven: 150 °C, 20 °C/min. to 350 °C (15 min.)  
 det.: FID, 400 °C  
 carrier gas: helium, 15 mL/min.  
 injection: 1 µL, cool on-column  
 sample: lard extract

- |        |        |
|--------|--------|
| 1. T44 | 4. T50 |
| 2. T46 | 5. T52 |
| 3. T48 | 6. T54 |



The MET-Biodiesel, in combination with cool on-column injection, fast carrier gas flow rates, rapid oven temperature ramp rates, and high final oven temperatures, was able to provide significant resolution for both applications with relatively short run times. As evident, the triglyceride profile of butter is much more complex than that of lard. The profile differences can be attributed to the fact that these products are from different sources; butter is processed from cow milk or cream whereas lard is rendered from pig fatty tissues.

### Glycerides in Palm Oil

The mono-, di-, and triglyceride composition of vegetable oils can be used as a measurement of quality and purity. If the oil is adulterated with inferior oil, the peak patterns and/or ratios will not match up to historical data, potentially falling outside of acceptable control limits.

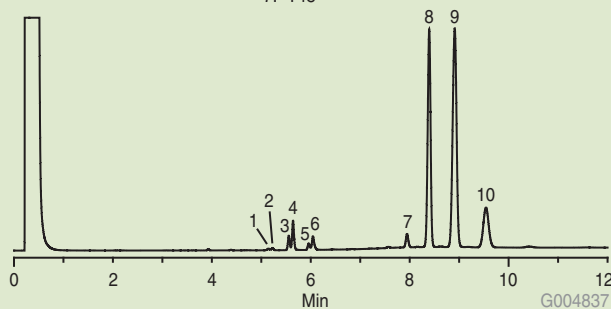
Because mono- and diglycerides have active hydroxyl functional groups, they must be derivatized prior to analysis to minimize analyte adsorption, thereby reducing peak tailing. An extract of palm oil was analyzed on the MET-Biodiesel after the mono- and diglycerides were converted to TMS-derivatives. As shown in Figure 3, the di- and triglycerides were separated with no overlap between groups. Additionally, peak shape of the triglycerides is an indication of good column inertness.

### Figure 3. Palm Oil Glycerides

Chromatogram courtesy of Dr. C. Mariani (SSOG, Milan, Italy)

column: MET-Biodiesel, 14 m x 0.53 mm I.D., 0.16 µm with integrated 2 m x 0.53 mm I.D. guard (28668-U)  
 oven: 200 °C, 20 °C/min. to 350 °C (15 min.)  
 det.: FID, 380 °C  
 carrier gas: helium, 10 mL/min.  
 injection: 1 µL, cool on-column  
 sample: palm oil extract (subsequent derivatization with TMS)

- |              |              |         |
|--------------|--------------|---------|
| 1. DAG32 1:2 | 4. DAG34 1:3 | 8. T50  |
| 2. DAG32 1:3 | 5. DAG36 1:2 | 9. T52  |
| 3. DAG34 1:2 | 6. DAG36 1:3 | 10. T54 |
|              | 7. T48       |         |



Triglyceride peak ID nomenclature is the same as discussed earlier. Diglyceride peak IDs follow the nomenclature that DAG## (Diacylglycerol Number) signifies the total number of carbons on the fatty acid chains, regardless of degree of saturation. The 1:2 and 1:3 then designate the position of the fatty acid substitution on the glycerol. For example, DAG36 1:2 has fatty acids at both the first and second positions on the glycerol backbone whereas DAG36 1:3 has fatty acids at both the first and third positions.

### Olive Oil

Olive oil is rich in monounsaturated fats, most notably oleic acid. This is of interest because a diet with a higher proportion of monounsaturated fats is linked to a reduction in the risk of coronary heart disease, due to

(continued on page 14)

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(continued from page 13)

favorable effects on cholesterol regulation and LDL cholesterol oxidation, as well as helping to build a more healthy balance between omega 3 and omega 6 fats. Therefore, it is desirable to substitute olive oil for other food products wherever possible.

Olive oil is used throughout the world, especially in the countries surrounding the Mediterranean Sea. The quality of the olive oil, influenced by the source of the olives and also how it is processed, determines what it can best be used for. Possible uses for various grades of olive oils are shown in Table 2.

Grade	Use
Extra-virgin olive oil	Salads, soups and stews, dipping bread
Virgin olive oil	Sautéing ingredients
Olive oil	Cosmetics, pharmaceuticals, soaps
Olive-pomace oil	Certain kinds of cooking in restaurants
Lampante oil	Fuel in traditional oil-burning lamps (non-edible)

Olive oil is regulated in much of the world to discourage the labeling of lower grade oils as higher grade oils. As such, detailed analytical work is performed to fully characterize the oil. This may include the determination of free fatty acids, peroxide index, wax content, sterol content, erythrodiol and uvaol, saturated fatty acids in position 2 of the triglyceride, spectrophotometric analysis, fatty acid composition, volatile halogenated solvents, organoleptic characteristics, stigmastadienes, triglyceride content, and aliphatic alcohol content.

### Olive Oil Waxes

One of the regulated characteristics of olive oil is its wax content, an indicator of both quality and purity. Because wax content varies among the various categories of edible oils, comparing peak patterns and ratios to known references and specifications can determine if the olive oil has been adulterated with inferior oil.

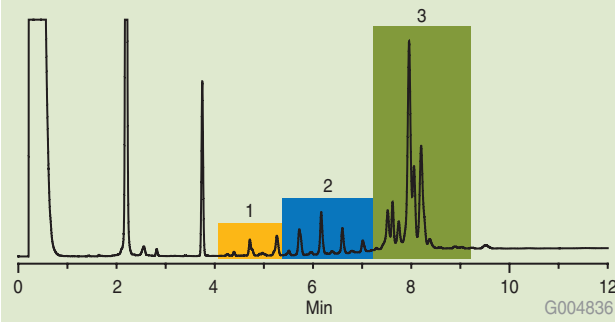
Because some of these wax compounds may have high boiling points, GC analysis using a column with a high temperature limit is desirable. This allows for elution of all analytes in a reasonable amount of time with good peak shape. Figure 4 shows the chromatogram obtained from the analysis of olive oil on the MET-Biodiesel column. The use of a 350 °C final oven temperature helps to produce sharp peaks, allowing for easy and accurate peak pattern and ratio comparisons to known references.

### Figure 4. Olive Oil Waxes

Chromatogram courtesy of Dr. C. Mariani (SSOG, Milan, Italy)

column: MET-Biodiesel, 14 m x 0.53 mm I.D., 0.16 µm with integrated 2 m x 0.53 mm I.D. guard (28668-U)  
 oven: 200 °C, 20 °C/min. to 350 °C (15 min.)  
 det.: FID, 380 °C  
 carrier gas: helium, 10 mL/min.  
 injection: 1 µL, cool on-column  
 sample: olive oil waxes

1. Diterpenes
2. C40-C46 esters
3. Sterol ester triterpene alcohols



### Conclusion

The MET-Biodiesel capillary column is a suitable choice for analysts who work on triglyceride and/or wax separations in which high column temperatures are required. The column's inertness, high thermal stability, and low bleed ensure good peak shape and resolution for the analytes that were evaluated. As shown, this column can be used to achieve optimal result for these applications.

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### Featured Products

Description	Cat. No.
MET-Biodiesel, 14 m x 0.53 mm I.D., 0.16 µm with integrated 2 m x 0.53 mm I.D. guard	28668-U

### Related Products

Description	Cat. No.
Ceramic scribe for cutting capillary columns, pack of 10	Z290254-1PAK
Needle-nose pliers, 7½ inch length	22437

# Extraction of Nitroimidazoles from Milk and Eggs using Molecularly Imprinted Polymers



## Contributed Article

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

**Olga Shimelis<sup>1</sup>, Anna-Karin Wihlborg<sup>2</sup>, Marcus Rudolfsson<sup>2</sup>, Brian Boyd<sup>2</sup>, and An Trinh<sup>1</sup>**

1. Supelco, Bellefonte, PA, USA  
2. MIP Technologies AB, Lund, Sweden

## Introduction

Nitroimidazoles are anti-bacterial and anti-coccidial drugs used in the treatment of cattle, poultry, and pigs. These compounds and their metabolites are suspected carcinogens and mutagens. Consequently, their use in veterinary practice is strictly regulated within European Union Countries (Council Regulation 2377/90, Annex IV Banned Compounds).

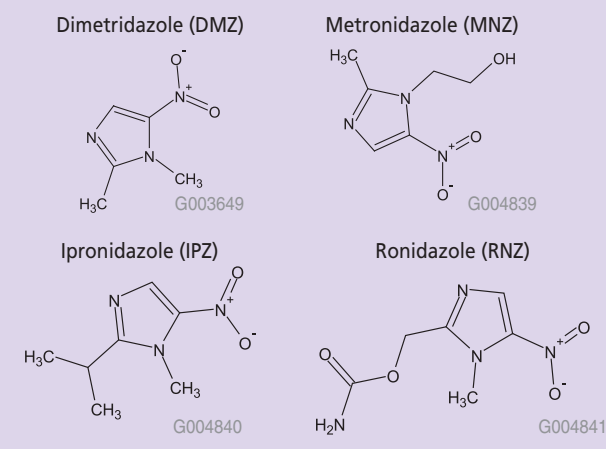
Metronidazole (MNZ), ipronidazole (IPZ) and ronidazole (RNZ) belong to a list of pharmacologically active substances for which no maximum residue limit (MRL) can be fixed. As a result, their use is prohibited in food-producing animals, and any residue of these compounds detected in animals (and their products) intended for human consumption is considered a violation of EU regulation. The on-going surveillance of nitroimidazole drug residues and their metabolites in animal products requires a highly selective and sensitive assay for trace detection.

In this report, a new molecularly imprinted polymer SPE phase (SupelMIP™) was evaluated for the extraction of nitroimidazoles: dimetridazole (DMZ), metronidazole (MNZ), ipronidazole (IPZ), ronidazole (RNZ), and their hydroxylated metabolites (DMZOH and MNZOH) (Figure 1). The nitroimidazole compounds were extracted from milk and egg samples and analyzed via LC-MS.

## What are Molecularly Imprinted Polymers?

Molecularly imprinted polymers (MIPs) are a class of highly cross-linked polymer-based molecular recognition elements engineered to bind one specific target compound or a class of structurally related compounds with high selectivity. The MIP material is designed with cavities that are sterically and chemically complementary to the target analyte(s). As a result, multiple interactions (e.g., hydrogen bonding, ionic, Van der Waals, hydrophobic) can take place between the MIP captivity and the analyte.

Figure 1. Structure of Nitroimidazoles



## SupelMIP SPE – Nitroimidazoles Extraction Procedure

One egg was homogenized. 10 g of the mix was spiked at levels of 1, 2, and 5 µg/kg nitroimidazoles: DMZ, IPZ, MNZ, RNZ, and metabolites DMZOH and MNZOH. Deuterated internal standards of each compound were added at the spike level of 2 µg/kg. For MNZ and MNZOH, DMZOH-d<sub>3</sub> was used as the internal standard.

10 g of the spiked samples were extracted with 10 mL acetonitrile followed by centrifugation for 5 minutes. 2 g sodium chloride was mixed with the isolated supernatant followed by centrifugation. The extract was evaporated to dryness in a silanized glass test tube. The residue was reconstituted in 2 mL DI water and sonicated for 3 minutes prior to SPE processing.

Similar sample pre-treatment was followed for milk samples; however, the acetonitrile and sodium chloride extraction process was conducted in one step.

After sample pre-treatment, the resulting samples were processed using the SupelMIP SPE procedure described in Figure 2 (page 16).

## LC-MS Analysis

After SupelMIP SPE, the samples were analyzed via LC-MS using the conditions described in Table 1 (page 16). Relative recovery was determined against internal standards for each of the spike levels tested. The data was also used to determine reproducibility and to estimate LLOD. Matrix (ionization) effects were assessed by spiking nitroimidazoles into post-SPE blank samples. The results were quantified against an external calibration curve diluted in buffer.

(continued on page 16)

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Sample Handling

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Analytical

## Figure 2. SupelMIP SPE - Nitroimidazoles Procedure

SPE Column: SupelMIP SPE – Nitroimidazoles, 50 mg/3 mL

↓	<b>Condition column with:</b> 1 mL toluene 1 mL acetonitrile 1 mL 10 mM ammonium acetate, pH 6.5
↓	<b>Load sample:</b> 2 mL sample
↓	<b>Wash:</b> <ul style="list-style-type: none"> <li>0.5 - 1 mL ultra-pure water  <i>Followed by a strong vacuum through cartridge for 5 min. to remove residual moisture and ensure a dry cartridge (-0.7 bar, -20 in Hg, or -70 kPa)</i></li> <li>2 x 1 mL hexane</li> <li>1 mL heptane:toluene 3:1 (v/v)  <i>Gentle vacuum (-0.4 bar or -12 in Hg for 5-10 s) between each wash volume</i></li> </ul>
↓	<b>Analyte elution:</b> Elute with 2 x 1 mL acetonitrile:water (60:40, v/v) with 0.5 % acetic acid <i>Gentle vacuum (-0.4 bar or -12 in Hg for 5-10 s) between each elution volume.</i> Eluted fraction evaporated to 50 µL at 45 °C under N <sub>2</sub> and reconstitute in 500 µL LC-MS mobile phase, filter prior to analysis if necessary.

## Table 1. LC-MS-MS Methodology

Recommended Analytical Technique: LC-MS-MS or LC-MS

column:	Ascentis C18, 15 cm x 2.1 mm I.D., 3 µm particle size (581302-U)		
instrument:	Sciex API 3200		
mobile phase A:	0.1% formic acid in LC-MS grade water		
mobile phase B:	0.1% formic acid in acetonitrile		
gradient:	Min.	A%	B%
	0.0	95	5
	1.0	95	5
	8.0	0	10.0
	12.0	0	10.0
	13.0	95	5
	18.0	95	5
flow rate:	0.3 mL/min.		
temp.:	ambient		
det.:	MS/MS, MRM transitions		
	DMZ (142/96)	IPZOH (186/168)	
	DMZ-d <sub>3</sub> (145/99)	IPZOH-d <sub>3</sub> (189/171)	
	DMZOH (158/140)	MNZ (172/128)	
	DMZOH-d <sub>3</sub> (161/143)	MNZOH (188/126)	
	IPZ (170/124)	RNZ (201/140)	
	IPZ-d <sub>3</sub> (189/171)	RNZ-d <sub>3</sub> (204/143)	
polarity:	Positive		
ion source:	Turbospray		
ion spray voltage:	1200 V		
source temp:	350 °C		
collision gas:	4 psi		
curtain gas:	50 psi		
inj.:	30 µL		

(continued from page 15)

## High Recoveries, Reproducibility, and Clean Extracts

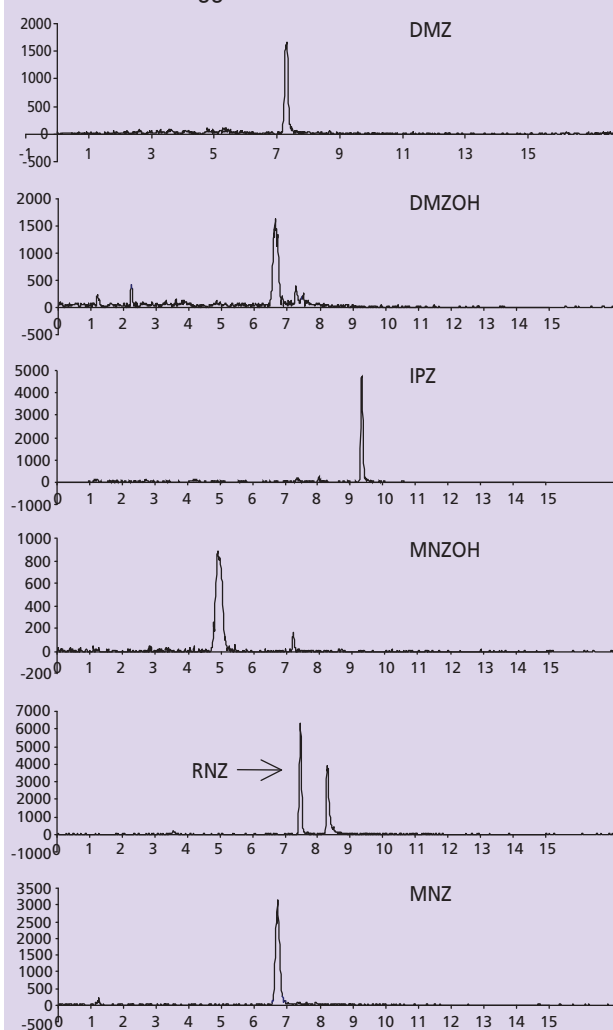
The relative recovery and RSDs for nitroimidazoles averaged at 95 ± 7%, and detailed results are described in Table 2. Representative ion-chromatograms for the spiked egg samples (1 ng/g) are presented in Figure 3 and illustrate low background.

Table 2. % Relative Recoveries (RSDs) of Nitroimidazoles from Spiked Egg and Milk Samples (n=5)

Sample	DMZ <sup>1</sup>	DMZOH <sup>1</sup>	IPZ <sup>1</sup>	MNZ <sup>2</sup>	MNZOH <sup>2</sup>	RNZ <sup>1</sup>
Egg 1 µg/kg	111(7)	106(5)	105(6)	109(3)	83(6)	105(7)
Egg 2 µg/kg	84(5)	90(11)	94(5)	91(9)	58(8)	113(20)
Egg 5 µg/kg	88(5)	92(8)	90(1)	92(8)	57(13)	134(3)
Milk 1 µg/kg	100(8)	107(6)	108(6)	105(2)	76(4)	104(3)
Milk 2 µg/kg	88(7)	94(3)	97(10)	92(14)	59(7)	111(10)
Milk 5 µg/kg	96(10)	101(10)	95(5)	107(9)	62(8)	123(4)

<sup>1</sup> Deuterated standard is available, <sup>2</sup> Used DMZOH-d<sub>3</sub> as an internal standard

Figure 3. Ion-Chromatograms of 1 ng/g Nitroimidazoles Extracted from Eggs



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Samples spiked into post-SPE blank extracts revealed good sample cleanup and low ion-suppression. The matrix ionization effects were minimal as evident from the 90-120% recovery values depicted in Table 3.

**Table 3. Determination of Matrix Effects – Absolute Recovery of Post-SPE Spiked Samples**

Sample	DMZ	DMZOH	IPZ	MNZ	MNZOH	RNZ
Egg	91	92	103	96	99	89
Milk	118	110	123	116	98	109

The estimated detection levels of nitroimidazoles ranged from 0.010-0.16 µg/kg in milk and 0.016-0.12 µg/kg in egg (Table 4). The LODs were estimated by analyzing the analyte and background response levels of the lowest spike concentration; and extrapolating a theoretic signal-to-noise ratio of 3:1.

**Table 4. Estimated LOD of Nitroimidazoles using SupelMIP SPE**

LOD (ng/g)	RNZ	MNZ	DNZ	DMZOH	MNZOH	IPZ
Egg	0.016	0.020	0.066	0.124	0.061	0.026
Milk	0.010	0.023	0.063	0.162	0.076	0.015

## Conclusion

In this report, SupelMIP SPE – Nitroimidazoles was evaluated for the extraction of nitroimidazoles and metabolites from milk and egg. Using molecularly imprinted polymer technology, high and reproducible recoveries were observed. Extraction was conducted with a high degree of selectivity minimizing ion-suppression, allowing researchers to achieve LODs at the ppt to low ppb levels. The SupelMIP method described in this report is highly robust and reproducible offering the necessary selectivity to achieve the stringent detection limit demands in food safety surveillance.

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Description	Oty.	Cat. No.
SupelMIP SPE - Nitroimidazoles, 50 mg/3 mL	50	52734-U
Ascentis C18 Column, 15 cm x 2.1 mm I.D., 3 µm particle size	1	581302-U

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# The Analysis of Resveratrol in Red Wine by On-Fiber Derivatization/SPME

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## Introduction

Resveratrol is a phytoalexin produced by grapes and other plants to increase resistance to fungal infection. Recent research suggests that consumption of resveratrol may reduce the risk of certain cancers, heart disease, and other age-related disorders (1). Red wine, which is produced by fermentation of juice on the crushed grapes, has been found to contain a greater amount of resveratrol than white wine, which is produced by fermentation of the juice alone.

In this study, the extraction and analysis of resveratrol from red wine is demonstrated using solid phase microextraction (SPME) and GC-MS. The presence of 3-hydroxyl (OH) groups make it necessary to derivatize this compound prior to GC analysis. Derivatization was conducted, after extraction, directly on the SPME fiber by exposing it to the vapors of a silylating reagent. The derivative of resveratrol was then analyzed directly from the fiber using GC-MS.

## Experimental

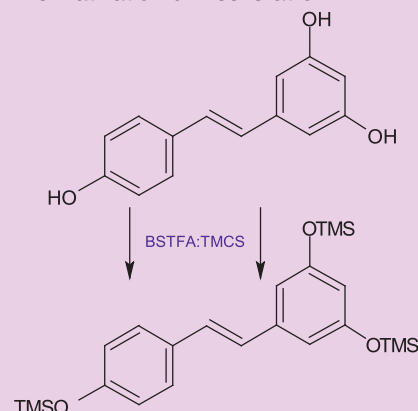
The extraction and derivatization conditions used are summarized in Table 1. The polyacrylate fiber, which is suitable for the extraction of polar semivolatiles and is more resistant to swelling than other types of fibers, was chosen based on recently published findings (2,3). The extraction conditions were based on these works as well, with some modifications made to extraction and desorption times to decrease matrix interference from the wine sample.

Table 1. SPME – On-Fiber Derivatization Conditions

sample/matrix:	3 mL of red wine (California merlot) diluted 3:1 in 12% ethanol:water
SPME fiber:	85 $\mu$ m polyacrylate
extraction:	immersion at room temperature, 15 min., with stirring at 400 rpm
derivatization:	20 min. in 4 mL vial containing 5 $\mu$ L of Sylon-BFT
desorption:	280 °C, 2 minutes

After sample extraction, the SPME fiber was gently blotted with a Kimwipe® to remove excess water. The fiber was then inserted into a 4 mL vial containing 5  $\mu$ L of Sylon™-BFT (BSTFA + 1% TMCS) reagent. For consistency, the vial containing the Sylon-BFT was allowed to equilibrate for 60 – 90 minutes prior to use, and a new reagent vial was used for each extraction. The fiber was desorbed at 280 °C for 2

Figure 1. Derivatization of Resveratrol



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minutes directly in the injection port of the GC-MS. The derivatization procedure resulted in silylation of all three –OH groups present in resveratrol (Figure 1). The resulting derivative has a molecular weight of 444, and subsequent GC-MS analysis showed a predominance of the molecular ion, which was used for quantification.

Calibration standards ranging from 10 – 300  $\mu$ g/L were prepared by spiking a solution of 12% ethanol in water with trans resveratrol. Red wine (California merlot), diluted 3:1 in 12% ethanol in water, was analyzed both “as is” and spiked with a known level of trans-resveratrol.

## Results/Discussion

A plot of the calibration standards as absolute response vs. concentration is presented in Figure 2. Linearity was very good, with a correlation coefficient of 0.9987, indicating that the extraction and derivatization procedure is quantitative. Peak responses obtained using the technique were adequate to allow for detection of the lowest calibration standard while using the MSD in full scan mode.

Chromatograms of the unspiked and spiked red wine samples are presented in Figures 3 and 4. Using the average response factor from the calibration, the levels of trans-resveratrol in each were calculated, along with a percent accuracy of this experimental value relative to the known spiking level of the wine sample (Table 2).

Table 2. Wine Spike Levels

	Unspiked Red Wine	Spiked Red Wine (100 $\mu$ g/L)
Conc. of trans-resveratrol ( $\mu$ g/L)	22.6	134.7
% Accuracy	---	110%

Figure 2. SPME Calibration Standards; 10-300 µg/L  
SPME - on-fiber derivatization analysis of trans-resveratrol

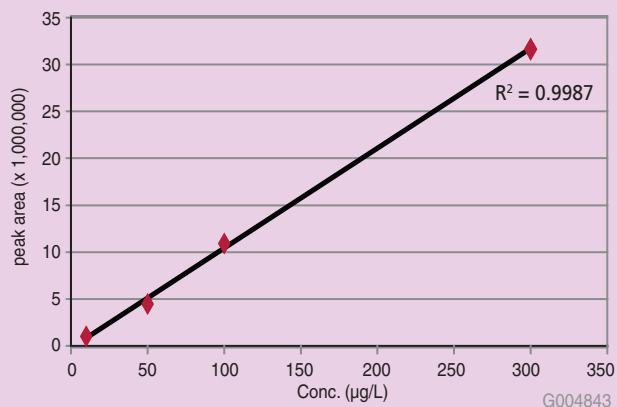


Figure 3. SPME On-Fiber Derivatization Analysis of Resveratrol in Unspiked Red Wine Sample

column: SLB-5ms, 30 m x 0.25 mm I.D., 0.25 µm (28471-U)  
oven: 100 °C (1 min.), 10 °C/min. to 325 °C (3 min.)  
MSD interface: 325 °C  
scan range: m/z 40-450  
carrier gas: helium, 1 mL/min, constant  
liner: 0.75 mm I.D. SPME

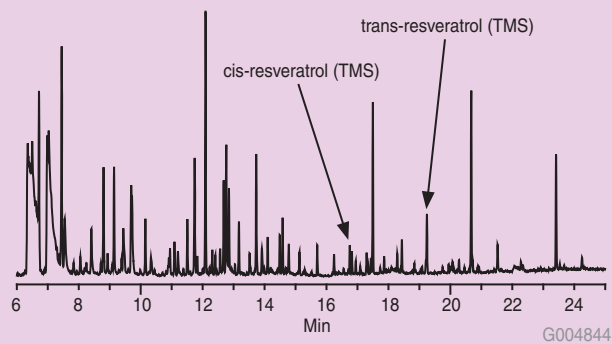
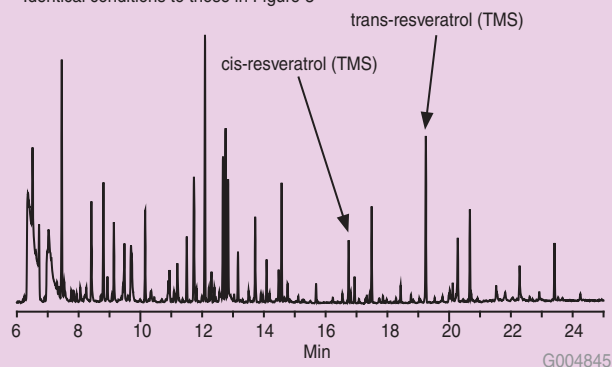


Figure 4. SPME On-Fiber Derivatization Analysis of Resveratrol in Spiked Red Wine Sample

Identical conditions to those in Figure 3



In addition to the trans form, a sizable cis-resveratrol peak was detected in the wine samples. The cis form is not naturally found in grapes, however it has been theorized that it can be formed from the trans during analysis, or the production and/or aging of wine (2).

## Conclusions

SPME when used in combination with on-fiber derivatization was found to be applicable to the extraction of resveratrol from red wine. The technique was found to be highly sensitive, simple, and quantitative. The polyacrylate fiber was also found to withstand exposure to the vapors of the silylating reagent without damage resulting from swelling.

## References

1. Red Wine Compound Resveratrol Demonstrates Significant Health Benefits. ScienceDaily. Retrieved June 18, 2009, from <http://www.sciencedaily.com/releases/2009/06/090611174052.htm>
2. Cai, Lingshuang, Koziel, Jacek A., Dharmadhikari, Murli, van Leeuwen, J. (Hans), Rapid Determination of trans-resveratrol in red wine by solid-phase microextraction with on-fiber derivatization and multidimensional gas chromatography-mass spectrometry. *J. Chrom. A* (2009), 1216, 281-287.
3. Vinas, Pilar, Campillo, Natalia, Martinez-Castillo, Nelson, Hernandez-Cordoba, Manuel, Solid-phase microextraction on-fiber derivatization for the analysis of some polyphenols in wine and grapes using gas chromatography-mass spectrometry. *J. Chrom A* (2009), 1216, 1279-1284.

## Featured Products

Description	Qty.	Cat. No.
<b>SPME Fibers</b>		
Manual, w/ 85 µm polyacrylate coating	3	57304
Auto, w/ 85 µm polyacrylate coating	3	57305
Auto, 23 GA w/ 85 µm polyacrylate coating	3	57294-U
<b>GC Column</b>		
Fused silica capillary column, SLB-5ms, 30 m x 0.25 mm I.D., 0.25 µm		28471-U
<b>Chemical Standards and Reagents</b>		
Resveratrol	100 mg	R5010
BSTFA + TMCS, 99:1 (Sylon BFT)	20 x 1 mL	33148
	25 mL	33155-U
	50 mL	33149-U

## Related Information

For more information on SPME products and applications request a copy of the *7th edition SPME applications CD*, T199925 (CJQ), or visit [sigma-aldrich.com/spme](http://sigma-aldrich.com/spme).



# Sigma-Aldrich Expands Environmental Product Line by Offering NSI Solutions Inorganic Products

**Vicki Yearick**

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Sigma-Aldrich Corporation and NSI Solutions, Inc. (Raleigh, NC) have partnered to provide US customers with a more complete selection of quality analytical products for environmental testing from a single vendor. NSI is an ISO registered, A2LA accredited provider of inorganic and microbiological proficiency testing samples, as well as quality control standards (QC) for oil and grease, turbidity, solids, and pH analyses.

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## Microbiology Proficiency Testing (PT) Samples

These microbiological PT samples are designed for use with all Most Probable Number (MPN) and Membrane Filter (MF) procedures. All PT and QC samples are packaged with a cold pack to maintain integrity and are drop shipped to preserve sample traceability.

Description	Cat. No.
<b>WS Microbiology Proficiency Testing</b>	
WS - Microbiological PT <i>Each set includes 10 samples and 10 vials of sterile diluent. Works with EPA promulgated methods for total coliform, fecal coliform and E. coli</i>	NSI-MIC-001
WS - Standards Plate Count <i>One vitroid sample containing a heterotrophic bacteria. Supplied with sterile diluent.</i>	NSI-MIC-002
WS Quantitative Coliforms <i>One vitroid sample in the range of 20-200 CFU/100mL, designed for LT2 Enhanced Surface Water Treatment Rule. Evaluated for total coliform, fecal coliform, and E. coli. Applicable for all SDWA quantitative methods. Supplied with hydration fluid.</i>	NSI-MIC-006
<b>Microbiological QC Known Standards</b>	
Drinking Water Set - Total and Fecal Coliform <i>Works with EPA approved methods. Each set contains one vitroid sample each of the following: total/fecal coliform positive, total coliform positive/fecal coliform negative, one total fecal and coliform negative, and one blank sample. Each set includes 4 x 100 mL bottles of sterile diluent.</i>	NSI-MIC-QC1
Standards Plate Count <i>Designed for use with SM9215-B Pour Plate Method. Set contains a single quantitative sample of heterotrophic bacteria and a 100 mL bottle of sterile diluent.</i>	NSI-MIC-QC3
WS - Microbiological QC <i>Each set includes 10 samples and 10 vials sterile diluent. Works with EPA promulgated methods for total coliform, fecal coliform, and E. coli.</i>	NSI-MIC-QC4
WS Quantitative Coliforms <i>One vitroid sample in the range of 20 -200 CFU/100mL, designed for LT2 Enhanced Surface Water Treatment Rule. Evaluated for total coliform, fecal coliform, and E. coli. Applicable for all SDWA quantitative methods. Supplied with hydration fluid.</i>	NSI-MIC-QC6

## Oil & Grease Standards for US EPA Method 1664



NSI Oil and Grease PAR (Precision and Recovery) Surrogate SNIPS are packaged in exact volumes in easy-to-use inert, thermo sealed PTFE tubes. Simply snip off the top of the tube and pour the entire contents into your extraction vessel. Also available are the more conventional bottles of oil and grease standards as well as recovery standards.

Products are supplied with a certificate of traceability listing certified concentrations and acceptance limits.

Description	Pkg. Size	Cat. No.
Method 1664 Oil and Grease PAR Surrogate (0.2%) <i>0.2% n-hexadecane, 0.2% stearic acid in acetone delivers 40 mg total oil &amp; grease per tube</i>	25 x 10 tubes	NSI-QC-003LSNIP
Method 1664 Oil and Grease PAR Surrogate (0.4%) <i>0.4% n-hexadecane, 0.4% stearic acid in acetone delivers 80 mg total oil &amp; grease per tube</i>	25 x 10 tubes	NSI-QC-003LSNIP
Oil and Grease, Ready-to-Use Bottles <i>Works with freon and hexane extraction. Oil and grease supplied as 20-100 mg/L. Minimum of 10 lots available per year.</i>	500 mL 4 x 500 mL	NSI-QCI-069 NSI-QCI-069C
Oil and Grease, Supplied in Boston Round Bottle <i>Designed for use with SPE. Oil and grease supplied as 20-100 mg/L. Minimum of 10 lots available per year.</i>	1 Liter 4 x 1Liter	NSI-QCI-069-33,400 NSI-QCI-069-33,400C
Oil and Grease Method 1664 Recovery Standards (0.4%)	25 mL	NSI-QC-003
0.4%(w/v) n-Hexadecane and 0.4% (w/v) Stearic acid in acetone	10 x 25 mL	NSI-QC-003TP
Oil and Grease Method 1664 Recovery Standards (0.2%)	25 mL	NSI-QC-0003L
0.2%(w/v) n-Hexadecane and 0.2% (w/v) Stearic acid in acetone	10 x 25 mL	NSI-QC-003LTP

## Turbidity QC Check Standards

These Turbidity Check Standard Solutions are ideal second-source check standards. The products are traceable to formazin, ready-to-use as received, and applicable to all turbidimeters. Products are supplied with certificates of traceability listing certified concentrations and acceptance limits.

Description	Pkg. Size	Cat. No.
100 NTU*	500 mL 4 x 500 mL 3.78 Liters	NSI-QCI-152 NSI-QCI-152C NSI-QCI-152L
50 NTU	500 mL 4 x 500 mL 3.78 Liters	NSI-QCI-153 NSI-QCI-153C NSI-QCI-153L
25 NTU	500 mL 4 x 500 mL 3.78 Liters	NSI-QCI-154 NSI-QCI-154C NSI-QCI-154L
10 NTU	500 mL 4 x 500 mL 3.78 Liters	NSI-QCI-155 NSI-QCI-155C NSI-QCI-155L
5 NTU	500 mL 4 x 500 mL 3.78 Liters	NSI-QCI-156 NSI-QCI-156C NSI-QCI-156L

\* NTU = Nephelometric Turbidity Units

## Solids Standards & Concentrates, pH Standards



Mid and high level solids standards are formulated to contain Total Dissolved Solids (TDS), Total Suspended Solids (TSS), and Total Solids (TS). They are stable at room temperature, non-hazardous, and easy-to-use. Simply shake the bottle well and measure

an appropriately sized aliquot for use. No pipeting, diluting or formulating is needed. The economical 4 x 1 liter case will provide up to 250 QC analyses for the high level concentrations.

The low level TSS standard is as easy-to-use as the mid and high level standards and is specifically formulated for Minimum Detectable Limit (MDL) validations.

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Description	Pkg. Size	Cat. No.
Low Level TSS Standard TSS 15-30 mg/L	4 x 1 Liter	NSI-QCI-057
Mid-Level Solids Standard TDS 500-1500 mg/L, TS 500-1600 mg/L, TSS 60-100 mg/L	4 x 1 Liter	NSI-QCI-055
High-Level Solids Standard TDS 1000-3000 mg/L, TS 1400-3500 mg/L, TSS 400-500 mg/L	4 x 1 Liter	NSI-QCI-055H
pH QC Standard pH 5-10 units, NIST traceable with multiple lots always available.	4 x 500 mL	NSI-QCI-112C

## Inorganic Water Supply (WS) and Non-Potable Water (NPW) PT Samples

No sample is released into a PT study unless the results meet NSI's acceptance limits, which is 30% more stringent than the NELAC standards. The purity of each analyte source material is certified, and then the PT sample assigned value is corrected for this purity. The accuracy of each formulation and the homogeneity of each batch are validated by instrumental analyses for each analyte in each of the nine samples taken from the production run.

All PT samples are supplied in duplicate and are drop shipped to maintain product traceability. All shipments include instructions for storage and preparation, as well as report forms.

Description	Pkg. Size	PT Samples Cat. No.	QC Known Cat. No.
<b>WS (Drinking Water) PT Samples</b>			
WS-Hardness For determination of: Calcium 30-90 mg/L Magnesium 2.0-20 mg/L Sodium 12-24 mg/L	250 mL	PEI-145	NSI-QCI-145
			Calcium Hardness 75-375 mg/L Total Hardness 83-307 mg/L
WS-Inorganic For determination of: Chloride 5-100 mg/L Fluoride 1-8 mg/L Nitrate as N 3-10 mg/L Nitrate/Nitrite-N 3.5-9.0 mg/L Potassium 10-40 mg/L	500 mL	PEI-041	NSI-QCI-041
			Sulfate 5-500 mg/L Alkalinity 25-200 mg/L Conductivity 250-2500 umhos/cm Tot. Dissolved Solids 200-450 mg/L
WS-pH For determination of pH without dilution. Formulated in the NELAC range of 5.0-10.0 units.	250 mL	PEI-083	NSI-QCI-083
WS-Metals Each ampul produces 2 liters of sample. For determination of the following elements: Aluminum 130-2500 µg/L Antimony 6-50 µg/L Arsenic 5-50 µg/L Barium 500-3000 µg/L Beryllium 1-10 µg/L Boron 800-2000 µg/L Cadmium 2-50 µg/L Chromium 10-200 µg/L Copper 50-2000 µg/L Iron 100-1800 µg/L	2 x 21 mL	PEI-016	NSI-QCI-016
			Lead 5-100 µg/L Manganese 40-900 µg/L Molybdenum 15-130 µg/L Nickel 10-500 µg/L Selenium 10-100 µg/L Silver 20-300 µg/L Thallium 2-10 µg/L Vanadium 315-2500 µg/L Zinc 400-2500 µg/L
WS-Nitrite Concentrate. Each ampul produces 2 liters of sample. Formulated in the NELAC range of 0.40-2.00 mg/L.	21 mL	PEI-140	NSI-QCI-140
WS-Orthophosphate Concentrate. Formulated in the NELAC range of 0.5-5.5 mg/L.	21 mL	PEI-141	NSI-QCI-141
WS-Residual Free Chlorine Concentrate for determination of residual free chlorine and total residual chlorine. Each ampul produces 2 liters of sample. Formulated in the NELAC range of 0.500-3.00 mg/L.	0.5 mL	PEI-012	NSI-QCI-012
<b>NPW (Wastewater) PT Samples</b>			
NPW-Demands Concentrate. Each ampul produces 2 liters of sample.	21 mL	PEI-026	NSI-QCI-026
NPW-Minerals Ready-to-use sample packaged in an HDPE bottle to be analyzed for: Chloride 35-275 mg/L Fluoride 0.3-4 mg/L Potassium 4.0-40 mg/L Sodium 6-100 mg/L Sulfate 5-125 mg/L	500 mL	PEI-136	NSI-QCI-136
			Alkalinity 10-120 mg/L Conductivity 200-930 umhos/cm TDS at 180 °C 140-650 mg/L
NPW-Hardness Ready-to-use sample packaged in an HDPE bottle to be analyzed for: Calcium 3.5-110 mg/L Magnesium 2.0-40 mg/L	250 mL	PEI-137	NSI-QCI-137
			Calcium Hardness 8.7-275 mg/L Total Hardness 17-440 mg/L
NPW-pH For pH analysis without dilution. Formulated in the NELAC range of 5.0-10.0 units.	250 mL	PEI-035	NSI-QCI-035

(continued on page 22)

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Standards

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(continued from page 21)

Description	Pkg. Size	PT Samples Cat. No.	QC Known Cat. No.
NPW-Residue Ready-to-use whole volume sample to be analyzed for total suspended solids in the NELAC range of 23-100 mg/L and total solids formulated in the NELAC range of 140-675 mg/L.	500 mL	PEI-079	NSI-QCI-079
NPW-Simple Nutrients Concentrate. Each ampul produces 2 liters of sample. <i>Ammonia as N 0.65-19.0 mg/L</i> <i>Nitrate as N 0.25-40.0 mg/L</i>	21 mL	PEI-138	NSI-QCI-138
			<i>Nitrate/Nitrite as N 0.25-40.0 mg/L</i> <i>Orthophosphate as P 0.50-5.50 mg/L</i>
NPW Complex Nutrients Concentrate. Each ampul produces 2 liters of sample. <i>TKN 1.50-35.0 mg/L</i>	21 mL	PEI-139	NSI-QCI-139
			<i>Total Phosphorus 0.50-10.0 mg/L</i>
NPW Trace Metals Each ampul produces 2 liters of sample. For analysis of the following elements: <i>Aluminum 200-4000 µg/L</i> <i>Antimony 95-900 µg/L</i> <i>Arsenic 70-900 µg/L</i> <i>Barium 100-2500 µg/L</i> <i>Beryllium 8-900 µg/L</i> <i>Cadmium 8-750 µg/L</i> <i>Chromium 17-1000 µg/L</i> <i>Cobalt 28-1000 µg/L</i> <i>Copper 40-900 µg/L</i> <i>Iron 200-4000 µg/L</i> <i>Lead 70-3000 µg/L</i>	2 x 21 mL	PEI-034	NSI-QCI-034
			<i>Manganese 70-4000 µg/L</i> <i>Molybdenum 60-600 µg/L</i> <i>Nickel 80-3000 µg/L</i> <i>Selenium 90-2000 µg/L</i> <i>Silver 26-600 µg/L</i> <i>Strontium 30-300 µg/L</i> <i>Thallium 60-900 µg/L</i> <i>Tin 1000-5000 µg/L</i> <i>Titanium 80-300 µg/L</i> <i>Vanadium 55-2000 µg/L</i> <i>Zinc 100-2000 µg/L</i>
NPW-Mercury A 21 mL concentrate for determination of Mercury. Each ampul produces 2 liters of sample. Formulated in the NELAC range of 2.0-30 µg/L.	21 mL	PEI-087	NSI-QCI-087
NPW Total Residual Chlorine Concentrate. Each ampul produces 2 liters of sample. Formulated in the NELAC range of 0.50-3.00 mg/L.	2.2 mL	PEI-033	NSI-QCI-033

Visit [sigma-aldrich.com/nsisolutions](http://sigma-aldrich.com/nsisolutions) for a complete list of NSI products now available through Sigma-Aldrich.

## Reference

1. American Public Health Association. Standard Methods for the Examination of Water & Wastewater. 20th ed.; Baltimore, MD, 1999.

## Characterized Reference Oils for Standardizing Lipid Procedures

We offer characterized reference oil samples for use as controls or check samples for fatty acid methyl ester (FAME) analyses. These samples provide an excellent means of standardizing your lipid procedures and comparing your results to other researchers. Each standard is packaged in an amber ampul under nitrogen. Package size is 1000 mg. A Certificate of Composition is provided with each oil sample.

Description	CAS No.	Cat. No.
Canola oil	120962-03-0	46961
Coconut oil	8001-31-8	46949
Corn oil	8001-30-7	47112-U
Cottonseed oil	8001-29-4	47113
Lard oil	8016-28-2	47115-U
Linseed (flaxseed) oil	8001-26-1	47559-U
Menhaden fish oil	8002-50-4	47116
Olive oil	8001-25-0	47118
Palm oil	8002-75-3	46962
Peanut oil	8002-03-7	47119
Safflower oil	8001-23-8	47120-U
Soybean oil	8001-22-7	47122
Sunflower seed oil	8001-21-6	47123

# Mono-, Di-, and Triglyceride Chemical Standards

Sigma-Aldrich offers numerous qualitative mono-, di-, and triglyceride standards for determining relative retention times and establishing approximate response factors when used in chromatographic applications. These standard mixtures are prepared by weight, and the composition verified by gas and/or thin layer liquid chromatography. A certificate of composition is provided with each standard.

A sampling of this product line is listed below. Additional glyceride standards can be found by visiting [sigma-aldrich.com](http://sigma-aldrich.com).

## Glyceride and Phospholipid Mixtures

Description	Pkg. Size	Cat. No.
Mono-, Di-, & Triglyceride Mix <i>1,3-Diolein, 10 mg</i> <i>1,2-Dioleoyl-rac-glycerol, 10 mg</i>	40 mg	1787-1AMP
	<i>Monolein, 10 mg</i> <i>Triolein, 10 mg</i>	
Triglyceride Mixtures <i>Tricaprin, ~20 mg</i> <i>Tricaprylin, ~20 mg</i> <i>Trilaurin, ~20 mg</i>	100 mg	17811-1AMP
Phospholipid Mixture for HPLC derived from soybeans, in chloroform (varied conc.) <i>L-α-Lysophosphatidylcholine, 0.3 mg/mL</i> <i>L-α-Phosphatidylcholine, 1.5 mg/mL</i> <i>L-α-Phosphatidylethanolamine Escherichia coli, 1.2 mg/mL</i> <i>L-α-Phosphatidylinositol ammonium salt from Glycine max (soybean), 0.9 mg/mL</i>	2 mL	P3817-1VL

## Glyceride Kits

Description	Cat. No.
Mono-, Di-, and Triglycerides Kit (individually packaged in 100 mg each)	MDT12-1KT
<i>Dilaurin</i>	<i>1-Monopalmitoyl-rac-glycerol</i>
<i>Dimyristin</i>	<i>1-Monostearoyl-rac-glycerol</i>
<i>Dipalmitin</i>	<i>Trilaurin</i>
<i>Distearin</i>	<i>Trimyristin</i>
<i>1-Monolauroyl-rac-glycerol</i>	<i>Tripalmitin</i>
<i>1-Monomyristoyl-rac-glycerol</i>	<i>Tristearin</i>
Triglycerides Kit (individually packaged in 100 mg unless otherwise indicated)	TRI19-1KT
<i>Triacetin</i>	<i>Trilaurin</i>
<i>Triarachidin</i>	<i>Trilinolein</i>
<i>Tribehenin</i>	<i>Trilinolenin</i>
<i>Tributyryl</i>	<i>Trimyristin</i>
<i>Tricaprin</i>	<i>Triolein</i>
<i>Tricaproin, 1 mL</i>	<i>Tripalmitin</i>
<i>Tricaprylin, 0.5 mL</i>	<i>Tripalmitolein</i>
<i>Trielaidin</i>	<i>Tripetroselinin</i>
<i>Tri-11-eicosenoic acid</i>	<i>Tristearin</i>
<i>Trierucin</i>	
Saturated, Even Carbon Triglycerides Kit (individually packaged in 100 mg unless otherwise indicated)	TRI11-1KT
<i>Triacetin</i>	<i>Tricaprylin, 1 mL</i>
<i>Triarachidin</i>	<i>Trilaurin</i>
<i>Tribehenin</i>	<i>Trimyristin</i>
<i>Tributyryl</i>	<i>Tripalmitin</i>
<i>Tricaprin</i>	<i>Tristearin</i>
<i>Tricaproin, 1 mL</i>	

# SGE Slim Line Syringes for CTC/Leap Autosamplers

A gas chromatograph (GC) autosampler syringe serves as the interface between the sample and the instrument; compatibility is critical for rapid and precise sampling. The wrong sized syringe can lead to inconsistent results and, more seriously, can damage the instrument. Syringes also must deliver accurate amounts with no-dead volume or carryover between injections.

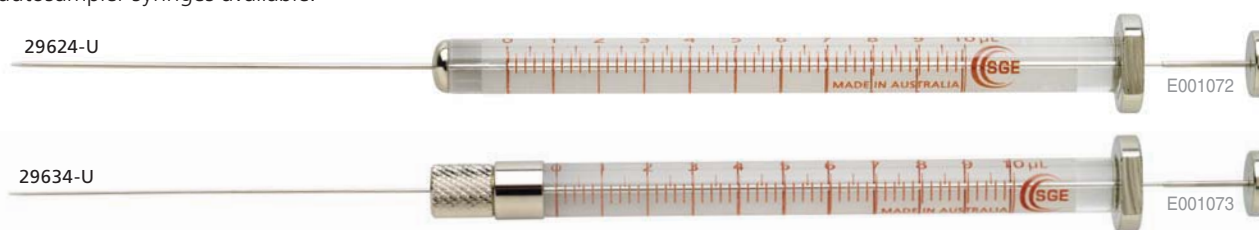
Sigma-Aldrich has introduced a new line of GC autosampler syringes from SGE™ that are made to be used with CTC CombiPAL™ and GC PAL autosamplers. SGE Slim Line syringes are designed with a unique plunger that eliminates bending and seizing and are tested to ensure optimal fit within autosampler specifications. The cone tip point style of the needle withstands multiple and fast septum injections, making it one of the most trouble-free autosampler syringes available.

When used with CombiPAL and GC PAL autosamplers, these syringes are installed into a syringe holder designed to accommodate the outside diameter of the syringe barrel.

SGE Slim Line autosampler syringes are available with both fixed and removable needle with the following specifications:

- Volumes from 0.5 to 100 µL
- Needle length of 50 mm
- Point style 1 (cone tip)
- Two gauge sizes: 23 (0.63 mm) and 26 (0.47 mm) O.D.

For more information on any of our syringes, email our Technical Service Department at [techservice@sial.com](mailto:techservice@sial.com) or visit us on the web at [sigma-aldrich.com/syringes](http://sigma-aldrich.com/syringes).



## + Featured Products

Slim Line (6.5 mm O.D. syringe barrel), 50 mm Cone Tip Needle SGE Syringes for CTC CombiPAL and GC PAL

Volume	Description	Needle	Pack Size	SGE Cat. No.	Supelco Cat. No.
<b>Slim Line, Fixed Needle</b>					
5 µL	5F-C/F-5/0.63	23 gauge (0.63 mm OD)	1	001981	29622-U
5 µL	5F-C/F-5/0.47	26 gauge (0.47 mm OD)	1	001982	29623-U
10 µL	10F-C/F-/0.63	23 gauge (0.63 mm OD)	1	002981	29624-U
10 µL	10F-C/F-5/0.47C	26 gauge (0.47 mm OD)	1	002980	29625-U
25 µL	25F-C/F-0.47C	26 gauge (0.47 mm OD)	1	003980	29628-U
<b>Slim Line, Fixed Needle, Gas Tight</b>					
10 µL	SK 10F-C/F-5/0.47C	26 gauge (0.47 mm OD)	6	002986	29626-U
10 µL	10F-C/F-GT-0.63	23 gauge (0.63 mm OD)	1	002987	29627-U
25 µL	25F-C/F-GT-0.63	23 gauge (0.63 mm OD)	1	003987	29629-U
100 µL	100F-C/F-GT-0.63	23 gauge (0.63 mm OD)	1	005335	29631-U
100 µL	100R-C/F-GT-0.47C	26 gauge (0.47 mm OD)	1	005333	29632-U
<b>Slim Line, Removable Needle</b>					
0.5 µL	0.5BNR-C/F-0.63	23 gauge (0.63 mm OD)	1	000492	29633-U
10 µL	10R-C/F-5/0.47C	26 gauge (0.47 mm OD)	1	002982	29634-U
10 µL	10R-C/F-0.63	23 gauge (0.63 mm OD)	1	002984	29635-U
<b>Slim Line, Removable Needle, Gas Tight</b>					
10 µL	10R-C/F-GT-0.47	26 gauge (0.47 mm OD)	1	002985	29636-U

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