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## Table of Contents

### Liquid Chromatography

Fast LC-MS-MS Analysis of  
25-Hydroxyvitamin D2 and  
25-Hydroxyvitamin D3 .....3

LC-MS Nitrogen: Generation and  
Purification.....6

Derivatization and Improved  
Detection of Estradiol with ESI-MS..... 18

Introduction of Novel  
Performance-Tested Solvents  
for UHPLC Applications.....22

High-Purity Headspace  
Grade Solvents .....23

### Sample Preparation

Highly Selective Separation of  
Vitamin D epi-Metabolites Using  
HybridSPE®-Phospholipid..... 14

Aldehydes and Ketones in Indoor  
Air using a Low Background LpDNPH  
Solvent Desorption Tube and  
Fused-Core® HPLC..... 16

### Gas Chromatography

EN 14103 FAMES in B100 Biodiesel  
on the Omegawax™ ..... 10

FAMES in B20 Biodiesel on the  
SLB™-IL111 ..... 12

### Standards

*Withania somnifera* Analytical Standards  
and an Improved HPLC Method .....20

### Chiral Chromatography

Chiral HPLC Analysis of Underivatized  
Amino Acid Enantiomers.....8

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Director of Marketing & R&D  
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# Fast LC-MS-MS Analysis of 25-Hydroxyvitamin D2 and 25-Hydroxyvitamin D3

## Contributed Article

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

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## Abstract

A robust method for analyzing 25-hydroxyvitamin D3 and 25-hydroxyvitamin D2 in serum using a QTRAP® 5500 and an MPX™-2 High Throughput multiplexed LC-MS-MS system is presented. Sample preparation was simplified in order to accommodate automated liquid handling systems and to minimize the time commitment needed by clinical staff. With multiplexing, sample results were achieved in less than 1.5 minutes per sample. Accuracy and linearity was demonstrated for 25-hydroxyvitamin D3 and 25-hydroxyvitamin D2 over a concentration range 0.5-100 ng/mL in serum. This dynamic range was achieved without the use of solid phase extraction, online extraction, or any other type of sample concentration steps.

## Introduction

Heightened interest in vitamin D is related to an observed trend of increased vitamin D insufficiency and its role in human health (1). Vitamin D is derived in vivo primarily from UVB radiation impacting the skin where 7-dehydrocholesterol is converted to cholecalciferol (vitamin D3) through a photolytic, nonenzymatic reaction. Smaller amounts of vitamin D are acquired through diet, including vitamin D3 (cholecalciferol) and vitamin D2 (ergocalciferol) from animal and plant sources, respectively. Vitamin D3 is metabolized to 25-hydroxyvitamin D3 in the liver and then to the active form 1,25-dihydroxyvitamin D3 in the kidney. Circulating levels of 1,25-dihydroxyvitamin D are tightly controlled and serve to modulate expression of specific genes through the vitamin D receptor.

Vitamin D acts to promote intestinal calcium and phosphate absorption and also controls their liberation from bone. In addition to rickets and osteomalacia, low serum 25-hydroxyvitamin D3 and D2, has been linked to hypertension, autoimmune diseases, and cancer (2,3).

The most abundant metabolite of vitamin D is 25-hydroxyvitamin D and it is considered one of the best indicators of vitamin D status. Accurately measuring 25-hydroxyvitamin D is necessary to adequately assess an individual's vitamin D levels and to help determine the role of vitamin D in various diseases. Immunoassays suffer from high reagent cost, narrow dynamic range, and poor selectivity (inability to distinguish 25-hydroxyvitamin D3 from 25-hydroxyvitamin D2) (4). These inadequacies of immunoassays make them unattractive for vitamin D analysis in patient samples.

Liquid chromatography tandem mass spectrometry (LC-MS-MS) is a highly sensitive and specific technique for the analysis of a wide range of compounds contained in biological matrices. The current generation of this technology is capable of reliably determining 25-hydroxyvitamin D concentrations across the entire clinical range. Coupled with liquid chromatography, accurate and precise measurements of both 25-hydroxyvitamin D3 and 25-hydroxyvitamin D2 can be routinely achieved using LC-MS-MS. The primary goal of this method was to use a simple sample preparation step while accommodating high throughput 25-hydroxyvitamin D runs, ~500/day, on a single LC-MS-MS system.

## Sample Preparation

Protein precipitation consisted of combining 50 µL of serum with 100 µL acetonitrile in a 1.5 mL centrifuge tube. The sample was then centrifuged at 5,000 g for 5 minutes before transferring the supernatant into a sample vial for analysis.

## MS/MS Conditions

The multiplexed LC system, MPX™, was coupled to an AB SCIEX QTRAP® 5500 LC-MS-MS system with a Turbo V™ source and Atmospheric Pressure Chemical Ionization (APCI) probe in positive ion mode. A total of 5 Multiple Reaction Monitoring (MRM) transitions were monitored, including 2 per compound (quantifier and qualifier) and one for the internal standard, 25-hydroxyvitamin D3-d6. Chromatographic conditions are listed in Figures 1 and 2.

(continued on page 4)

Figure 1. Initial Serum Extracted Sample of 350 Injections

column:	Ascentis Express C18, 2 cm x 2.1 mm I.D., 2.7 µm (53799-U)			
mobile phase A:	0.1% formic acid in water (v/v)			
mobile phase B:	0.1% formic acid in acetonitrile			
gradient:	Time (min)	%A	%B	Flow Rate (mL/ min)
	0.00	50.00	50.00	1.00
	0.85	7.50	92.50	1.00
	0.90	5.00	95.00	1.50
	1.00	0.00	100.00	1.50
	1.45	0.00	100.00	1.50
	1.50	0.00	100.00	1.00
	1.60	50.00	50.00	1.00
	2.00	50.00	50.00	1.00
flow rate:	see flow program in gradient table			
temp.:	40 °C			
det.:	MS, APCI (+) mode			
inj:	5 µL			
sample:	as indicated in method			

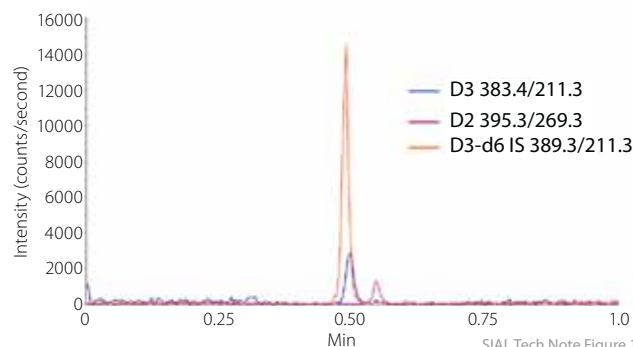
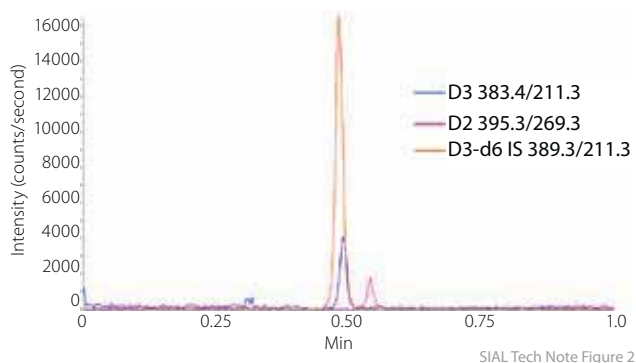


Figure 2. Re-Injection of the First Sample after 350 Injections of Serum Extracted Samples Analyzed

Same conditions as Figure 1.



SIAL Tech Note Figure 2

(continued from page 3)

## Results and Discussion

A method suitable for quantification of 25-hydroxyvitamin D2 and D3 required only two minutes, prior to multiplexing. **Figure 1** shows the separation of 25-hydroxyvitamin D2 and D3 and the internal standard. This is the first injection of an extracted serum sample on the Ascentis Express C18 column. **Figure 2** shows the result of re-injecting this first sample after 350 serum extracted samples were run. Note that peak shape, retention, and signal response have not changed after the 350 injections. This indicates a very robust method which employed simple sample preparation (protein precipitation) prior to analysis.

Quantitative results for serum samples are presented in **Tables 1 and 2**. These results show satisfactory precision and accuracy for a high-throughput method requiring minimal sample preparation.

## Conclusion

A robust and reliable assay for 25-hydroxyvitamin D capable of handling high sample throughput observed in a clinical research laboratory was presented. Through the use of the AB SCIEX MPX™ system and the QTRAP® 5500 LC-MS-MS system in combination with Supelco's Ascentis Express C18 column, laboratories can achieve 25-hydroxyvitamin D sample analysis times of <1.5 minutes per sample. This streamlined method can be implemented without the need for UHPLC systems or advanced knowledge of separation chemistry and achieves Limits of Quantitation (LOQ) of 1 ng/mL for vitamin D2 and D3.

## References

1. Ginde AA, Liu MC, Camargo Jr CA, Demographic differences and trends of vitamin D insufficiency in the US population, 1988-2004, Arch Intern Med 2009;169:626-632
2. Borradaile D, and Kimlin M, Vitamin D and disease: an insight into traditional functions and new roles for the 'sunshine vitamin', Nutr Res Rev 2009;22:118-136.
3. Adams, JS, Hewison M, Update in Vitamin D J Clin Endocrinol Metab 2010 95:471-478.
4. Lai JKC, Lucas RM, Clements MS, Harrison SL, Banks E, Assessing vitamin D status: Pitfalls for the unwary, Mol. Nutr. Food Res. 2010 54:1-10

Table 1. Quantitative Results 25-Hydroxyvitamin D3 Extracted from Serum (MRM Transition 383.4/229.2)\*

Concentration	Mean	Std Deviation	Percent CV	Accuracy
0.50	0.5599	5.758E-02	10.28	111.97
1.00	1.0830	2.351E-01	21.71	108.31
2.00	1.8610	3.026E-01	16.26	93.05
5.00	4.8210	3.549E-01	7.36	96.41
10.00	9.3020	6.114E-01	6.57	93.02
20.00	18.7700	1.373E+00	7.32	93.83
50.00	51.2900	2.005E+00	3.91	102.59
100.00	100.8000	3.707E+00	3.68	100.81

\*n= 8 injections/concentration (ng/mL), results were compiled from a 461 injection data set including matrix samples, standards, and controls.

Table 2. Quantitative Results 25-Hydroxyvitamin D2 Extracted from Serum (MRM Transition 395.3/269.3)\*

Concentration	Mean	Std Deviation	Percent CV	Accuracy
0.50	0.5817	1.208E-01	20.77	116.34
1.00	1.0170	1.307E-01	12.84	101.75
2.00	1.9210	1.982E-01	10.32	96.04
5.00	4.7210	4.682E-01	9.92	94.43
10.00	9.5080	1.030E+00	10.83	95.08
20.00	18.4400	1.513E+00	8.20	92.21
50.00	51.8400	2.886E+00	5.57	103.69
100.00	100.5000	6.646E+00	6.61	100.46

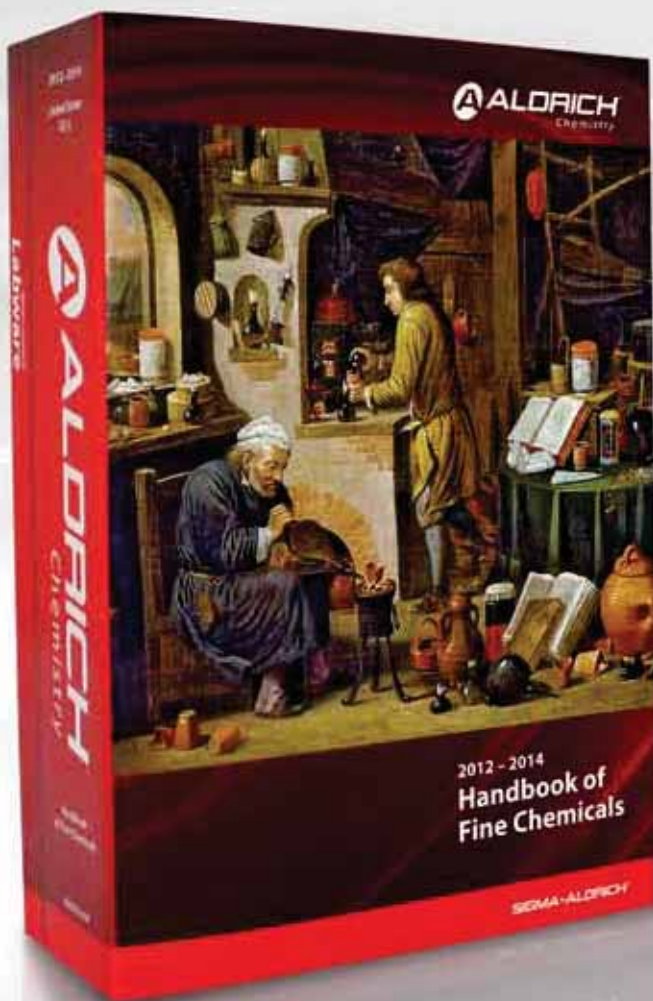
\*n= 8 injections/concentration (ng/mL), results were compiled from a 461 injection data set including matrix samples, standards, and controls.

### + Featured Product

Description	Cat. No.
Ascentis Express C18, 2 cm x 2.1 mm I.D., 2.7 µm	53799-U

### + Related Products

Description	Cat. No.
Ascentis Express C18, 3 cm x 2.1 mm I.D., 2.7 µm	53802-U
Ascentis Express C18, 5 cm x 2.1 mm I.D., 2.7 µm	53822-U
Ascentis Express C18, 7.5 cm x 2.1 mm I.D., 2.7 µm	53804-U
Ascentis Express C18, 10 cm x 2.1 mm I.D., 2.7 µm	53823-U



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# LC-MS Nitrogen: Generation and Purification

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The current trends in High Performance Liquid Chromatography (HPLC) are switching to more efficient columns, and applications that require higher sensitivity and higher selectivity. Mass Spectrometry (MS) is a technique being used to help achieve some of these targets. An important consideration for analytical accuracy is an uninterrupted supply of a

relatively large volume of high-purity nitrogen to the MS unit.

Many laboratories that utilize LC-MS may purchase their nitrogen in high-priced certified gas cylinders in an effort to ensure gas purity. A better strategy is to generate and purify sufficient volumes of nitrogen to the high-grade necessary for LC-MS use. This can be accomplished by installing an on-site nitrogen generator coupled to contaminant-specific purifiers that are installed downstream.

## Nitrogen Generation

Parker® offers several nitrogen generators that are specifically designed to meet the gas flow, purity, and pressure requirement needed to supply single or multiple LC-MS instruments. These units are compatible with both atmospheric pressure chemical ionization (APCI) and electrospray ionization (ESI) interfaces. Oil-free compressed air is first filtered then passed through a bed of carbon molecular sieve which selectively removes oxygen and other contaminants. Some models are offered with an integral oil-free air compressor, while other models require a separate oil-free air compressor to be installed upstream. Pressure swing adsorption technology is then employed to produce a continuous supply of high-purity nitrogen. This technology works by separating gas species in a mixture under pressure according to their molecular characteristics and their affinity for an adsorbent material operating near ambient temperatures. Two adsorbent beds alternate between purification and regeneration modes to ensure a continuous supply of nitrogen at the specified purity levels.

Laboratory gas generators are a great alternative to gas cylinders. In addition to being a much more sensible source of gas from a cost standpoint, generators take up less space, are safer, and do not require the labor needed to transport bulky cylinders into the lab.

All generator models offered by Parker are available through Sigma-Aldrich. Simply contact one of our Technical Service chemists at [techservice@sial.com](mailto:techservice@sial.com), specifying the make, model, and electrical requirements.

## Nitrogen Purification

Whether using nitrogen gas cylinders or nitrogen generators, removing trace contaminants is critical to the proper operation of an LC-MS instrument. We recommend Super Clean™ LC-MS purifiers for this purpose. These two-cartridge base-plate systems are specifically designed to meet the high-flow and high-purity requirements of LC-MS units. Two versions are available, one for hydrocarbon removal, and one for moisture removal.

Independent of the original gas quality, these purifiers can reduce contaminants to reach nitrogen purity greater than 99.9999% quality. Cartridges are inert, comprised of an adsorbent in a non-diffusive glass tube, a strong polycarbonate shell, and an inert stainless steel fitting. Base plates are a special two-position design with an inert gas path that is split equally between the two cartridges and rejoined afterwards before leaving the base plate. Therefore, each cartridge handles half the flow, providing longer contact with the gas stream. This special design is what allows these purifiers to produce such high-quality gas at flow rates up to 20 L/min and pressures up to 150 psi (11 bar). Note that each base plate must contain the same type cartridge (either both hydrocarbon removal, or both moisture removal).

Some other features/benefits of Super Clean LC-MS purifiers are:

- Permanent connections
  - After installation, connections to the gas line never need to be broken
  - Reduces the risk of leaks from kinked tubing
- Continuous operation
  - Needle valves instantly close to provide a diffusion-proof seal when cartridges are removed
  - Eliminates the need to suspend operation during cartridge change-out
- Quick cartridge change-out and no tools required
  - Install in seconds without exposing the gas lines to room air
  - Only needs held in place on the base-plate while the retaining ring is hand-tightened
- Vertical design requires very little bench space
- Indicator capability for moisture

As a safeguard, it is recommended to install Super Clean LC-MS purifiers just before the gas line enters the LC-MS unit. This will protect against contaminants that may have entered through fittings.

TRADEMARKS: Agilent – Agilent Technologies; Ascentis, Astec, CHIROBIOTIC, CHROMASOLV, Discovery, Fluka, HybridSPE, Omegawax, SLB, Supel, Supelco, Thermogreen – Sigma-Aldrich Co. LLC; FocusLiner – SGE Analytical Science Pty Ltd; Fused-Core – Advanced Materials Technology, Inc.; Parker – Parker Hannifin Corporation; QTRAP, MPX – AB SCIEX; Radiello – Fondazione Salvatore Maugeri IRCCS; Super Clean – Scientific Glass Technology B.V.; Tedlar – E. I. duPont de Nemours & Co.

## Conclusion

A nitrogen generator is a great alternative for supplying gas to the LC-MS instrument. If you are unsure of the economic advantage of replacing gas cylinders with gas generators, look at the convenience, safety, and reliability. Generators eliminate the labor involved and the need to shut down the entire system when changing empty cylinders. Additionally, installing gas purifiers downstream of the nitrogen source helps guarantee that the quality of the gas going to the LC-MS instrument will be free of contaminants, allowing the highest quality analytical results to be achieved.

## + Featured Products

Description	Qty.	Cat. No.
<b>LC-MS High-Flow Base-Plate Design Kits</b>		
Hydrocarbon Removal Kit (SU861029 + 28879-U)	1 kit	SU861046
Moisture Removal Kit (SU861028 + 28879-U)	1 kit	SU861045
<b>LC-MS High-Flow Base-Plate Design Replacement Items</b>		
Hydrocarbon Removal Cartridges, w/out indicator	2 ea	SU861029
Moisture Removal Cartridges, w/indicator	2 ea	SU861028
LC-MS High-Flow Two Position Base Plate	1 ea	28879-U
Replacement O-Ring Set (10 small and 10 large)	1 ea	SU861050
Base-Plate Wall Mounting Bracket	1 ea	SU861016

## + Related Information

Additional information regarding generators and purifiers for nitrogen can be found at [sigma-aldrich.com/hplc](http://sigma-aldrich.com/hplc)



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# Chiral HPLC Analysis of Underderivatized Amino Acid Enantiomers

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In this brief article, we report a single, simple LC-MS-compatible mobile phase system that resolves the enantiomers of the common underderivatized amino acids on an Astec CHIROBIOTIC® T column. Some experiments to provide insight into the variables that control retention and selectivity will also be touched upon.

Although L-amino acids dominate in nature, D-amino acids have been found in almost all species of bacteria, plants, and animals. Their presence has implications in physiology, nutrition, pharmacology, and toxicology that have spawned development of chromatographic methods to resolve in order to identify and quantify amino acid enantiomers (1,2). Resolving the enantiomers on polysaccharide-based chiral stationary phases (CSPs) is a challenge because native (underderivatized) amino acids are zwitterionic and poorly soluble in non-polar solvents. Derivatization prior to separation can be used to improve solubility, or to create diastereomers that are resolvable by achiral HPLC (3). Derivatization however, adds an additional step and potential impurities. Direct analysis is preferred, and possible on macrocyclic glycopeptide-based CSPs.

Unlike polysaccharide-based CSPs, macrocyclic glycopeptides possess ionic groups (4) and are compatible with both organic and aqueous mobile phases. This makes them ideal CSPs for separating enantiomers of polar and ionic compounds, like amino acids. One such CSP that is particularly successful for resolving the enantiomers of underderivatized amino acids is Astec CHIROBIOTIC T, which employs the macrocyclic glycopeptide teicoplanin as the chiral selector (5,6). The goal of this study was to develop a single mobile phase system that would resolve the majority of common amino acids on Astec CHIROBIOTIC T.

## Approach

The retention of four representative amino acids, DL-arginine (positively charged), DL-aspartic acid (negatively charged), DL-threonine (polar, uncharged), and DL-tyrosine (hydrophobic) versus percentage of methanol in a water:methanol:formic acid mobile phase was measured and plotted (Figure 1). The mobile phase composition that gave the best overall enantioselectivity was applied to the remaining amino acids.

## Results

A simple mobile phase comprising water:methanol:formic acid (30:70:0.02) gave baseline resolution of most of the twenty chiral amino acids enantiomers on the Astec CHIROBIOTIC T column (Table 1). The small amount of formic acid was necessary to produce elution of the charged acidic and basic amino acids. Enantiomers of histidine, cysteine, and proline were not resolved under these conditions, but could be resolved on the same column under slightly different conditions (see Table 1 footnote). Representative chromatograms of DL-tryptophan and DL-methionine appear in Figures 2 and 3, respectively.

**Table 1. Screen of Underderivatized Amino Acids Under Optimized Mobile Phase Conditions**

column: Astec CHIROBIOTIC T, 25 cm x 4.6 mm I.D.,  
5 µm particles (12024AST)  
mobile phase: water:methanol:formic acid (30:70:0.02)  
flow rate: 1.0 mL/min.  
temp.: 25 °C

Amino Acid*	Rt1 (min.)	Rt2 (min.)	Selectivity	Resolution
DL-Arginine	7.367	9.623	1.31	3.78
DL-Histidine	9.132	9.737	1.07	0.67
DL-Lysine	7.128	9.464	1.33	4.12
DL-Aspartic Acid	4.477	5.165	1.15	2.68
DL-Glutamic Acid	4.340	5.321	1.23	4.59
DL-Serine	4.441	4.896	1.10	2.05
DL-Threonine	4.234	4.619	1.09	2.09
DL-Asparagine	5.267	6.835	1.30	4.64
DL-Glutamine	4.934	6.033	1.22	4.51
DL-Alanine	4.744	6.156	1.30	5.54
DL-Isoleucine	4.349	5.662	1.30	5.83
DL-Leucine	4.421	5.938	1.34	6.39
DL-Methionine	4.811	6.674	1.39	6.56
DL-Phenylalanine	4.994	6.170	1.24	6.17
DL-Tryptophan	5.095	6.275	1.23	3.90
DL-Tyrosine	4.578	5.594	1.88	4.25
DL-Valine	4.472	5.385	1.20	3.93

\* Optimized conditions for histidine, cysteine, and proline on Astec CHIROBIOTIC T:

DL-Histidine: 160 mM sodium phosphate:ethanol, pH 4.5 (40:60)  
DL-Cysteine: water:acetonitrile (30:70)  
DL-Proline: water:acetonitrile (95:5)

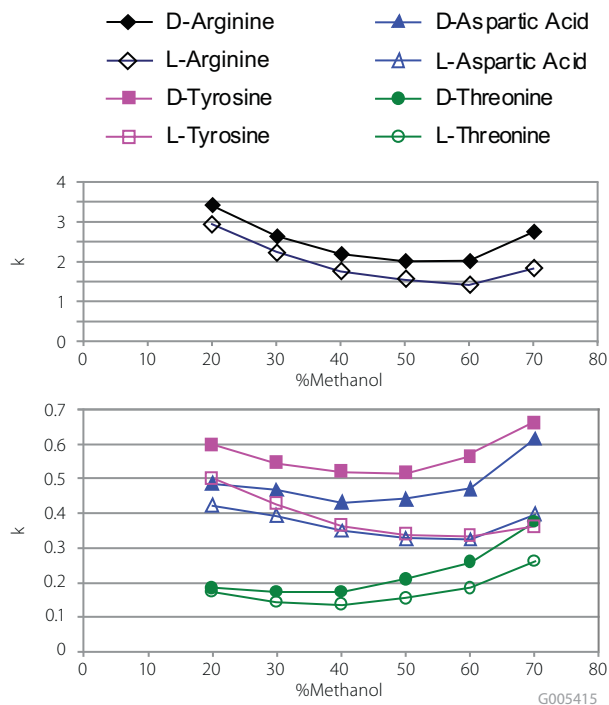
For all amino acids tested, enantioselectivity increased with organic modifier concentration. Retention versus organic modifier concentration exhibited a U-shaped profile (Figure 1). This observation has been well documented in small and large molecule achiral separations, and has also been reported for chiral compounds in methanol and acetonitrile on teicoplanin-based CSPs. It is likely due to the combined effects of analyte solubility and conformational changes in the CSP as a function of organic modifier content (7). The effect has also been reported on cyclodextrin (CD)-based CSPs, but only in acetonitrile. It is thought to be related to the accessibility of the CD cavity by the acetonitrile molecule (8).

It is interesting to note that the D enantiomer is always more strongly retained than the corresponding L enantiomer on macrocyclic glycopeptide CSPs. This is no coincidence since these molecules exert their antibiotic activity by interacting with terminal D-alanyl-D-alanine residues in bacterial cell membrane peptides (9).



**Figure 1. Effect of Organic Modifier Concentration on Amino Acid Retention**

column: Astec CHIROBIOTIC T, 25 cm x 4.6 mm I.D.,  
5 µm particles (12024AST)  
mobile phase: methanol:water:formic acid (20:70:0.02)  
flow rate: 1.0 mL/min.  
temp.: 25 °C  
det.: UV at 205 nm  
injection: 10 µL  
sample: 0.2 mg/mL in 50:50, water:ethanol



## Conclusion

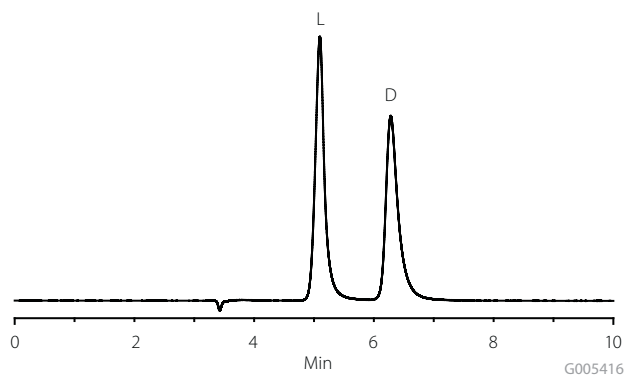
Because Astec CHIROBIOTIC T columns possess ionic functional groups and operate in mobile phase systems that favor polar analyte solubility, they are uniquely able to separate underivatized D- and L-amino acids. A simple LC-MS-compatible mobile phase system was found that resolved most amino acid pairs, and provides a foundation for future studies in this area.

## References

- Schieber, A.; Brückner, H.; Rupp-Classen, M.; Specht, W.; Nowitzki-Grimm, S.; Classen, H.-G. J. Chromatogr. B, 1997, 691(1), 1-12.
- Péter, A.; Árki, A.; Vékes, E.; Tourwé, D.; Lázár, L.; Fülöp, F.; Armstrong, D. W. J. Chromatogr. A, 2004, 1031(1-2), 159-170, 171-178.
- Ilisz, I.; Berkecz, R.; Péter, A. J. Pharm. Biomed. Anal. 2008, 47, 1-15.
- Xiao, T. L.; Tesarova, E.; Anderson, J.L.; Egger, M.; Armstrong, D. W. J. Sep. Sci. 2006, 29(3), 429-445. Berthod, A.; Liu, Y.; Bagwill, C.; Armstrong, D. W. J. Chromatogr. A, 1996, 731(1), 123-137.
- Berthod, A.; Liu, Y.; Bagwill, C.; Armstrong, D. W. J. Chromatogr. A, 1996, 731(1), 123-137.
- Péter, A.; Töröka, G.; Armstrong, D. W. J. Chromatogr. A, 1998, 793, 283-296.
- Armstrong, D. W.; Liu, Y.; Ekborg-Ott, K. H. Chirality, 1995, 7, 474-497.
- Seeman, J.; Secor, H.; Armstrong, D. W.; Timmons, K.; Ward, T. Anal. Chem. 1988, 60(19), 2120-2127.
- Wade, D.; Boman, A.; Wahlin, B.; Drain, C.; Andreui, D.; Boman, H.; Merrifield, R. Proc. Natl. Acad. Sci. USA, 1990, 87, 4761-4765.

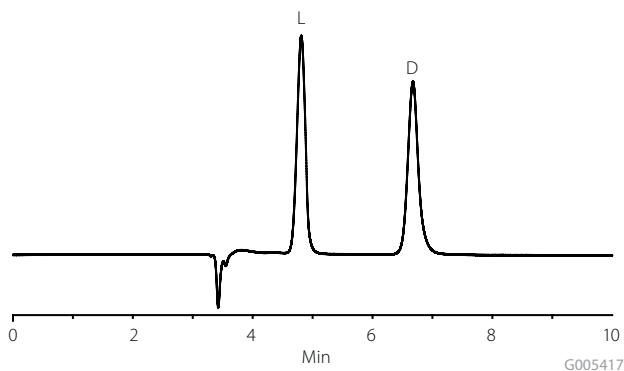
**Figure 2. Representative Chromatogram of DL-Tryptophan on Astec CHIROBIOTIC T**

Conditions same as Figure 1 except:  
mobile phase: water:methanol:formic acid (30:70:0.02)



**Figure 3. Representative Chromatogram of DL-Methionine on Astec CHIROBIOTIC T**

Conditions same as Figure 1 except:  
mobile phase: water:methanol:formic acid (30:70:0.02)



## + Featured Products

Description	Cat. No.
Astec CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles	12024AST
Astec CHIROBIOTIC T Chiral HPLC Guard Cartridge, 2 cm x 4.0 mm I.D., 5 µm particles	12100AST
Stand-Alone HPLC Guard Column Holder	21150AST

## + Related Information

Visit [sigma-aldrich.com/chiral](http://sigma-aldrich.com/chiral) for a complete listing of all products for chiral chromatography and chemistry.

# EN 14103 FAMEs in B100 Biodiesel on the Omegawax

Katherine K. Stenerson and Michael D. Buchanan  
mike.buchanan@sial.com

## Introduction

Biodiesel is a renewable, alternative diesel fuel produced from vegetable oils, animal fats, or recycled restaurant grease. This non-toxic, biodegradable liquid fuel consists of mono-alkyl esters of long chain fatty acids (also known as fatty acid methyl esters, or FAMEs) and may be used alone or blended with petroleum-based diesel fuels. The most common process for producing biodiesel involves two steps:

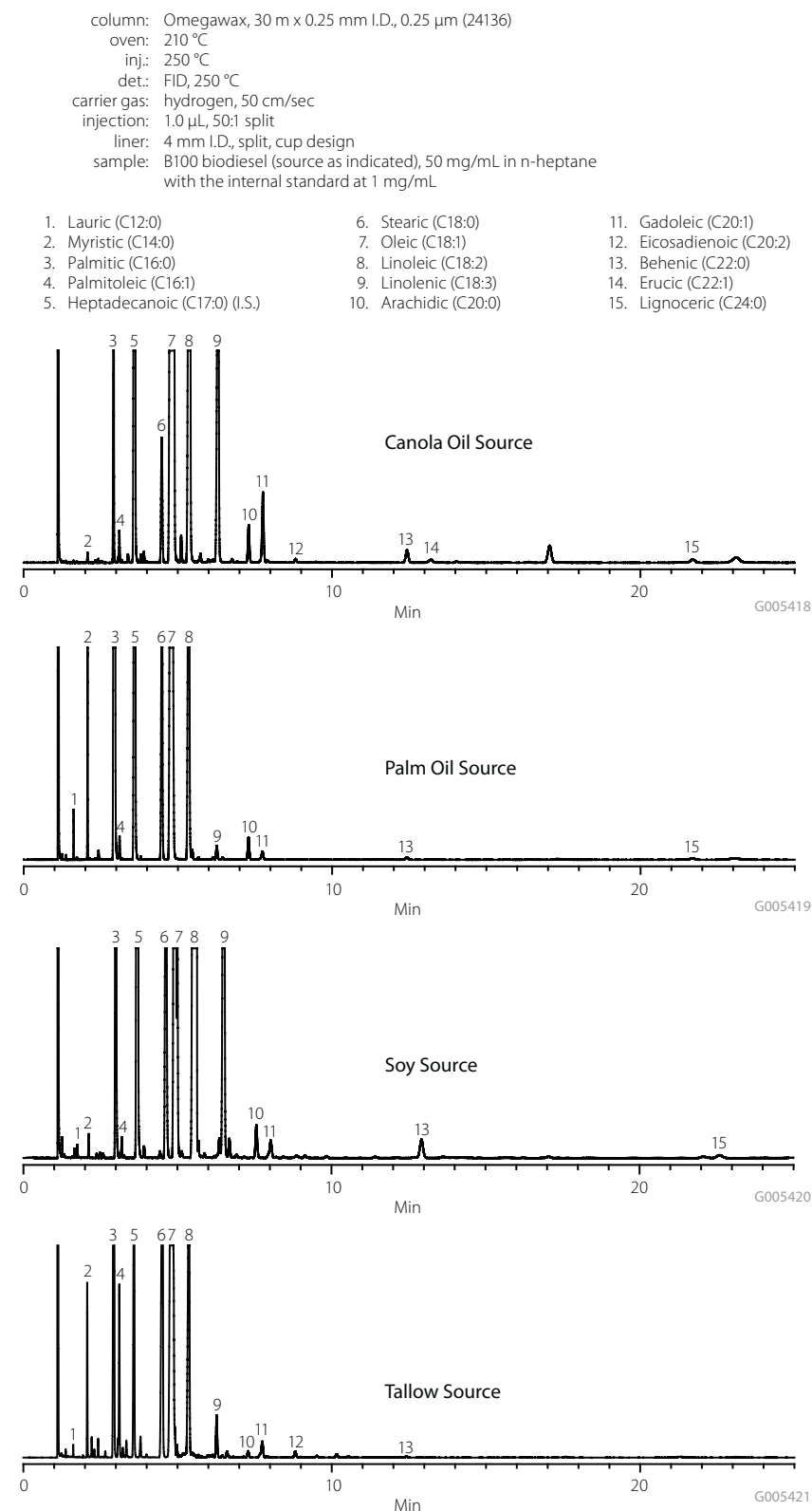
- Through the transesterification reaction, triglycerides (i.e. oils or fats) are chemically reacted with an alcohol, usually methanol, in the presence of a catalyst, like sodium or potassium hydroxide, yielding fatty acid methyl esters (FAMEs) and glycerin by-product.
- The FAMEs and glycerin by-product are then separated and purified. Biodiesel is the name given to the FAME fraction retained for use as fuel. The glycerin fraction is sold for use in soaps and other products.

The resulting biodiesel contains little-to-no sulfur or fossil fuel aromatics. Biodiesel is almost 10% oxygen, making it an oxygenated fuel, which aids combustion in fuel-rich circumstances. Biodiesel can be used pure (B100 biodiesel = 100% biodiesel) or blended (for example, B20 biodiesel = 20% biodiesel and 80% petroleum diesel).

## FAME Profile as a Measure of Purity

Biodiesel is produced around the world using a variety of biomass starting materials, including oils from canola, palm, soy, and tallow. The biomass starting material used by a given manufacturing facility depends on how plentiful it is in their region of the world. As an indicator of the amount of useable fuel in the final B100 biodiesel product, many bulk biodiesel producers measure the FAME profile. DIN Method EN 14103 specifies a gas chromatography (GC) procedure for determining the FAME profile in B100 biodiesel samples. (1)

Figure 1. B100 Biodiesel from Various Sources on the Omegawax





## GC Analysis

Four B100 biodiesel samples, manufactured from various biomass starting materials, were each diluted to 50 mg/mL in n-heptane. Additionally, heptadecanoic acid methyl ester was added to a level of 1 mg/mL in each for use as an internal standard. Each mix was then analyzed on an Omegawax™ capillary GC column. The resulting chromatograms are shown in **Figure 1** on the same time scale for ease of comparing patterns.

The “soy source” mix was analyzed five months prior to the analyses of the other mixes. The slightly longer retention times for this run can be attributed to small differences in the carrier gas linear velocity and/or the fact the column was slightly longer (it was subsequently trimmed several times prior to the later analyses). FAME peak identifications were done by comparing retention times to Characterized Reference Oils as well as AOCS Animal and Vegetable Reference Mixes that were also analyzed on the Omegawax column under identical conditions during each period of analysis.

## Results and Discussions

As expected, the starting biomass material used influences the FAME profile of the final product. Pattern recognition (a useful QA/QC tool) can be performed, as each mix exhibits its own unique FAME profile. The Omegawax column separates unsaturated FAMEs primarily by degree of unsaturation, with no separation by the cis/trans orientation of the double bonds. This results in a fairly clean chromatogram (not cluttered with numerous cis/trans peaks), making the Omegawax column ideal for this application.

## Conclusion

The Omegawax column was originally designed for, and is specifically tested for, the analysis of omega 3 and omega 6 fatty acids (as methyl esters). As shown here, it can also be used for determining the FAME profile of B100 biodiesel samples.

## References

1. DIN EN 14103, “Fat and Oil Derivates - Fatty Acid Methyl esters (FAME) - Determination of Ester and Linolenic Acid Methyl Ester Contents”
2. AOCS Method Ce 1-62, “Fatty Acid Composition by Gas Chromatography” AOCS Official Methods (2005) American Oil Chemists Society.

### + Featured Product

Description	Cat. No.
Omegawax, 30 m x 0.25 mm I.D., 0.25 µm	24136

### + Related Information

Additional information about this, or other analytical methodologies used for biofuel (biodiesel or bioethanol) can be found at [sigma-aldrich.com/biofuels](http://sigma-aldrich.com/biofuels)

### + Related Products

Description	Cat. No.
Omegawax, 15 m x 0.10 mm I.D., 0.10 µm	23399-U
Omegawax, 30 m x 0.32 mm I.D., 0.25 µm	24152
Omegawax, 30 m x 0.53 mm I.D., 0.50 µm	25374

### Characterized Reference Oils

Characterized Reference Oils are useful to assist in identifying biomass starting materials, and to identify if pure or blended materials were used. Each standard is packaged in an amber ampul under nitrogen. A Certificate of Composition is provided with each.

Description	Qty.	Cat. No.
Canola oil	1 g	46961
Coconut oil	1 g	46949
Corn oil	1 g	47112-U
Cottonseed oil	1 g	47113
Lard oil	1 g	47115-U
Linseed (Flaxseed) oil	1 g	47559-U
Menhaden fish oil	1 g	47116
Olive oil	1 g	47118
Palm oil	1 g	46962
Peanut oil	1 g	47119
Safflower oil	1 g	47120-U
Soybean oil	1 g	47122
Sunflower oil	1 g	47123

### AOCS animal and vegetable reference mixes

AOCS animal and vegetable reference mixes are also available. Each quantitative mix is similar to the fatty acid distribution of specific oils and conforms to the requirements of AOCS Method Ce 1-62. (2) A lot-specific Certificate of Analysis is supplied with each mix. Compositional specifications for each mix is available at [sigma-aldrich.com/fame](http://sigma-aldrich.com/fame).

Description	Qty.	Cat. No.
AOCS No. 1 Corn, cottonseed, kapok, poppyseed, rice, safflower, soybean, sunflower, and walnut	100 mg	O7006-1AMP
AOCS No. 2 Hempseed, linseed, perilla, and rubberseed	100 mg	O7131-1AMP
AOCS No. 3 Mustard seed, peanut, and rapeseed	100 mg	O7256-1AMP
AOCS No. 4 Neatsfoot, olive, and teaseed	100 mg	O7381-1AMP
AOCS No. 5 Babassu, coconut oil, ouri-curi, and plam kennel	100 mg	O7506-1AMP
AOCS No. 6 Lard, beef tallow, mutton tallow and palm	100 mg	O7631-1AMP
AOCS for Low Erucic Rapeseed Oil	100 mg	O7756-1AMP

# FAMEs in B20 Biodiesel on the SLB-IL111

Katherine K. Stenerson and Michael D. Buchanan  
mike.buchanan@sial.com



## B100 Biodiesel vs. B20 Biodiesel

In its undiluted, just-manufactured state, biodiesel is known as B100 biodiesel. Following several tests to determine purity and the absence of impurities, this material is typically blended with petroleum-based diesel prior to consumer use. A common blend is B20 biodiesel, which contains 20% biomass-based diesel and 80% petroleum-based diesel.

One test performed on biodiesel is to measure its FAME profile as a measure of purity. As shown in the previous article, an Omegawax column is suitable for performing this analysis with B100 biodiesel samples. Is it possible to also perform this test with a B20 biodiesel sample, maybe as a stability check? Will the petroleum-based biodiesel component interfere with the analysis?

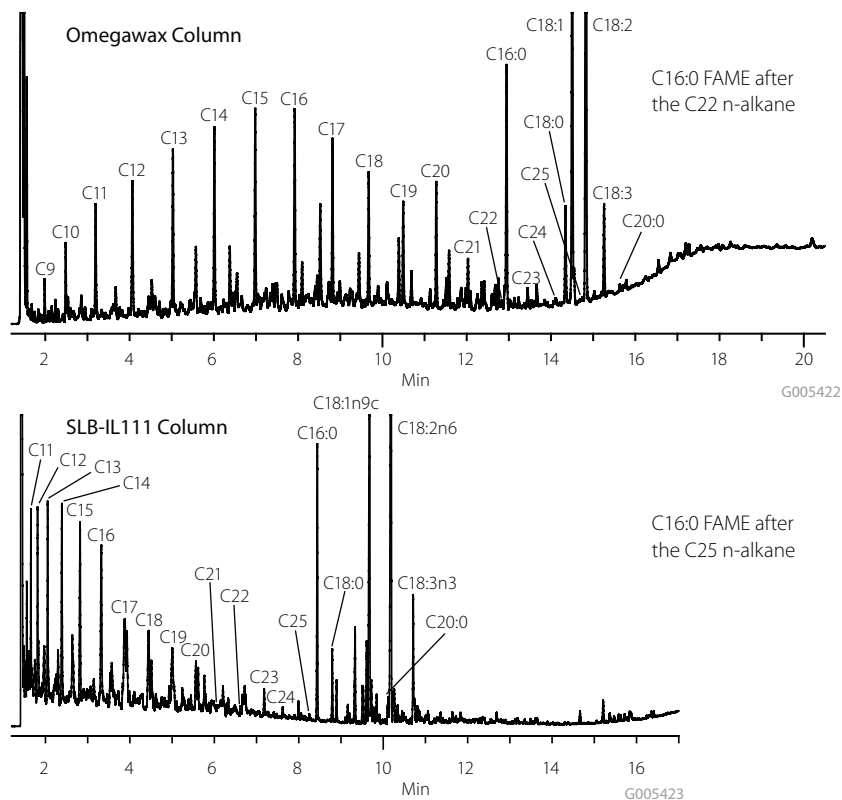
## GC Analysis, Results, and Discussion

A B20 biodiesel sample was made by mixing biomass-based diesel (soy source) and petroleum-based diesel to a 20:80 ratio. This mixture was diluted 1:20 with hexane prior to analyses on Omegawax and SLB-IL111 columns. Resulting chromatograms are shown in **Figure 1**, displayed using the same time scale for ease of comparison.

The Omegawax uses a polar phase, poly(ethylene glycol), as the stationary phase. This column was originally designed for, and is specifically tested for, the analysis of omega 3 and omega 6 fatty acids (as methyl esters). As evident, there is some overlap of the n-alkane and FAME fractions. Specifically, the C16:0

**Figure 1. B20 Biodiesel n-Alkanes and FAMEs**

columns: Omegawax, 30 m x 0.25 mm I.D., 0.25  $\mu$ m (24136)  
SLB-IL111, 30 m x 0.25 mm I.D., 0.20  $\mu$ m (28927-U)  
oven: 50  $^{\circ}$ C, 13  $^{\circ}$ C/min. to 270  $^{\circ}$ C (5 min.)  
inj.: 250  $^{\circ}$ C  
det.: FID, 270  $^{\circ}$ C  
carrier gas: helium, 40 cm/sec  
injection: 1  $\mu$ L, 100:1 split  
liner: 4 mm I.D. FocusLiner inlet liner, no taper  
sample: B20 biodiesel (soy source) diluted 1:20 in n-hexane



FAME (the first major FAME in the sample) elutes after the C22 n-alkane. Modifications to these analysis conditions did not result in a decrease in the overlap of the n-alkane and FAME fractions.

The SLB-IL111 uses an extremely polar ionic liquid as the stationary phase. Due to its higher polarity, it was predicted that this column would exhibit less overlap of the n-alkane and FAME fractions, compared to the Omegawax. Analysis confirmed this. Specifically, the C16:0 FAME elutes after the C25 n-alkane (the last significant n-alkane in the sample). These chromatograms also demonstrate the phenomenon that analytes will elute at lower temperatures on a more polar phase than on a less polar phase. The benefit is that the SLB-IL111 not only has the selectivity necessary to separate these n-alkanes and FAMEs by class, it also does it quicker (11 minutes compared to 16 minutes).

## Conclusion

Bulk biodiesel producers regularly determine the FAME profile of their final product as a measure of purity. As B100 biodiesel, the Omegawax column is well-suited to perform this application. However, once blended, the SLB-IL111 is better able to perform this analysis. It results in better class separation in addition to an overall shorter analysis.



**+** Featured Products

Description	Cat. No.
SLB-IL111, 30 m x 0.25 mm I.D., 0.20 µm	28927-U
Omegawax, 30 m x 0.25 mm I.D., 0.25 µm	24136

**+** Related Products

Description	Cat. No.
SLB-IL111, 15 m x 0.10 mm I.D., 0.08 µm	28925-U
SLB-IL111, 100 m x 0.25 mm I.D., 0.20 µm	29647-U

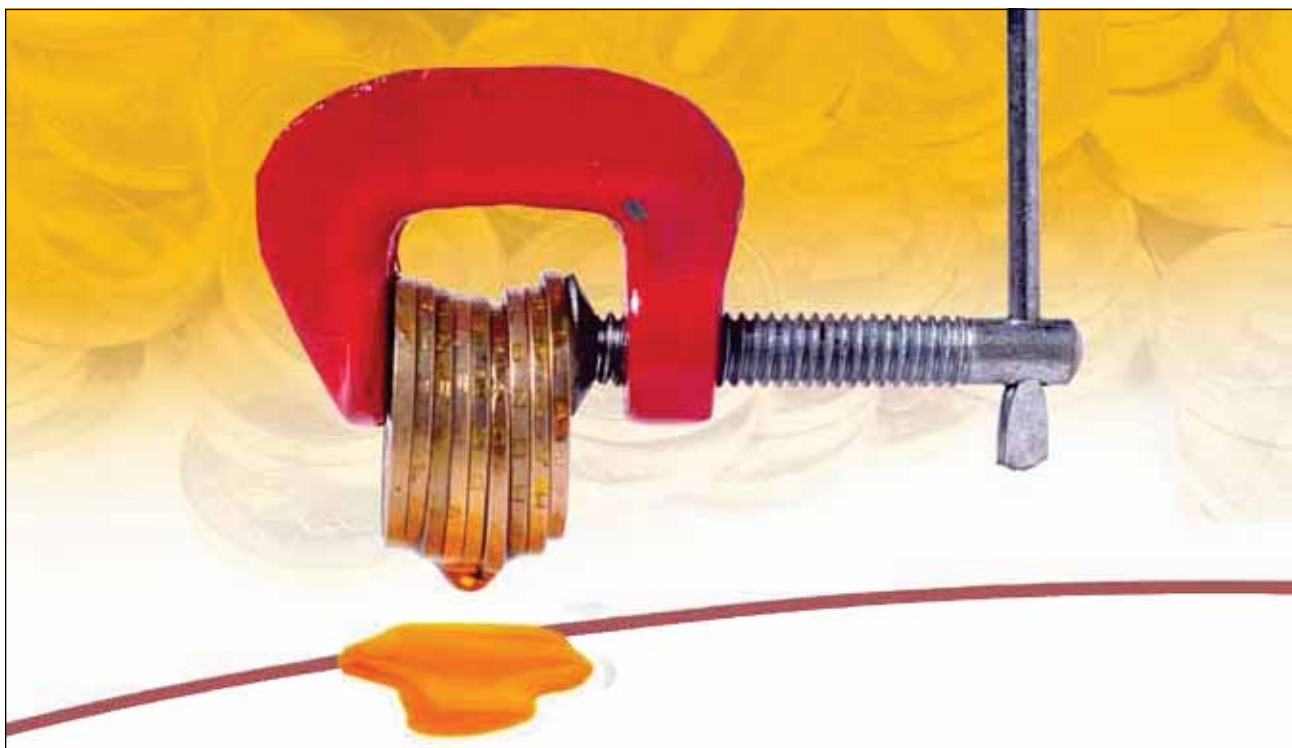
Introductory Offer:

**40% off SLB-IL111 Columns**

Use **Promo Code 962** when placing your order. Offer expires December 31, 2011. Offer not valid in Argentina, Brazil, China, India, and Japan.

**+** Related Information

Additional information regarding the SLB-IL111, or any of our ionic liquid GC columns, can be found at our website: [sigma-aldrich.com/il-gc](http://sigma-aldrich.com/il-gc)



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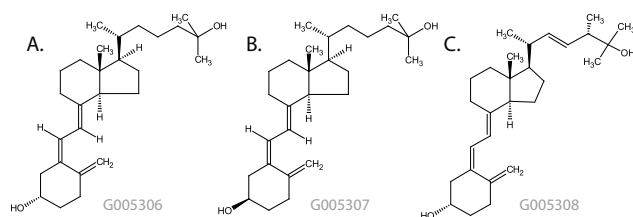
# Highly Selective Separation of Vitamin D epi-Metabolites Using HybridSPE-Phospholipid

Craig Aurand, David Bell, and Anders Fridstrom  
emily.barrey@sial.com

## Introduction

Vitamin D deficiency has become a topic of interest in recent publications (1-3). Vitamin D, along with calcium, promotes proper bone growth in children and aids in the prevention of osteoporosis in older adults. Vitamin D is present in two forms, Vitamin D3 and Vitamin D2. Both D2 and D3 vitamins are metabolized in the liver to form 25-hydroxyvitamin D2 (25-OH D2) and 25-hydroxyvitamin D3 (25-OH D3), respectively. In addition, biologically inactive 3-epi analogs of 25-OH D2 and 25-OH D3 have been reported, especially in young children (3). Recent studies have indicated that separation from the inactive 3-epi analogs may provide more accurate information for treatment and prevention. Analytical methods that can accurately quantitate both of the 25-hydroxyvitamin D analytes in the presence of 3-epi analogs may become essential for diagnosis and monitoring of patients with vitamin D disorders. The structures of the vitamin D analytes are shown in **Figure 1**.

**Figure 1. Vitamin D Metabolite Structures**



**A. 25-Hydroxyvitamin D3**

Monoisotopic Mass = 400.334131 Da; Molecular Formula =  $C_{27}H_{44}O_2$

**B. 3-epi-25-Hydroxyvitamin D3**

Monoisotopic Mass = 400.334131 Da; Molecular Formula =  $C_{27}H_{44}O_2$

**C. 25-Hydroxyvitamin D2**

Monoisotopic Mass = 412.334131 Da; Molecular Formula =  $C_{28}H_{44}O_2$

HPLC analysis of 25-OH D2 and 25-OH D3 is traditionally performed using C18 stationary phases. Under such conditions, the 3-epi analogs are poorly resolved, and thus are included in the overall reported value. Recently, Phinney, et al., reported the use of a cyano column for the effective separation of the 25-OH and the 3-epi forms for use in reference measurement procedures (1). Although effective, the conditions necessitate a run time greater than 40 minutes limiting its utility for routine high throughput analyses. LC-MS analysis methodologies for 25-hydroxyvitamin D3 are not without disadvantages as well. Sample preparation from serum requires protein precipitation with organic solvents or strong acids. This technique results in gross depletion levels of proteins from the sample, but also results in high levels of matrix interference from the still present phospholipids. LC-MS methods using standard protein precipitation are susceptible to ion

suppression caused by the PLs or require gradient elution to wash this co-extracted matrix from the column resulting in longer cycle times for sample analysis.

## Discussion

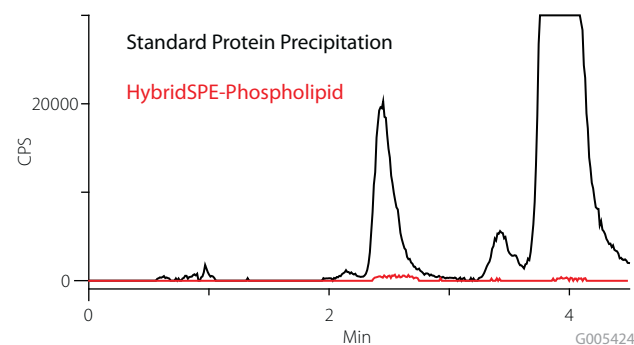
This study continues to detail new methodology for the analysis of Vitamin D metabolites which was introduced in a previous Reporter (4). In this study, the HybridSPE-Phospholipid was utilized to selectively extract the phospholipids from the serum sample. This technique combines the simplicity of standard protein precipitation with the added benefit of additional matrix removal. The combination of this novel sample preparation technique along with the unique selectivity of the Ascentis Express F5 HPLC column provides a fast and simplified bioanalytical method for the associated Vitamin D metabolites.

Rat serum purchased from Lampire Biological Laboratories (Pipersville PA) was spiked at 300 ng/mL with 25-hydroxyvitamin D2, 25-hydroxyvitamin D3 and epi-25-hydroxyvitamin D3. An internal standard was not included as part of this method. Protein precipitation was performed offline by adding 100  $\mu$ L of spiked serum into a 500  $\mu$ L 96-well collection plate followed by 300  $\mu$ L of 1% formic acid acetonitrile. Samples were mixed by performing five 300  $\mu$ L draw/aspiration cycles using a digital pipetter, then set for 5 minutes before transferring 200  $\mu$ L of precipitate into the HybridSPE-Phospholipid 96-well plate. Samples were passed through the HybridSPE-Phospholipid plate by applying 10" Hg vacuum for 4 minutes, the resulting filtrate was analyzed directly.

As a comparison, spiked rat serum was also processed using standard protein precipitation by adding 100  $\mu$ L of serum into 2 mL centrifuge vials followed by 300  $\mu$ L of 1% formic acid acetonitrile. Samples were vortexed and centrifuged, and the resulting supernatant was collected and analyzed directly.

**Figure 2** depicts the phospholipid monitoring chromatograms of the resulting co-extracted matrix from standard protein precipitation and from using the HybridSPE-Phospholipid technique. The HybridSPE-Phospholipid selectively depleted the

**Figure 2. Comparison of Phospholipid Monitoring on Standard Protein Precipitation and HybridSPE-Phospholipid**





phospholipid matrix resulting in no matrix interference from this source. The standard protein precipitation technique shows a large amount of co-extracted phospholipids resulting in interferences that co-elute in the retention range of 25-hydroxyvitamin D2, 25-hydroxyvitamin D3 and epi-25-hydroxyvitamin D3. This co-elution has the potential to cause sensitivity loss and reproducibility issues resulting in irregularities in quantitation.

**Figures 3a and 3b** demonstrate the selectivity of the Ascentis Express F5 phase enabling isocratic resolution of 25-OH D3, 25-OH D2 and epi-25-OH D3 in less than four minutes, enabling and allowing for quantitation of all three components in one chromatographic analysis. **Table 1** details a comparison of the average analyte response after HybridSPE-Phospholipid sample preparation for the three Vitamin D metabolites versus protein precipitation. The responses for the metabolites were 10-70% greater using HybridSPE-Phospholipid and a significant improvement was observed with the analysis of 25-OH D2 in which more reproducible results were achieved. By removing the interfering phospholipid matrix, enhanced sensitivity and precision were demonstrated for the three Vitamin D metabolites.

### Summary

Separation of the biologically inactive 3-epi analog serves to provide improved data in support of vitamin D related clinical diagnostics and treatment. The pentafluorophenyl stationary phase has been shown to provide superior selectivity for the separation of the closely related 25-OH D3 and 3-epi-25-OH D3 as compared to methods reported in the literature. The unique selectivity of the Ascentis Express F5 combined with the selective phospholipid depletion of the HybridSPE-Phospholipid 96-well plate enable a fast and efficient analysis of 25-hydroxyvitamin D and related forms, that would otherwise be unattainable with traditional sample prep and reversed-phase HPLC approaches.

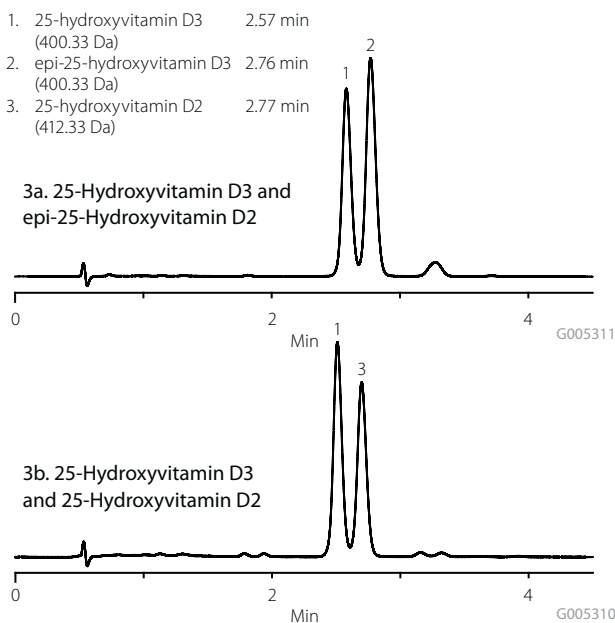
### References

1. Tai, S. S.-C.; Bedner, M.; Phinney, K. W. *Analytical Chemistry* 2010, 82, 1942-1948.
2. Higashi, T.; Homma, S.; Iwata, H.; Shimada, K. *Journal of Pharmaceutical and Biomedical Analysis* 2002, 29, 947-955.
3. Higashi, T.; Shimada, K.; Toyo'oka, T. *Journal of Chromatography B* 2010, 878, 1654-1661.
4. Aurand, Craig R., Bell, David S., *Reporter* 29.2, 3-4.

**Figure 3. Analysis of 25-Hydroxyvitamin D Using Ascentis Express F5**

column: Ascentis Express F5, 10 cm x 2.1 mm I.D., 2.7 μm (53569-U)  
 mobile phase A: 5 mM ammonium formate:water  
 mobile phase B: 5 mM ammonium formate:methanol  
 mobile phase mixing ratio: A:B = 25:75  
 flow: 0.4 mL/min.  
 temp.: 40 °C  
 injection: 1 μL  
 ms detection: 100-1000 m/z

1. 25-hydroxyvitamin D3 (400.33 Da) 2.57 min
2. epi-25-hydroxyvitamin D3 (400.33 Da) 2.76 min
3. 25-hydroxyvitamin D2 (412.33 Da) 2.77 min



### + Featured Products

Description	Qty.	Cat. No.
96-well Plate, 50 mg/well	1	575656-U
Ascentis Express F5, 10 cm x 2.1 mm I.D., 2.7 μm	1	53569-U

### + Related Products

Description	Qty.	Cat. No.
96-well Plate, 15 mg/well	1	52794-U
<b>SPE Tubes</b>		
30 mg/1 mL	100	55261-U
500 mg/6 mL	30	55267-U
<b>SPE Tube for In-tube Precipitation</b>		
HybridSPE Ultra 30 mg/1 mL	100	55269-U

**Table 1. Average Vitamin D Peak Areas for Rat Plasma Spiked at 300 ng/mL**

	25-OH Vit D3 Results	epi-25-OH Vit D3 Results	25-OH Vit D2 Results
<b>HybridSPE-Phospholipid</b>			
Average Area (cps)	12729	19875	4466
Standard Deviation	1045	1146	542
Relative Standard Deviation (%)	8.2	5.8	12.1
<b>Protein Precipitation</b>			
Average Area (cps)	9583	16965	2638
Standard Deviation	1160	1345	3130
Relative Standard Deviation (%)	12.1	7.9	118.6

# Aldehydes and Ketones in Indoor Air using a Low Background LpDNPH Solvent Desorption Tube and Fused-Core HPLC

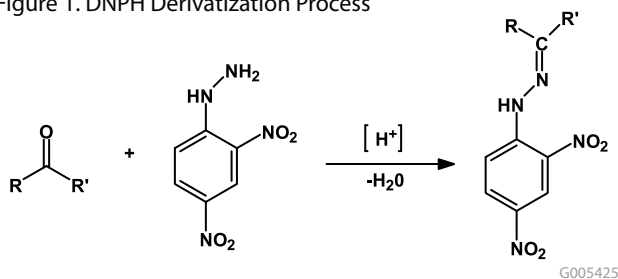
James Desorcie and Kristen Schultz  
kristen.schultz@sial.com

## Introduction

Aldehydes are well-known and routinely measured pollutants due to their toxic properties and their role as ozone precursors. Human exposure to aldehydes in both commercial and residential environments continues to be a hot topic of interest for regulatory agencies such as the OSHA, NIOSH, EPA, and ASTM. Materials testing have also become an important factor for Green Building Council Initiatives (GBCI) and Leadership in Energy and Environmental Design (LEED) Programs whose goal is to create a system to promote sustainable building practices. The demand for low aldehyde background and detection limits is important for successful sampling protocols in both indoor and ambient environments.

A widely accepted method for capturing airborne aldehydes and ketones is via their reaction with 2,4-dinitro-phenylhydrazine (DNPH) coated on a silica support. The reaction produces non-volatile, hydrazone derivatives as depicted in **Figure 1**. These products are readily measured using HPLC analysis.

Figure 1. DNPH Derivatization Process



## Experimental

Indoor laboratory air was sampled using an LpDNPH Cartridge (21014) with a DNPH loading of 1 mg and a PAS-500 Micro Air Sampling Pump (24865) powered by a 9-volt replaceable battery. Air was sampled at a rate of 200 mL/min for 20 hours. Following sampling, the cartridge was gravity eluted with acetonitrile (3 mL). HPLC analysis was used to measure the amounts of hydrazone derivatives in the eluent solution.

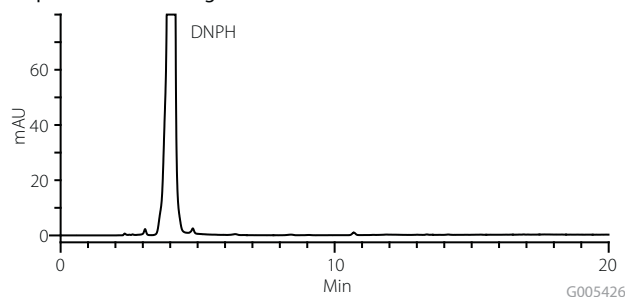
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Sigma-Aldrich/Supelco has the widest selection of air sampling media available for sampling aldehydes – 12 different products in all, suitable for OSHA, NIOSH, EPA and ASTM methods. For more information, go to [sigma-aldrich.com/air\\_monitoring](http://sigma-aldrich.com/air_monitoring)

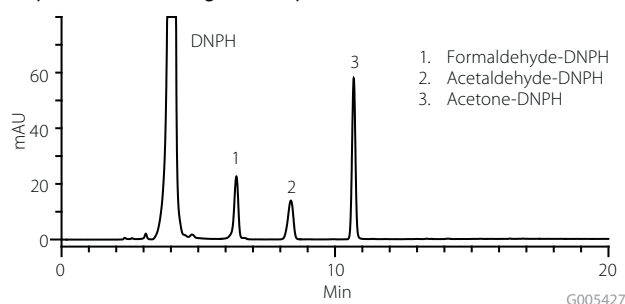
Figure 2. Acetonitrile Eluent Solutions

column:	Ascentis Express C18, 15 cm x 4.6 mm I.D., 2.7 μm (53829-U)		
mobile phase A:	water		
mobile phase B:	acetonitrile		
gradient:	Time (min)	%A	%B
	0	45	55
	4	45	55
	13	10	90
	20	10	90
flow rate:	0.5 mL/min		
temp.:	ambient		
det.:	UV at 360 nm		
injection:	10 μL		

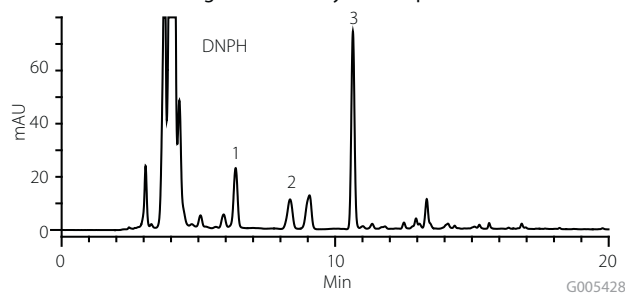
LpDNPH S10 Cartridge Blank



LpDNPH S10 Cartridge Blank Spiked with DNPH Standards



LpDNPH S10 Cartridge – Laboratory Air Sample





## Results and Discussion

Chromatograms from the elution of a blank LpDNPH cartridge, the elution blank spiked with DNPH derivative standards, and elution of an LpDNPH cartridge used to sample laboratory air are shown in **Figure 2**. The low background of the LpDNPH S10 cartridge (clean blank extract) permits measurement of airborne aldehydes and ketones at low concentrations. The standards are used to identify and quantify the DNPH derivatives in the sample chromatogram. The measured airborne concentrations were formaldehyde: 3.8 µg/m<sup>3</sup> (3.1 ppb), acetaldehyde: 3.2 µg/m<sup>3</sup> (1.8 ppb) and acetone: 16.8 µg/m<sup>3</sup> (7.1 ppb).

The Ascentis Express C18 HPLC column utilizes innovative Fused-Core particle technology. Compared to conventional HPLC columns with 5 µm particle silica, the Ascentis Express shows much higher column efficiency, allowing the use of shorter columns and still achieve similar or better separations. To maintain similar DNPH derivative retention times, lower flow rates than traditional methods can be applied (0.5 mL/min flow rate instead of the 1.2 mL/min) resulting in significant mobile phase savings. Because the column backpressure is similar to that achieved with 5 µm silica columns at higher flow rates (approx. 1,500 psi), the

Ascentis Express column can be readily used with conventional HPLC instrumentation. It should be noted that another method modification is the need to reduce the injection volume from 20 to 10 µL in order to avoid column overload. However, there is no sacrifice in method sensitivity due to the inherent higher chromatographic efficiency of the Fused-Core particles.

## Summary

Low background LpDNPH S10 air sampling cartridges are ideally suited for measuring low concentrations of airborne aldehydes and ketones. The Ascentis Express C18 column maintains the quality characteristics of the HPLC analysis while providing significant cost and environmental savings through the reduction of mobile phase consumption.

### + Featured Products

Description	Qty.	Cat. No.
LpDNPH S10, 3 mL/350 mg	50	21014
Ascentis Express C18, 15 cm x 4.6 mm I.D., 2.7 µm	1	53829-U
PAS-500 Micro Air Sampling Pump	1	24865



[sigma-aldrich.com/air\\_monitoring](http://sigma-aldrich.com/air_monitoring)

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P001383

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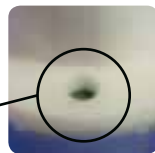
E001172

Valve is opened and closed by turning only the cap a ½ turn and not the valve body. The ideal choice if your application requires replacing septa.

### Push Lock Valve (PLV)



E001171



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# Derivatization and Improved Detection of Estradiol with ESI-MS

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Steroid hormones are derivatives of cholesterol and play an important role in a large variety of organisms, as they can have a direct control on the gene expression. 17 $\beta$ -Estradiol (E2) controls the growth and the function of female secondary sexual characteristics. High blood concentrations inhibit the formation of further regulatory factors responsible for ovulation and pregnancy. E2 and its derivatives, e.g. ethinyl estradiol, are part of combined contraceptive pharmaceuticals, which have become widespread and common in use, thus leading so far to an unconsidered environmental problem: increased concentrations of estradiol and its metabolites in waste water (1-2). Now both, clinical and environmental laboratories have a vital interest in finding the most sensitive method for analysis of E2 and other steroid hormones mostly in matrices, which are difficult to remove. E2 is a nonpolar compound and hard to detect by ESI.

Fortunately, the analyte can be extracted very efficiently with solvents like methylene chloride or acetone. Additionally, this procedure reduces negative effects of the matrix, e.g. signal suppression by alkali salts. But only the introduction of ionizable moieties by derivatization can enhance the detection limits significantly. Dansyl chloride is the most common agent and reacts selectively and quantitatively with E2, testosterone and their derivatives (3-4). The detection is limited to APCI and APPI sources, which have some disadvantages regarding availability, dopant usage and lower sensitivity of the APCI source (Figure 1). Only a short pre-column (Ascentis Express C18, 5 cm x 2.1 mm, 2.7  $\mu$ m) is necessary to separate the analyte from excessive reagent and byproducts (BPC, magenta). The MS/MS spectra results in a large number of fragments and a lower sensitivity on the quantifier.

Figure 1. Separation and detection of 55 pg E2 as dansyl derivative (EIC, peak 1). The inset shows the MS/MS spectrum of [M+H]<sup>+</sup>=506.235 Da (APCI+)

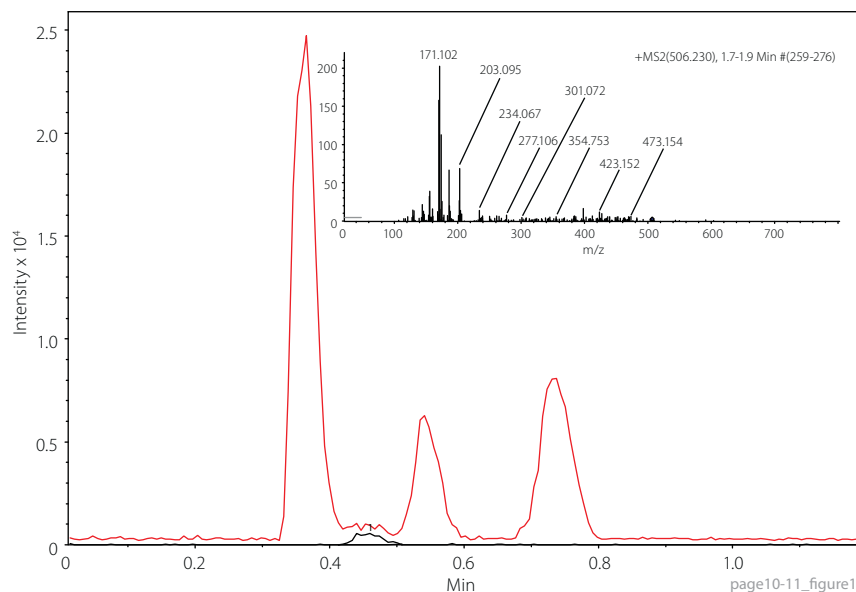
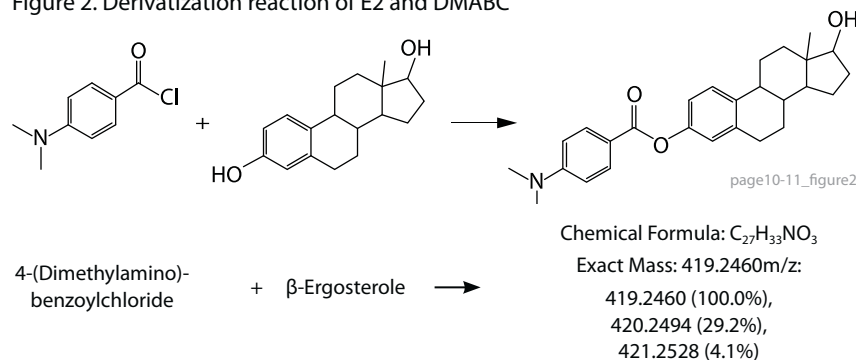


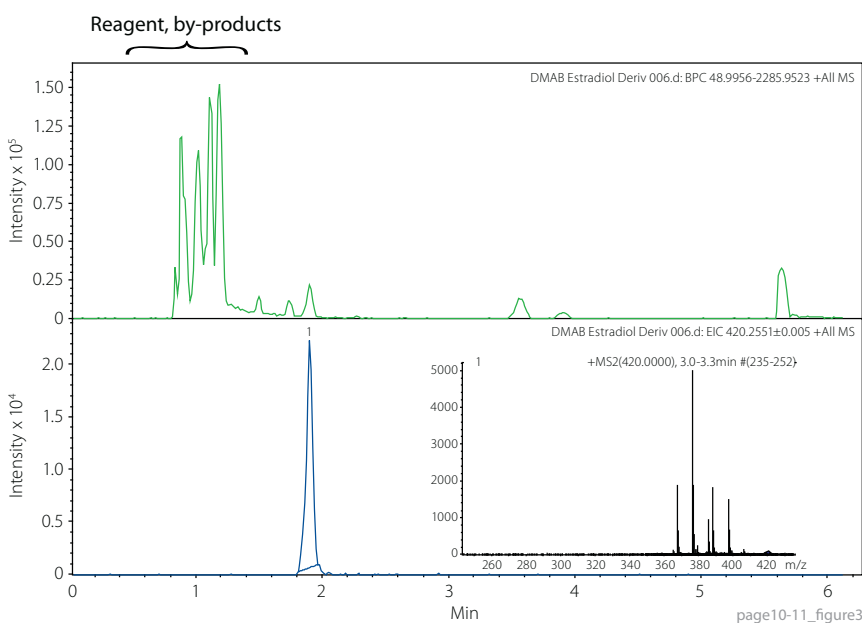
Figure 2. Derivatization reaction of E2 and DMABC



A more sensitive and versatile derivatization agent for ESI sources is 4-(Dimethylamino)benzoyl chloride (DMABC Cat. No. 67954-1G). The reagent can be dissolved in acetone and applied on the dried residue of the sample extract. An adjustment of the pH is not necessary, only an anhydrous reaction medium is needed. At a high E2 level of 5 ppm only 0.2 % (rel. area fraction) of DAMBC react with the 2nd hydroxyl moiety (2:1 adduct). At 5 ppb E2 concentration the 2:1 adduct is below the detection limit. The high purity of DMABC guarantees a good solubility, very selective and quantitative reaction at a moderate temperature between 55-60  $^{\circ}$ C (5 min). The reagent and possible byproducts can be separated from the analytes by a standard reversed-phase HPLC column and detected down to very low concentrations (Figure 3). The MS/MS

**Figure 3. Injection of 5 pg DMAB-E2 derivative and separation on a UHPLC system**

column: Ascentis Express C18, 5 cm x 2.1 mm I.D., 2.7  $\mu$ m (53822-U)  
 mobile phase: water:formic acid:acetonitrile, 30:0.01:70  
 flow rate: 0.4 mL/min.



spectrum (inset) shows only 4 major peaks, which is ideal for the quantification and identification using triple quadrupole mass spectrometer.

## References

1. Jason W. Birkett, John Norman Lester (eds.), Endocrine disruptors in wastewater and sludge treatment processes, CRC Press, 2003.
2. M. Metzler (ed.), The handbook of environmental chemistry: Endocrine Disruptors, Vol. Part 1, Springer Verlag, Heidelberg, 2001.
3. R. E. Nelson, S. K. Grebe, D. J. O'Kane, R. J. Singh, Clinical Chemistry 50: 373-384, 2004.
4. F. Zhang et al, Rapid Commun. Mass Spectrom. 2009; 23: 3637-3646.



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# New *Withania somnifera* Analytical Standards and an Improved HPLC Method

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Fresh Plant



Dried Root

The dried roots of *Withania somnifera* are the source for Ashwagandha, one of the most popular remedies in traditional Indian medicine (Ayurveda). This traditional Indian herbal medicine is becoming increasingly popular in the western world as reflected by the existence of a United States Pharmacopeia (USP) monograph for powdered Ashwagandha roots (1) and a British Pharmacopeia (BP) monograph for *W. somnifera* root for use in Traditional Herbal Medicinal Products (THMP) (2).

The traditionally benign health effects are very diverse and include aphrodisiac, sedative, rejuvenative and life-prolonging properties (2). Beneficial effects are also supported by recent bioactivity studies: Withaferin A has been shown to have significant anticancer activity in animals (3).

Sigma-Aldrich recently added several standards of *W. somnifera* constituents to our herbal medicinal product portfolio for the characterization and quantification of Ashwagandha. An HPLC column was also identified for providing a fast and efficient separation of the key constituents.

## Withania Constituents

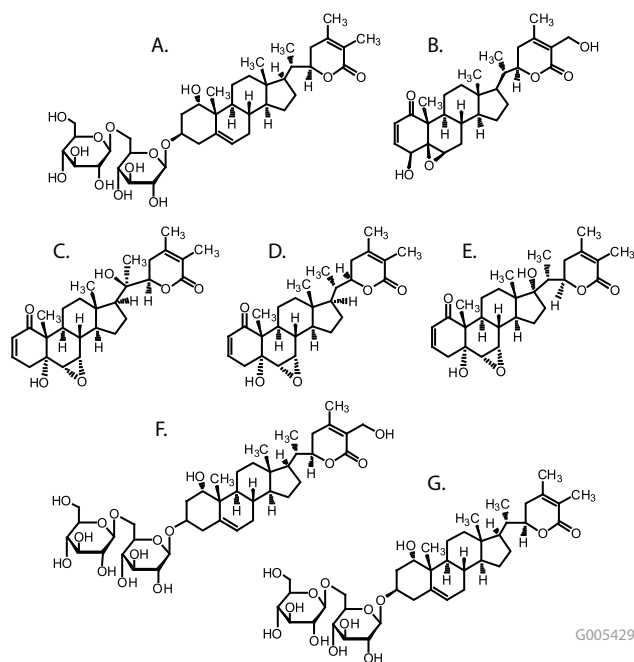
The characteristic constituents belong to a group of steroidal lactones consisting of a steroidal scaffold attached to a six membered lactone ring. The structures are shown in Figure 1.

## HPLC Analysis of Withania Constituents Using Ascentis Express Columns

As part of a large number of natural health product studies recently conducted, many of the main constituents of Withania were screened on several modern Fused-Core stationary phases. The results from the screening effort showed that the Ascentis Express F5 and the Ascentis Express Phenyl-Hexyl phases provided improved selectivity over the C18 stationary phase. Both phases, presumably due to their intrinsic rigidity, are known to provide enhanced shape selectivity. The shape selectivity component is often found useful for the separation of closely related compounds with rigid structures.

Figure 2 shows a comparison of Withania standard constituents separated using the USP method to an optimized separation using Ascentis Express Phenyl-Hexyl. The USP method calls for

Figure 1. Structures of Selected Withania Constituents



A. 12-Deoxywithastramonolide  
B. Withaferin A  
C. Withanolide A  
D. Withanolide B

E. Withanone  
F. Withanoside IV  
G. Withanoside V

G005429

a long, 40-minute gradient and the use of a 25 cm x 4.6 mm C18 column. Even with this lengthy system, only marginal separation of Withanolide A and Withanone is obtained. Conversely, a 15-minute gradient utilizing a shorter 10 cm x 2.1 mm phenyl-hexyl phase provides baseline separation of all components.

Figure 3 shows the separation of Ashwagandha extract constituents using both systems. The use of the phenyl hexyl column is again shown to provide improved resolution in a shorter period of time and with approximately 3X greater sensitivity. Note that only those components that could be confidently identified are noted. Similar results were obtained utilizing the Ascentis Express F5 stationary phase (data not shown).

## Conclusions

Analyses of complex matrices such as the assay of natural product components may be greatly facilitated through the availability and use of quality standards and modern analytical separation tools. In this case, a fast method with full resolution of 7 constituents from the Ashwagandha root was achieved on an Ascentis Express Phenyl-Hexyl Fused Core particle column.



Figure 2. Comparison of Withania Standard Separation Using a Standard C18 vs Optimized Ascentis Express Phenyl-Hexyl Method

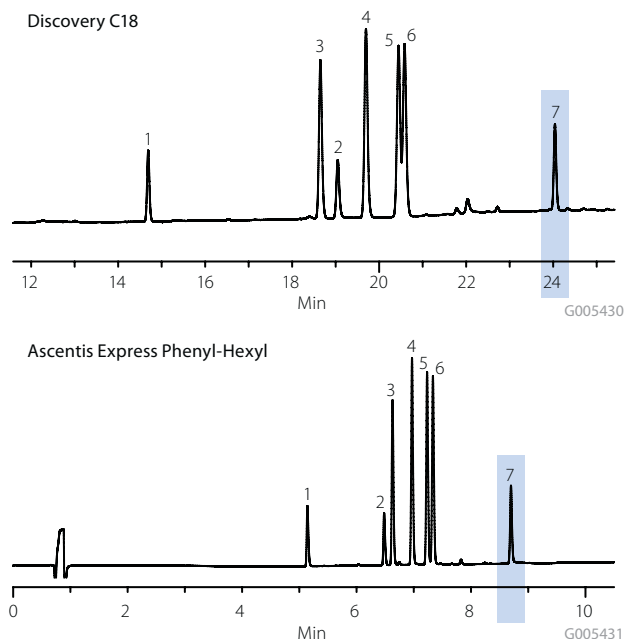
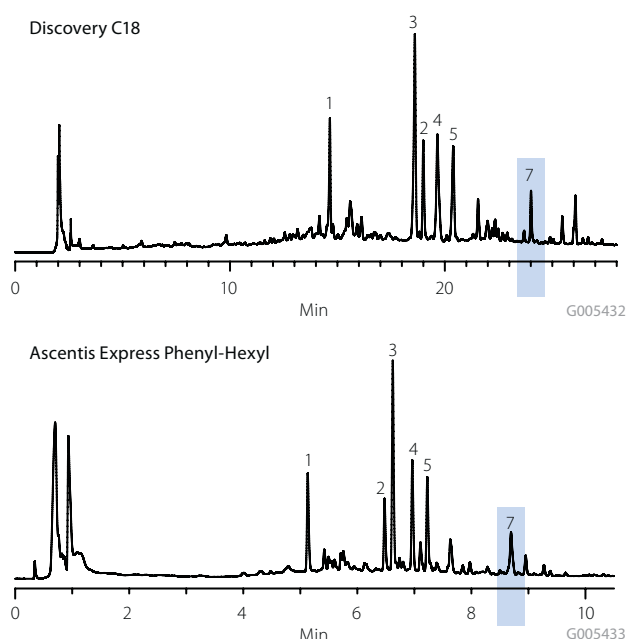


Figure 3. Comparison of Ashwagandha Extract Component Separation Using a Standard C18 vs Optimized Ascentis Express Phenyl-Hexyl



### Discovery C18 Conditions for Figures 2 and 3

column: Discovery C18, 25 cm x 4.6 mm I.D., 5 µm (504971)  
 mobile phase A: phosphate buffer\*  
 mobile phase B: acetonitrile  
 gradient: 

Min	%A	%B
0.0	95.0	5.0
18.0	55.0	45.0
25.0	20.0	80.0
28.0	20.0	80.0
30.0	95.0	5.0
40.0	95.0	5.0

  
 flow rate: 1.5 mL/min  
 temp.: 27 °C  
 det.: 227 nm  
 injection: 20 µL  
 samples: Standard: 20 µg/mL each in 80:20 water:methanol  
 Extract: As per USP (1)

\* Dissolve 0.14 g of potassium dihydrogen phosphate in 900 mL water, add 0.5 mL of phosphoric acid, dilute with water to 1000 mL, and mix.

### Ascentis Express Phenyl-Hexyl Conditions for Figures 2 and 3

column: Ascentis Express Phenyl-Hexyl, 10 cm x 2.1 mm I.D., 2.7 µm (53336-U)  
 mobile phase A: water  
 mobile phase B: acetonitrile  
 gradient: 

Min	%A	%B
0.0	80.0	20.0
10.0	0.0	100.0
10.5	0.0	100.0
11.0	20.0	80.0
15.0	20.0	80.0

  
 flow rate: 0.3 mL/min  
 temp.: 35 °C  
 det.: 227 nm  
 injection: 5 µL  
 samples: same as Discovery C18  
 1. Withanoside IV                      5. Withanolide A  
 2. Withanoside V                      6. Withanone  
 3. Withaferin A                         7. Withanolide B  
 4. Withastramonolide

### Acknowledgement

Photographs of *W. somnifera* were provided by Dr. Amit Agarwal, Director, Natural Remedies Pvt. Ltd., Bangalore, India.

### References

1. USP 34, Dietary Supplements, Ashwagandha 1079.
2. BP 2011, Withania somnifera roots for THMP, 3674
3. A. Agarwal; B. Murali; "Quality Assessment of Selected Indian Medicinal Plants"; Volume 1.
4. Singh, G.; Sharma, P. K.; Dudhe, R.; Singh, S; Annals of Biological Research, 1 (3); 56-63.

### + Featured Products

Description	Pkg. Size	Cat. No.
12-Deoxywithastramonolide	10 mg	94187
Withaferin A	10 mg	89910
Withanolide A	10 mg	74776
Withanolide B	10 mg	94284
Withanone	10 mg	90896
Withanoside IV	10 mg	94186
Withanoside V	10 mg	66042
Ascentis Express Phenyl-Hexyl Column 10 cm x 2.1 mm I.D., 2.7 µm	1	53336-U

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# Introduction of Novel Performance-Tested Solvents for UHPLC Applications

## New LC-MS Ultra CHROMASOLV® Grade Solvents and Additives

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Recent innovations in HPLC and mass spectrometry (MS), including Fused-Core® particles and ultra high performance/pressure liquid chromatography (UHPLC) systems, have pushed the limits of speed (throughput), efficiency and sensitivity. They have greatly increased the amount and quality of data obtained from HPLC and LC/MS experiments. While the technical “buzz” is usually around columns and instruments, the solvents used for mobile phases, sample preparation, and sample dissolution are also critical components of the system. Their influence on reducing background noise and baseline instability, extending column lifetime, and maintaining system integrity cannot be underestimated nor overlooked.

Solvent-derived impurities are one major issue facing chromatographers today. Background noise is typically caused by impurities that enter the system from the sample, by leaching from system components (bottles, tubes, valves, vials, etc.), and from solvents and additives. To reduce the latter contribution, Sigma-Aldrich developed and recently introduced Fluka®-brand LC-MS Ultra CHROMASOLV grade solvents and additives. During their development, several factors were taken into consideration when looking into UHPLC applications:

1. Impurities (types and concentrations)
2. Concentration of impurities under weak gradient conditions, with resulting elution later in the chromatogram or in subsequent runs
3. Baseline under gradient elution
4. Reduction of system down-time for cleaning

This new product line is tested specifically for UHPLC performance, including special designed gradient tests for UV and MS UHPLC separations with short columns, typically 5 cm to 10 cm in length, gradient applications. High flow rates under these condition types permit detection of as many impurities as possible in short run times and MS experiments are performed in both ESI(-) and ESI(+) modes. These detection limits differ considerably from standard HPLC gradient or LC-MS grade solvents, and pay tribute to the higher demands of UHPLC. In particular, the improvements in detection systems (UV, MS) reveal the smallest amounts of impurities. In order to obtain very low detection limits, reliable instrument performance and to lessen the amount of system maintenance, it is important to always use the highest quality solvent. The LC-MS Ultra CHROMASOLV solvents meet and exceed these criteria for UHPLC gradient separations in UV, as well as positive and negative ion mode MS detection

In conclusion, the advent of UHPLC and other high-efficiency HPLC and LC-MS techniques have set new standards for sensitivity, efficiency, and throughput. In order for the UHPLC system to provide this reliable data and high performance and eliminate system down-time, it is critical to use solvents that are as carefully developed, prepared and tested as other components of the system. The solvents must be tested *in situ* under demanding UHPLC conditions and under various detection modes. Fluka's new LC-MS Ultra CHROMASOLV grade solvents fulfill these requirements. For more information, visit us at [sigma-aldrich.com/lc-ms](http://sigma-aldrich.com/lc-ms).

### + Featured Products

Name	Description	Package Size	Cat. No.
Acetonitrile	LC-MS Ultra CHROMASOLV, ≥99.9%, gradient tested for UHPLC, UV & MS	1 L, 2 L	14261
Methanol	LC-MS Ultra CHROMASOLV, ≥99.9%, gradient tested for UHPLC, UV & MS	1 L, 2 L	14262
Water	LC-MS Ultra CHROMASOLV, gradient tested for UHPLC	1 L, 2 L	14263
Trifluoroacetic acid	LC-MS Ultra eluent additive, ≥ 99.0% suitable for UHPLC-MS	1 mL, 2 mL	14264
Formic acid LC-MS	Ultra eluent additive, ≥ 98% suitable for UHPLC-MS	1 mL, 2 mL	14265
Ammonium formate	LC-MS Ultra eluent additive, suitable for UHPLC-MS	25 g	14266
Ammonium acetate	LC-MS Ultra eluent additive, suitable for UHPLC-MS	25 g	14267

# New High-Purity Headspace Grade Solvents

Michael Kiselewsky

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When developing a GC-HS method, parameters such as sample solvent, extraction temperature, extraction time, sample volume and headspace volume are optimized. Sigma-Aldrich has developed solvents specifically for GC-HS applications. The purity and handling specifications for these solvents meet the requirements of the European Pharmacopoeia (Ph.Eur.) and United States Pharmacopeia (USP), as well as ICH guidelines. The new GC-HS product line includes water and three of the most commonly used organic solvents: dimethyl sulfoxide (DMSO), N,N-dimethylformamide, and N,N-dimethylacetamide. N,N-dimethylformamide and dimethyl sulfoxide are specified in Ph.Eur. and USP for water-insoluble substances. Water is the preferred solvent for water-soluble solutions, as described in

Ph.Eur. and USP monographs. In addition, Sigma-Aldrich has now expanded its GC-HS portfolio with two new products, GC-HS Cyclohexanone and 1-Methyl-2-pyrrolidinone.

All solvents are microfiltered at 0.2 µm and packed under inert gas for extended shelf life.

For more information, visit us at [sigma-aldrich.com/gc-hs](http://sigma-aldrich.com/gc-hs)

## Literature

United States Pharmacopeia, 31st Edition (2008), <467> Residual Solvents. Ph.Eur. 6.0 (2008) Method 2.4.24, Identification and control of residual solvents.

ICH Guideline Q3C, Impurities: Guideline for Residual Solvents, The Fourth International Conference on Harmonization, July 17, 1997.

## + Featured Products

Product name	Abbreviation	BP	Package Size	Cat. No.
<b>Solvents for GC Headspace Analysis</b>				
Cyclohexanone, for GC-HS	-	155 °C	1 L	68809
N,N-Dimethylacetamide, for GC-HS	DMA	166 °C	1 L	44901
N,N-Dimethylformamide, for GC-HS	DMF	153 °C	1 L	51781
1,3-Dimethyl-2-imidazolidinone, for GC-HS	DMI	225 °C	100 mL, 1 L	67484
Dimethyl sulfoxide, for GC-HS	DMSO	189 °C	1 L	51779
1-Methyl-2-pyrrolidinone, for GC-HS	NMP	202 °C	1 L	69337
Water, for GC-HS	-	100 °C	1 L	53463



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AAPS 2011	Oct. 23-27, 2011	Washington, DC
EAS 2011	Nov. 14-17, 2011	Somerset, NJ
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