REPORTER

Applications Newsletter Volume 33.4

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Cover Photo:
Supelco has released three new capillary GC column product lines: 200 m FAME columns, SLB-35ms, and SLB-ILD3606. An article featuring each product line appears in this issue.

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The Hype on Hyphenation

Dave Bell

Manager HPLC Surface Chemistry
and Health Sciences Research

Dear Colleague,

When one or more separation techniques are performed on-line with spectroscopic detection techniques, the combination is termed a hyphenated analytical technique. Although the instrument investment can be large, in financial terms and in operator expertise, the improvement in speed, sensitivity, and specificity can be dramatic. Analytical samples are becoming more and more complex, and target analytes are being measured at lower and lower levels. Some analytical applications, like proteomics, metabolomics, disease biomarker identification, and chemical fingerprinting benefit in particular from the enhancement in analytical performance provided by hyphenated analytical techniques. Indeed, one only need read the scientific literature to see how the use of hyphenated techniques has facilitated a great many discoveries in these fields and others.

A requirement of the front-end of the hyphenated technique, whether LC/MS, GC/MS, LCxLC/MS/MS, GCxGC/MS or any technique that uses chromatography, is that the column provide high peak capacity: A high number of baseline-resolved peaks per unit time in the chromatogram. Such columns need high resolving power, high efficiency, and optimal selectivity. They also must be robust to stand up to repeated injections of complex sample extracts. In addition, they must perform their function as fast as possible to enable high lab throughput. In keeping with the growing importance of hyphenated techniques, efficiency, selectivity, resolving power, robustness, and speed, have been hallmark features of the GC, HPLC, and UHPLC columns Supelco has introduced over the past several decades. The results of our development efforts have been columns that give users the best chance of maximizing the information from experiments using hyphenated techniques.

Several of the articles featured in this edition of The Reporter take advantage of the power of hyphenated analytical techniques utilizing Supelco HPLC and GC columns. It is not a coincidence that the work behind two of these articles was carried out in the laboratories of Prof. Luigi Mondello, a pioneer in the development and application of hyphenated analytical techniques for the characterization of complex samples. Supelco has been collaborating with Prof. Mondello, utilizing his and his team's expertise and laboratory instrumentation, toward providing solutions to many complex samples of current analytical interest.

Best regards,

Dave Bell
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NEW! 200 m GC Columns for Detailed Analysis of *cis/trans* FAME Isomers

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Over the last half of the previous century, the use of partially hydrogenated vegetable oil (PHVO) replaced the use of animal fats for baking purposes in most western countries. Initially developed for supply/demand and economic reasons, it was discovered that the use of PHVO could increase a food's shelf-life and/or increase its taste. It was also suggested that the unsaturated fatty acids in PHVO were healthier than the saturated fatty acids in animal fat.

In nature, the overwhelming majority of unsaturated fatty acids occur in the *cis* orientation. As such, humans evolved metabolic pathways to break down *cis* fatty acids. However, the process to make PHVO converts *cis* fatty acids into *trans* fatty acids. Scientific research over the last decade has shown that this situation (the increased intake of *trans* fatty acids coupled with our inability to properly metabolize them) can increase the risk of coronary disease. This is most evident by the proliferation of this disease in countries where the use of PHVO has replaced the use of animal fats. To help combat this trend, in June 2015 the US FDA mandated that food manufacturers must eliminate the use of all artificial *trans* fats (i.e. they can no longer use PHVO) within three years.[1]

The qualitative and quantitative testing of *cis/trans* fatty acids is best accomplished using gas chromatography (GC) after conversion of the fatty acids to fatty acid methyl esters (FAMEs). To assist with this testing, Supelco® recently developed two new capillary GC columns. These 200 m versions of SP™-2560 and SLB®-IL111 are specifically designed for and specially tested for the detailed analysis of *cis/trans* FAME isomers. Specifications for both columns are shown in **Table 1**. This article will show the suitability of these columns for analysis of *cis/trans* FAME isomers as well as other FAME isomer applications.

C18 FAME Isomer Mix

Some of the most studied fatty acids are the C18 family. A custom mixture was made by combining a C18:0 FAME standard, a custom C18:1 PHVO sample (containing multiple C18:1 FAME isomers), a 4-component C18:2 FAME isomer standard, and an 8-component C18:3 FAME isomer standard. This mixture was injected on each column, and run conditions were adjusted to achieve maximize resolution. The optimized chromatograms are shown in **Figure 1**. Peak identification was assigned based on previous work.

While neither column can separate every isomer, both columns provide a high degree of separation of *trans* FAME isomers from *cis* FAME isomers. Of interest is that with SLB-IL111, no *trans* C18:1 FAME isomer co-elutes with C18:1Δ9c, one of the most abundant naturally occurring unsaturated fatty acids. It often results in a very large peak area when analyzing food extracts. This is significant because this entire peak area must be considered as being contributed by the *trans* FAME if there is a co-elution, resulting in *trans* fat values that are biased high.

Table 1. Column Specifications

SP-2560

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

SLB-IL111

- **Application:** World's first commercial column to rate over 100 on our GC column polarity scale. Selectivity most orthogonal to non-polar and intermediate polar phases, resulting in very unique elution patterns. Maximum temperature of 270 °C is very impressive for such an extremely polar column. Great choice for separation of polarizable analytes (contain double and/or triple C-C bonds) from neutral analytes. Also a good GCxGC column choice. Launched in 2010.
- **USP Code:** None
- **Phase:** Non-bonded; 1,5-di(2,3-dimethylimidazolium)pentane bis(trifluoromethylsulfonyl)imide
- **Temp. Limits:** 50 °C to 270 °C (isothermal or programmed)

CLA FAME Isomer Mix

The stomachs of several mamalian species have four compartments. These mammals are known as ruminants, and include cows, sheep, goats, and deer. Ruminant fat contains conjugated linoleic acid (CLA) isomers, which are C18:2 fatty acids in which a single carbon-carbon bond separates the two double bonds. A custom mixture containing four CLA FAME isomers was prepared and injected on each column. Run conditions were adjusted to achieve maximize resolution. The optimized chromatograms are shown in **Figure 2**. Peak identification was assigned by injecting each isomer individually. Both columns were able to provide resolution, although with slightly different elution patterns.

Rapeseed Oil FAMEs with CLA FAME Isomers

Rapeseed oil is a simple vegetable oil that contains a series of saturated and unsaturated fatty acids ranging from C14 through C24 in carbon number. A custom mixture containing rapeseed oil FAMEs plus four CLA FAME isomers was prepared and injected on each column. Run conditions were identical to those previously used. The resulting chromatograms are shown in **Figure 3**. Peak identification was assigned based on previous work.

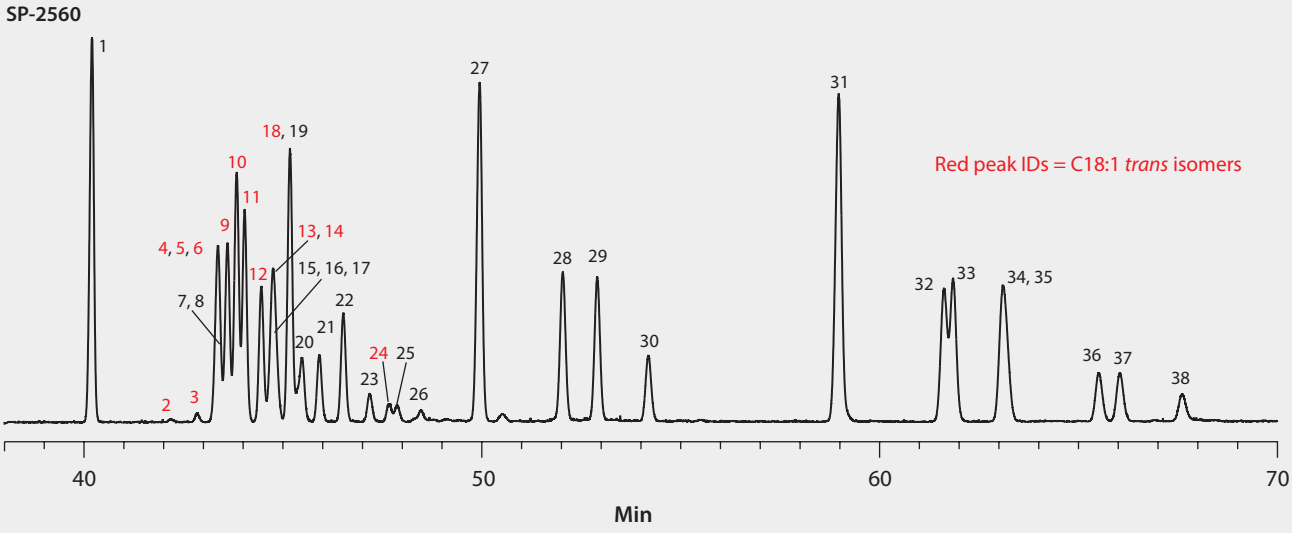
Monitoring the elution locations of the polyunsaturated C18 FAME isomers (peaks 5-10) relative to the saturated and monounsaturated FAME isomers is an indication of a column's ability to undergo dipole-induced dipole interactions. Both columns exhibited great relative retention of these isomers. In fact, the SLB-IL111 retained the CLA FAME isomers (C18:2 species) after C22:0. Also of note is that the co-elutions on one column are fully resolved on the other, providing complementary data.

(continued on next page)

Figure 1. C18:0, C18:1, C18:2, and C18:3 FAME Isomers

column: SP-2560, 200 m × 0.25 mm I.D., 0.20 µm (29688-U)
column: SLB-IL111, 200 m × 0.25 mm I.D., 0.20 µm (29689-U)
oven: 175 °C (SP-2560), 150 °C (SLB-IL111)
inj. temp.: 250 °C
carrier gas: hydrogen
detector: FID, 250 °C
injection: 1.0 µL, 50:1 split
liner: 4 mm I.D., split type, cup design
sample: Mix of C18:0, C18:1 (from partially hydrogenated vegetable oil [PHVO]), C18:2, and C18:3 FAME isomers

1. C18:0
2. C18:1Δ4t
3. C18:1Δ5t
4. C18:1Δ6t
5. C18:1Δ7t
6. C18:1Δ8t
7. C18:1Δ4c
8. C18:1Δ5c
9. C18:1Δ9t
10. C18:1Δ10t
11. C18:1Δ11t
12. C18:1Δ12t
13. C18:1Δ13t
14. C18:1Δ14t
15. C18:1Δ6c
16. C18:1Δ7c
17. C18:1Δ8c
18. C18:1Δ15t
19. C18:1Δ9c
20. C18:1Δ10c
21. C18:1Δ11c
22. C18:1Δ12c
23. C18:1Δ13c
24. C18:1Δ16t
25. C18:1Δ14c
26. C18:1Δ15c
27. C18:2Δ9t,12t
28. C18:2Δ9c,12t
29. C18:2Δ9t,12c
30. C18:2Δ9c,12c
31. C18:3Δ9t,12t,15t
32. C18:3Δ9t,12t,15c
33. C18:3Δ9t,12c,15t
34. C18:3Δ9c,12c,15t
35. C18:3Δ9c,12t,15t
36. C18:3Δ9c,12t,15c
37. C18:3Δ9t,12c,15c
38. C18:3Δ9c,12c,15c



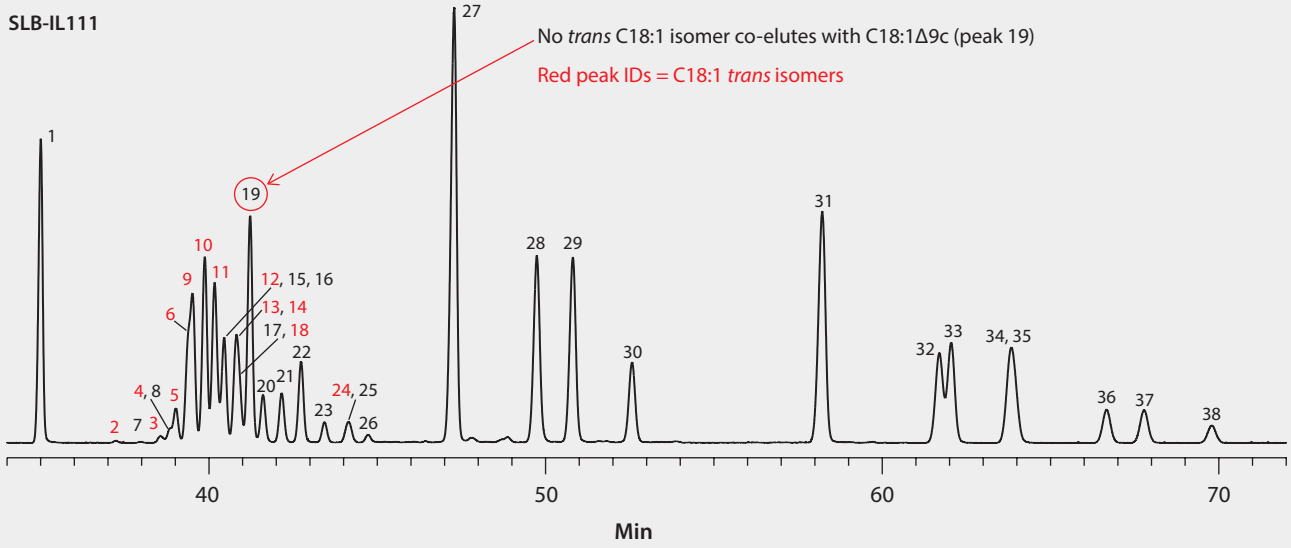
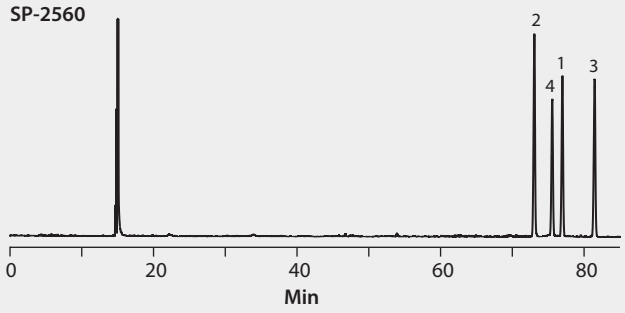
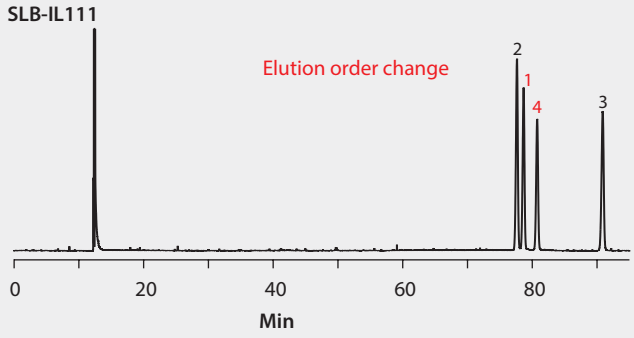


Figure 2. CLA FAME Isomers

column: SP-2560, 200 m × 0.25 mm I.D., 0.20 µm (29688-U)
column: SLB-IL111, 200 m × 0.25 mm I.D. , 0.20 µm (29689-U)
oven: 175 °C (SP-2560), 150 °C (SLB-IL111)
inj. temp.: 250 °C
carrier gas: hydrogen
detector: FID, 250 °C
injection: 1.0 µL, 50:1 split
liner: 4 mm I.D., split type, cup design
sample: 4-component CLA FAME isomer mix, 0.5 mg/mL in methylene chloride

1. C18:2Δ9t,11t (methyl 9-*trans*,11-trans octadecadienoate)
2. C18:2Δ9t,11c (methyl 9-*trans*,11-cis octadecadienoate)
3. C18:2Δ9c,11c (methyl 9-*cis*,11-cis octadecadienoate)
4. C18:2Δ10c,12t (methyl 10-*cis*,12-trans octadecadienoate)





38-Component FAME Isomer Mix

Determining the degree of fatty acid unsaturation of a food product is difficult because foods can contain a complex mixture of saturated, monounsaturated, and polyunsaturated fatty acids with a variety of carbon chain lengths.

The Supelco 37-Component FAME Mix contains methyl esters of fatty acids ranging from C4 to C24, including key monounsaturated and polyunsaturated fatty acids, making this standard very useful to food analysts since it can be used to identify fatty acids in many different types of foods. A custom standard comprised of this mix plus C22:5n3 FAME was prepared, and analyzed on each column under identical conditions. **Figure 4** shows the chromatograms obtained from both columns.

Discussion

The SP-2560/SLB-IL111 pairing allows the most comprehensive fatty acid composition information possible, able to provide accurate results (qualitative and quantitative) for both saturated and *trans* fatty acids. Observations include:

- While not shown, increased resolution was achieved when comparing chromatograms from 200 m versions to chromatograms from 100 m versions.
- Analytes tend to elute from the SLB-IL111 at a lower oven temperature (see **Figures 1-4**)
- SLB-IL111 provides resolution of C18:1Δ9c (one of the most abundant naturally occurring unsaturated fatty acids) from all *trans* FAMEs (see **Figure 1**).
- SP-2560 and SLB-IL111 provide different elution patterns for the CLA FAME isomers analyzed (see **Figures 2** and **3**).
- SLB-IL111 provides increased retention of unsaturated FAME isomers (see **Figures 3** and **4**).
- SP-2560 provides better resolution of saturated FAME isomers from unsaturated FAME isomers (see **Figure 4**).

Conclusion

The SP-2560 chemistry was first introduced in 1983, and the SLB-IL111 chemistry was first introduced in 2010. The new 200 m versions of these chemistries indicate our commitment to remain at the forefront of detailed analysis of *cis/trans* FAME isomers. The SP-2560/SLB-IL111 pairing allows the most comprehensive fatty acid composition information possible.

Reference

1. FDA Cuts *Trans* Fat in Processed Foods. http://www.fda.gov/ForConsumers/ConsumerUpdates/ucm372915.htm (accessed August 18, 2015)

Featured Products

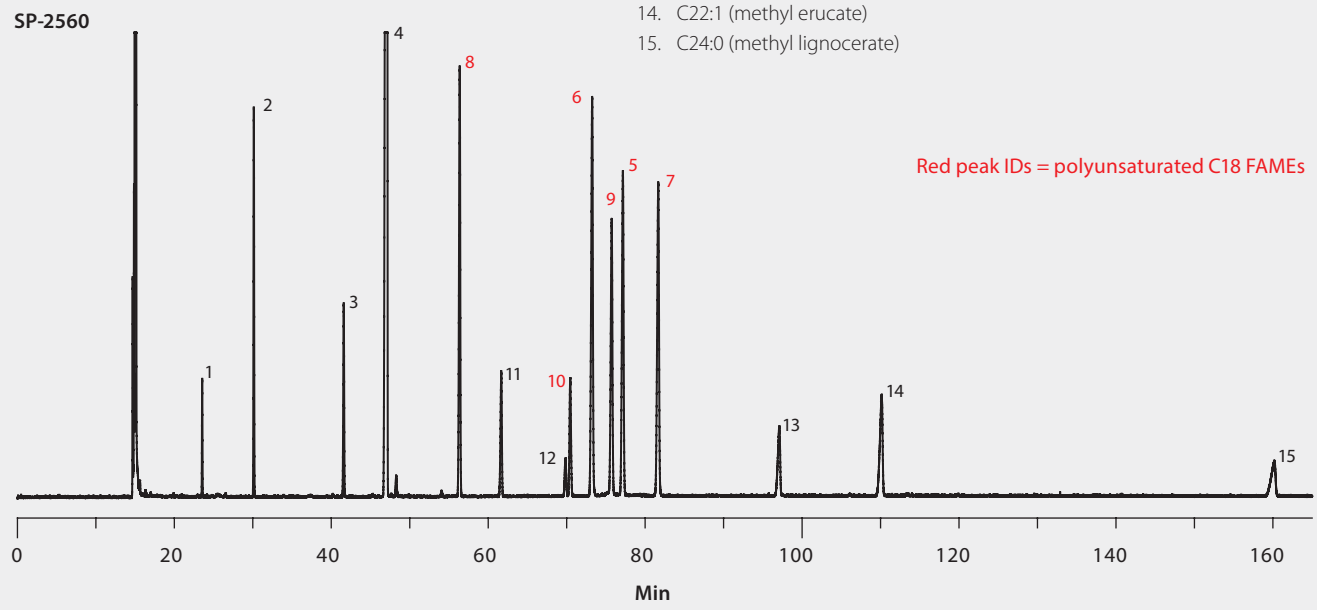
Description	Cat. No.
SP-2560, 200 m × 0.25 mm I.D., 0.20 µm	29688-U
SLB-IL111, 200 m × 0.25 mm I.D., 0.20 µm	29689-U
37-Component FAME Mix	CRM47885

(continued on next page)

Figure 3. Rapeseed Oil FAMEs with CLA FAME Isomers

column: SP-2560, 200 m × 0.25 mm I.D., 0.20 µm (29688-U)
column: SLB-IL111, 200 m × 0.25 mm I.D., 0.20 µm (29689-U)
oven: 175 °C (SP-2560), 150 °C (SLB-IL111)
inj. temp.: 250 °C
carrier gas: hydrogen
detector: FID, 250 °C
injection: 1.0 µL, 50:1 split
liner: 4 mm I.D., split type, cup design
sample: Mix of rapeseed oil FAME isomers and CLA FAME isomers

1. C14:0 (methyl myristate)
2. C16:0 (methyl palmitate)
3. C18:0 (methyl stearate)
4. C18:1n9c (methyl oleate)
5. C18:2Δ9t,11t (methyl 9-*trans*,11-trans octadecadienoate)
6. C18:2Δ9t,11c (methyl 9-*trans*,11-cis octadecadienoate)
7. C18:2Δ9c,11c (methyl 9-*cis*,11-cis octadecadienoate)
8. C18:2n6c,9c (methyl linoleate)
9. C18:2Δ10c,12t (methyl 10-*cis*,12-trans octadecadienoate)
10. C18:3n3c,6c,9c (methyl linolenate)
11. C20:0 (methyl arachidate)
12. C20:1 (methyl eicosenoate)
13. C22:0 (methyl behenate)
14. C22:1 (methyl erucate)
15. C24:0 (methyl lignocerate)



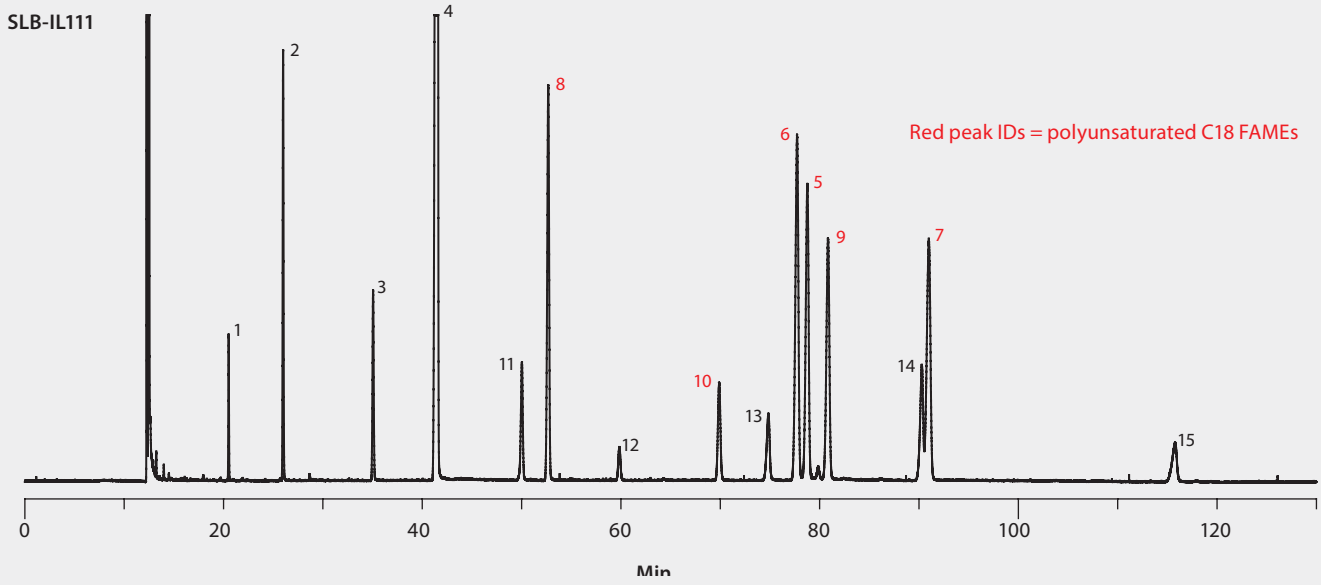
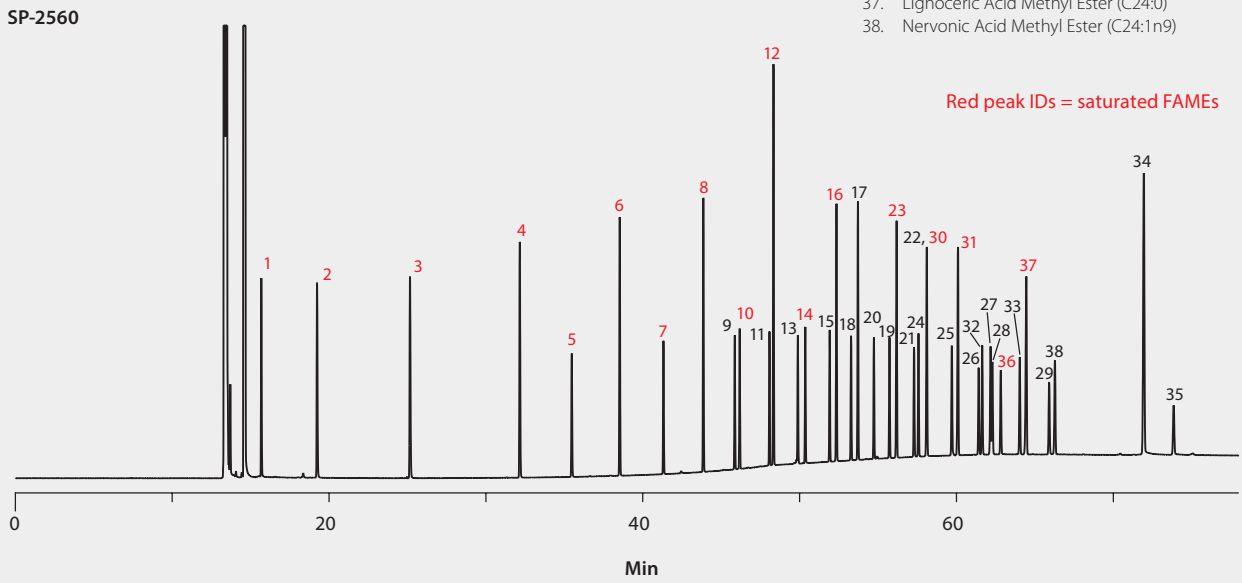
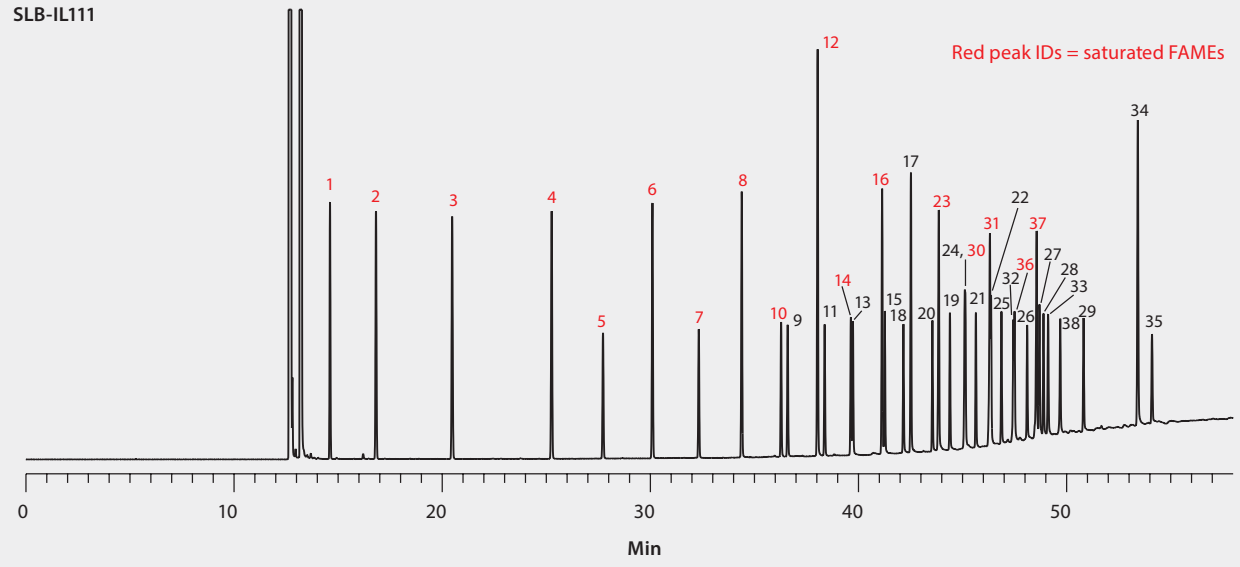


Figure 4. 38-Component FAME Isomers

column: SP-2560, 200 m × 0.25 mm I.D., 0.20 µm (29688-U)
column: SLB-IL111, 200 m × 0.25 mm I.D., 0.20 µm (29689-U)
oven: 100 °C (13 min), 4 °C/min to 240 °C (30 min)
inj. temp.: 250 °C
detector: FID, 250 °C
carrier gas: hydrogen, 27 cm/sec
injection: 1.0 µL, 50:1 split
liner: 4 mm I.D., split type, cup design
sample: Supelco 37-Component FAME Mix (47885-U) + C22:5n3 FAME

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





Supelco SLB-IL60 Ionic Liquid GC Columns: Stability to Loss of Carrier Gas Flow

Leonard M. Sidisky, R&D Manager; Katherine K. Stenerson, Applications Chemist; and Michael D. Buchanan, Product Manager

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The SLB®-IL60 gas chromatography (GC) column is based on an ionic liquid stationary phase platform, and displays desirable features that existing non-ionic liquid columns do not. This is the fifth of several Reporter articles which explore various aspects of this column.[1-4]

Hydroxyl (-OH) functional groups are abundant in polyethylene glycol (PEG) stationary phases. These active moieties can be oxidized when exposed to low levels of moisture and/or oxygen, contributing to degradation of the phase. The rate of phase degradation is accelerated with elevated temperatures. These phenomena have a direct impact on the maximum temperature limits of commercially available PEG columns.

The SLB-IL60 column is stable to 300 °C for both programmed and isothermal use. Compared to PEG columns, it exhibits both lower FID bleed, and better high temperature stability. These features are possible because the structure of the ionic liquid stationary phase does not contain any hydroxyl groups. Complete specifications of SLB-IL60 columns are shown in **Table 1**.

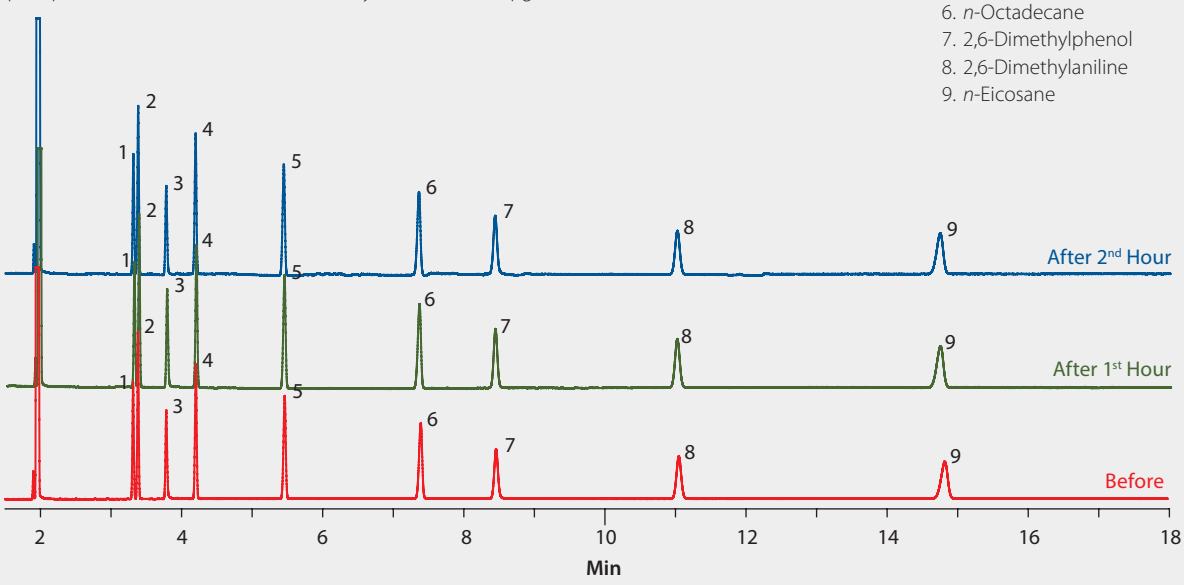
Table 1. SLB-IL60 Column Specifications

- **Application:** Modified (deactivated) version of SLB-IL59 provides better inertness. Selectivity more polar than PEG/wax phases, resulting in unique elution patterns. Higher maximum temperature than PEG/wax columns (300 °C compared to 270-280 °C). Excellent alternative to existing PEG/wax columns. Also a good GCxGC column choice. Launched in 2012.
- **USP Code:** None
- **Phase:** Non-bonded; 1,12-Di(tripropylphosphonium)dodecane bis(trifluoromethylsulfonyl)imide
- **Temp. Limits:** 35 °C to 300 °C (isothermal or programmed)

Figure 1. Performance Before/After Stop Flow Test

column: SLB-IL60, 30 m × 0.25 mm I.D., 0.20 µm (29505-U)
oven: 130 °C (25 min)
inj. temp.: 250 °C
carrier gas: helium, 25 cm/sec
detector: FID, 250 °C
injection: 1 µL, 100:1 split
liner: 4 mm I.D., split/splitless type, single taper wool packed FocusLiner™ design
sample: polar column test mix (47302), 9 analytes, each at 500 µg/mL in dichloromethane

1. 2-Octanone
2. *n*-Pentadecane
3. 1-Octanol
4. *n*-Hexadecane
5. *n*-Heptadecane
6. *n*-Octadecane
7. 2,6-Dimethylphenol
8. 2,6-Dimethylaniline
9. *n*-Eicosane



Carrier Gas Stop Flow Test

To illustrate the robust nature of the SLB-IL60 column, it was subjected to a brutal carrier gas stop-flow test. A polar column test mix was analyzed to establish initial chromatographic performance. This mix contains several analyte types, and can be used to measure key attributes of polar columns.

- The normal alkanes (pentadecane, hexadecane, heptadecane, octadecane, and eicosane) are used to measure column efficiency
- The alcohol (1-octanol) and ketone (2-octanone) are used to measure the presence of hydrogen-bonding sites (exposed silanols)
- The acid/base pair (2,6-dimethylphenol/2,6-dimethylaniline) are used to measure the acid/base characteristic of the phase surface

The carrier gas flow was then turned off, and the oven heated to 250 °C. After 1 hour, carrier gas flow was re-established, followed by the analysis of the polar column test mix. This cycle was then repeated a second time. The degree that column performance changed was determined by comparing the resulting chromatography from the three polar column test mix runs, which are shown in **Figure 1**. No significant change in chromatographic performance was observed, even after the column was held at 250 °C for a total of 2 hours with no carrier gas flow. Peak shapes, absolute retention times, and relative retention times are virtually identical for the all three chromatograms. This is impressive.

Conclusion

Columns based on polyethylene glycol phase chemistry are widely used for a variety of applications (such as solvents and FAMEs), but are limited to use below 260-280 °C oven temperature. The SLB-IL60 column is similar in selectivity to PEG columns, but can be used to 300 °C without degradation of chromatographic performance. This column also exhibits substantial robustness, able to withstand the temporary loss of carrier gas flow without damaging the stationary phase.

References

1. Sidisky, L. M.; Stenerson K. K.; Buchanan, M. D. *Supelco SLB-IL60 Ionic Liquid Columns: Unique Selectivity*; Supelco Reporter Volume 32.2: 29-31.
2. Sidisky, L. M.; Stenerson K. K.; Buchanan, M. D., *Supelco SLB-IL60 Ionic Liquid Columns: Improved Resolution*; Supelco Reporter Volume 33.1: 21-22.
3. Sidisky, L. M.; Stenerson K. K.; Buchanan, M. D. *Supelco SLB-IL60 Ionic Liquid Columns: Better High Temperature Stability*; Supelco Reporter Volume 33.2: 6-9.
4. Sidisky, L. M.; Stenerson K. K.; Buchanan, M. D. *Supelco SLB-IL60 Ionic Liquid Columns: Lower FID Bleed*; Supelco Reporter Volume 33.3: 30-31.

Featured Products

Description	Cat. No.
SLB-IL60 Ionic Liquid Capillary GC Columns	
15 m × 0.10 mm I.D., 0.08 µm	29503-U
20 m × 0.18 mm I.D., 0.14 µm	29504-U
30 m × 0.25 mm I.D., 0.20 µm	29505-U
60 m × 0.25 mm I.D., 0.20 µm	29506-U
30 m × 0.32 mm I.D., 0.26 µm	29508-U
60 m × 0.32 mm I.D., 0.26 µm	29509-U
Polar Column Test Mix	
500 µg/mL, dichloromethane, 1 × 2 mL	47302

Related Information

For more information on the SLB-IL60 and other ionic liquid columns, visit
sigma-aldrich.com/il-gc

Molded Thermogreen LB-2 Septa

Jaime Martain
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Introduction

Injection port septa used in gas chromatographic analyses should exhibit low bleed, resist leaks, and be easy to penetrate. For years, Thermogreen® LB-2 septa have been considered by many as the benchmark of GC septa. A new generation septum, molded Thermogreen LB-2, continues the Supelco tradition of setting the benchmark in high performance.

Features and Benefits

Molded Thermogreen LB-2 septa are manufactured from high-quality, low-bleed material using the same exclusive rubber formulation as the popular Thermogreen LB-2 septa. The difference is that the molded septa, unlike traditional die cut septa, offer easier installation and also provide a better seal inside the injection port. With a liquid injection molding process, every septum conforms to the same mold shape with crisp, clean sides.

A liquid injection molding process allows injection holes to be incorporated into the septa. An injection hole allows needle penetration through the same location, time-after-time. This helps reduce septum coring, and prevents septum fragments from entering the injection port. The high puncture tolerance makes these septa ideal for autosamplers as well as users of solid phase microextraction (SPME).

Low Bleed Profile

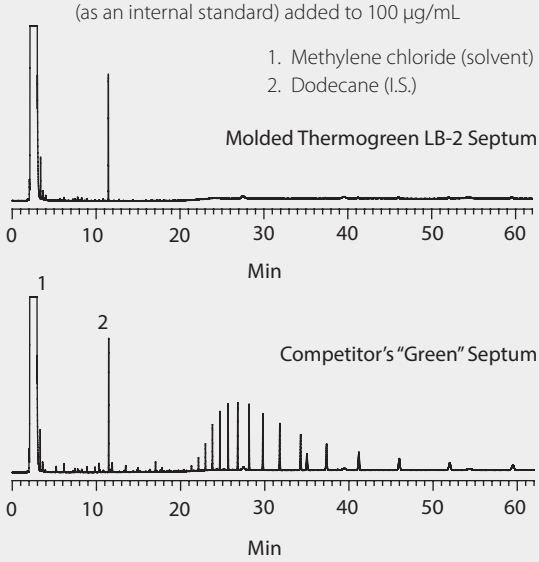
A molded Thermogreen LB-2 septum and a popular molded septum from a competitor were solvent extracted. Both molded septa tested were a standard design without injection hole. The resulting chromatograms are shown in **Figure 1**. As seen, the competitor's "green" septum performed very poorly when subjected to this bleed test. The unlabeled peaks represent silicone oils extracted from the septum. When placed on an injection port and heated, these silicone oils would inevitably bleed off, collect on the head of the column, and appear as contamination peaks in chromatograms.

Conclusion

The strict tolerances resulting from the constant dimensions of the mold itself result in septa that are easier to install and consistently fit better. Using a rubber formulation exclusive to Supelco, molded Thermogreen LB-2 septa exhibit an ultra low bleed profile, are very resistant to both slivering and coring, and have a high puncture tolerance when used in autosampler applications.

Figure 1. Bleed Profiles of High Performance Septa

column: SLB-5ms, 30 m × 0.25 mm I.D., 0.25 µm (28471-U)
oven: 40 °C (3 min), 15 °C/min to 325°C (15 min)
inj. temp.: 250 °C
carrier gas: helium, 25 cm/sec constant
detector: FID, 325 °C
injection: 1 µL, splitless (1 min)
liner: 4 mm I.D., single taper, unpacked
sample: solvent extracts of septa, dodecane (as an internal standard) added to 100 µg/mL



Featured Products

Diameter (mm)	Qty.	Cat. No.
Molded Thermogreen LB-2 Septa		
9.5	50	28670-U
9.5	250	28671-U
9.5 with injection hole	50	28331-U
9.5 with injection hole	250	28332-U
10	50	28673-U
10	250	28675-U
10 with injection hole	50	28333-U
10 with injection hole	250	28334-U
11	50	28676-U
11	250	28678-U
11 with injection hole	50	28336-U
11 with injection hole	250	28338-U
Diameter × L (mm)	**Qty.**	**Cat. No.**
Molded Thermogreen LB-2 Cylindrical Septa for Shimadzu™ Instruments		
6 × 9	10	20608
6 × 9	50	20633

To learn more about the molded Thermogreen LB-2 septa, visit us on the web at
sigma-aldrich.com/moldedsepta

High Resolution and High Efficiency Separations of mAbs and ADCs Using Proteomix HIC Columns

Stacy Shollenberger, Product Manager and Hillel Brandes, Principal R&D Scientist
stacy.shollenberger@sial.com

Introduction

A variety of products derived from monoclonal antibodies (mAbs), including mAb fragments and antibody-drug conjugates (ADCs), are being developed for the treatment of cancer and other diseases due to their increased potency combined with reduced toxicity. However, the efficacy of these molecules is highly dependent upon the target site-specificity and binding properties of the mAb, the linker stability, the potency of the drug, and both the distribution and number of drug species on the mAb.[1] These requirements highlight the importance of characterizing these highly heterogeneous products using appropriate analytical techniques in order to assess and monitor them during manufacturing and subsequent storage.

Hydrophobic interaction chromatography (HIC) is a technique for protein separations and has been commonly used as an orthogonal method to size exclusion chromatography (SEC) and ion exchange (IEX) chromatography for the characterization of mAbs. Here we introduce Proteomix® HIC columns which have been designed for high resolution and high efficiency separations of proteins, oligonucleotides, and peptides.

General Description

Utilizing proprietary surface technologies, Proteomix HIC-NP resin is made of non-porous polystyrenedivinylbenzene (PS/DVB) beads with narrow-dispersed particle size distribution. As shown in **Figure 1**, the PS/DVB bead is modified with alkyl groups or an aryl group that provides hydrophobic interaction with analytes. Proteomix HIC-NP resin is highly rigid and mechanically stable. In comparison to silica based HIC phase media, Proteomix HIC-NP phases have advantages for biomolecule separations with wide pH range (2-12) and high thermal stability. The nonporous structure and narrow particle distribution offer special selectivity, high resolution separation of proteins such as mAbs, ADCs, and related protein fragments, as well as DNA and oligonucleotides. Proteomix HIC-NP media are applicable at laboratory discovery, laboratory-scale purification, and process chromatography for the production of a few mgs to kilogram of proteins.

Figure 1. Structure of Proteomix HIC-NP5 Resin

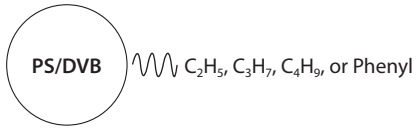


Table 1. Technical Specifications

Resin Matrix	Spherical, highly cross-linked PS/DVB
Pore Size	Nonporous
Particle Size	5 µm and 10 µm
Phase Structure	Ethyl, propyl, butyl, or phenyl
Separation Mechanism	Hydrophobic interaction (HIC)
pH Stability	2-12
Operating Temperature	Up to 80 °C
Operating Pressure	Up to 6,000 psi
Mobile Phase Compatibility	Compatible with aqueous solution, a mixture of water and acetonitrile, acetone, methanol, or THF

Featured Characteristics

- Highest capacity and resolution
- Consistent lot-to-lot reproducibility
- Improved protein recovery with intact biological activity
- Negligible non-specific interactions
- Ideal for separation and analysis of hydrophobic proteins, mAbs, and ADCs
- Suitable for separation and analysis of general biological samples

High Stability and Lot-to-Lot Reproducibility

Proteomix HIC columns are based on PS/DVB resin and all the surface coatings are chemically bonded onto PS/DVB support, which provides exceptionally high stability. The columns are compatible with most aqueous buffers, such as ammonium sulfate, sodium acetate, phosphate, Tris, and a mixture of water and acetone, methanol, acetonitrile and THF. When 25 mM sodium phosphate buffer, at pH 7.0, was used as the mobile phase to run the Proteomix HIC Butyl-NP5 column, 400 injections or 3 months of usage has negligible deterioration of the column.

Proteomix HIC columns provide high lot-to-lot consistency on ADC, mAb, and protein separations as shown in **Figures 2-4**.

(continued on next page)

Figure 2. Proteomix HIC Butyl-NP5 for Herceptin-cysteine ADC Separation-Lot Consistency Testing

column: Proteomix HIC Butyl-NP5, 4.6 × 35 mm, 5 µm (61864-U)
mobile phase: [A] 2 M ammonium sulfate in 0.025 M sodium phosphate, pH 7.0; [B] 0.025 M sodium phosphate, pH 7.0; [C] 100% IPA
flow rate: 0.8 mL/min
column temp.: 25 °C
detector: UV, 214 nm
injection: 10 µL
sample: ADC, 1 mg/mL in 1M ammonium sulfate

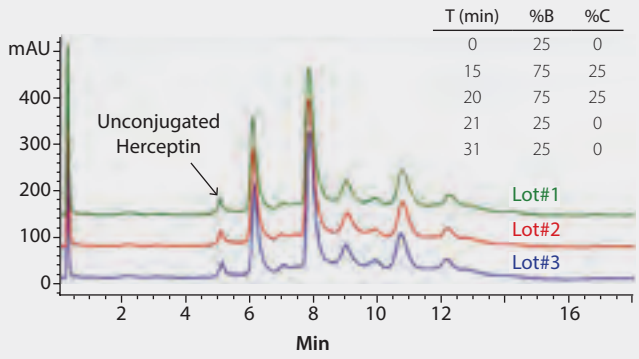


Figure 3. Proteomix HIC Butyl-NP5 for Herceptin-mAb Separation - Lot Consistency Testing

column: Proteomix HIC Butyl-NP5, 4.6 × 35 mm, 5 µm (61864-U)
mobile phase: [A] 2 M ammonium sulfate in 0.025 M sodium phosphate, pH 7.0; [B] 0.025 M sodium phosphate, pH 7.0; [C] 100% IPA
flow rate: 0.8 mL/min
column temp.: 25 °C
detector: UV, 214 nm
injection: 10 µL
sample: herceptin, 1 mg/mL in 1 M ammonium sulfate

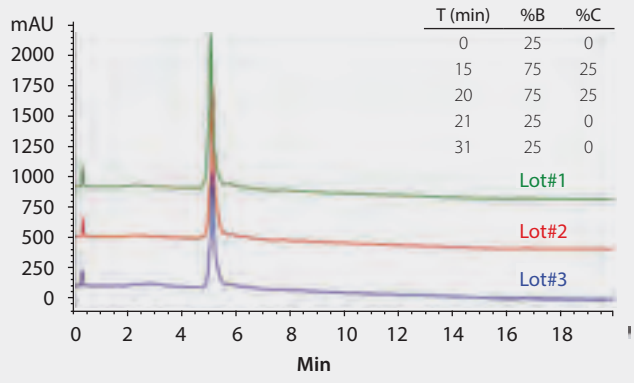
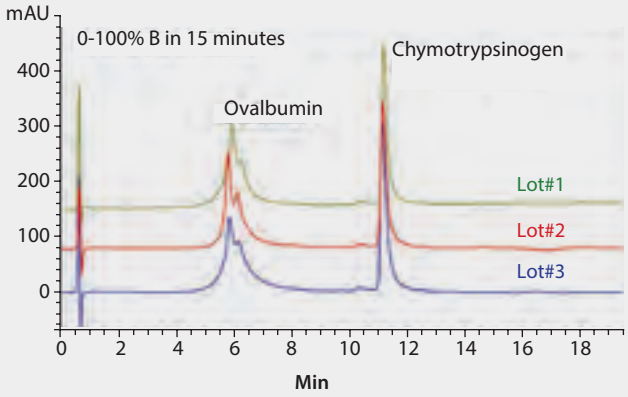


Figure 4. Proteomix HIC Butyl-NP5 for Protein Separation - Lot Consistency Testing

column: Proteomix HIC Butyl-NP5, 4.6 × 35 mm, 5 µm (61864-U)
mobile phase: [A] 2 M ammonium sulfate in 0.1 M sodium phosphate, pH 7.0; [B] 0.1 M phosphate, pH 7.0
flow rate: 0.4 mL/min
detector: UV, 214 nm
column temp.: 25 °C
injection: 4 µL
sample: ovalbumin 1.0 mg/mL, Chymotrypsinogen 0.5 mg/mL

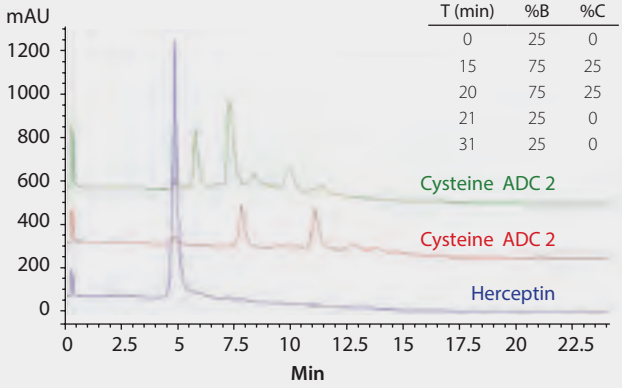


Additional Applications

In **Figure 5**, the *Proteomix* HIC Butyl-NP5 column was used for the characterization of the distribution of drug-linked species and the determination of the average drug to antibody ratio (DAR) after peak integration. Because HIC separates molecules based on their hydrophobicity, the Proteomix HIC Butyl-NP5 column is very effective for this type of separation due to the fact that hydrophobicity increases with the number of attached payloads.

Figure 5. Herceptin and its ADCs Separation on Proteomix HIC Butyl-NP5 Column

column: Proteomix HIC Butyl-NP5, 4.6 × 35 mm, 5 µm (61864-U)
mobile phase: [A] 2 M ammonium sulfate in 0.025 M sodium phosphate, pH 7.0; [B] 0.025 M sodium phosphate, pH 7.0; [C] 100% IPA
flow rate: 0.8 mL/min
column temp: 25 °C
detector: UV, 214 nm
injection: 10 µL
sample: herceptin/ADC1/ADC2, 1 mg/mL in 25 mM sodium phosphate



Reference

1. Wu, A. M.; Senter, P. D. Arming Antibodies: Prospects and Challenges for Immunoconjugates. *Nat. Biotechnol.* **2005**, *23(9)*, 1137-1146.

Featured Products

Description	Cat. No.
Proteomix HIC Butyl-NP5 Columns	
Proteomix HIC Butyl-NP5, NP, 5 cm × 2.1 mm, 5 µm	61862-U
Proteomix HIC Butyl-NP5 guard cartridge with holder, NP, 1 cm × 4 mm I.D.,5 µm	61863-U
Proteomix HIC Butyl-NP5, NP, 3.5 cm × 4.6 mm I.D., 5 µm	61864-U
Proteomix HIC Butyl-NP5, NP, 10 cm × 4.6 mm I.D., 5 µm	61865-U
Proteomix HIC Butyl-NP5, NP, 15 cm × 4.6 mm I.D., 5 µm	61866-U
Proteomix HIC Butyl-NP5, NP, 5 cm × 7.8 mm I.D., 5 µm	61867-U
Proteomix HIC Ethyl-NP5 Columns	
Proteomix HIC Ethyl-NP5 guard cartridge with holder, NP, 1 cm × 4 mm I.D.,5 µm	61868-U
Proteomix HIC Ethyl-NP5, NP, 3.5 cm × 4.6 mm I.D., 5 µm	61869-U
Proteomix HIC Ethyl-NP5, NP, 10 cm × 4.6 mm I.D., 5 µm	61870-U
Proteomix HIC Ethyl-NP5, NP, 5 cm × 7.8 mm I.D., 5 µm	61871-U
Proteomix HIC Phenyl-NP5 Columns	
Proteomix HIC Phenyl-NP5 guard cartridge with holder, NP, 1 cm × 4 mm I.D.,5 µm	61873-U
Proteomix HIC Phenyl-NP5, NP, 3.5 cm × 4.6 mm I.D., 5 µm	61874-U
Proteomix HIC Phenyl-NP5, NP, 10 cm × 4.6 mm I.D., 5 µm	61876-U
Proteomix HIC Phenyl-NP5, NP, 5 cm × 7.8 mm I.D., 5 µm	61878-U
Proteomix HIC Propyl-NP5 Columns	
Proteomix HIC Propyl-NP5 guard cartridge with holder, NP, 1 cm × 4 mm I.D.,5 µm	61879-U
Proteomix HIC Propyl-NP5, NP, 3.5 cm × 4.6 mm I.D., 5 µm	61881-U
Proteomix HIC Propyl-NP5, NP, 10 cm × 4.6 mm I.D., 5 µm	61883-U
Proteomix HIC Propyl-NP5, NP, 5 cm × 7.8 mm I.D., 5 µm	61884-U

Development of a Chiral Method for Levamisole and its Metabolite, Aminorex, for Monitoring Abuse in Horse Racing and Cocaine Adulteration

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Denise Wallworth, Tactical Marketing Manager, Clinical & Forensic Analysis
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Background

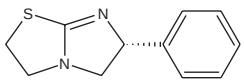
Concern over the abuse of amphetamines and other stimulants goes beyond its prevalence in the human population. Unscrupulous and unethical animal handlers administer drugs to their charges to enhance performance through stimulation, cardiovascular augmentation, reduction of pain or inflammation, increasing muscle mass, and other means. Improvements in analytical technologies have facilitated the detection of illicit drugs and other substances and continue to be a significant underpinning to criminal investigations into animal abuse.[1]

Illicit Uses of Levamisole

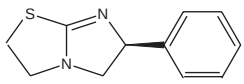
Levamisole, a tetramisole enantiomer, is sometimes given to horses as an anthelmintic treatment. Concern has arisen because levamisole is metabolized to the amphetamine-like drug aminorex (**Figure 1**). Investigation into race horses that tested positive for aminorex suggested that its source was from the administration of levamisole.[2] Because of the potential for abuse, the therapeutic use of levamisole is banned in some countries. The presence of aminorex in the animal may be from the administration of levamisole or from synthetic, racemic aminorex. Studies have shown that these two administration routes result in significantly different ratios of the aminorex enantiomers.[3] Additionally, levamisole is thought to play a role in the *in vivo* production of a significant performance enhancing drug, pemoline. Levamisole is also used as an adulterant in cocaine; its addition is likely intended to extend the effect of cocaine by metabolizing to aminorex just as the effect of cocaine diminishes.[4]

Figure 1. Analyte Chemical Structures

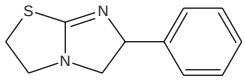
Dexamisole



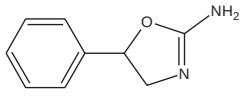
Levamisole



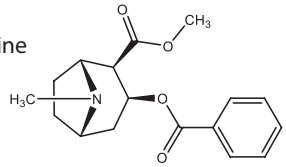
Tetramisole (racemate)



Aminorex (levamisole metabolite)



Cocaine



Importance of Chiral Discrimination

Chiral HPLC analysis can play a significant role in understanding whether detected levamisole or aminorex is due to legal administration of drugs or illicit use to enhance performance. A simple chiral HPLC method for levamisole and related compounds may therefore be of utility in both the determination of illicit use within the horse racing industry as well as in investigations into cocaine abuse and tracing studies.

Objectives of the Study

The aim of this study was to develop a method to differentiate the enantiomers of tetramisole (levamisole and dexamisole) for drug product analysis and performance enhancing activities in horse racing. An added bonus would be to have a method that could simultaneously separate aminorex and cocaine from the tetramisole enantiomers which would be useful in cocaine-related studies.

Experimental

In previous work, Astec® CYCLOBOND® cyclodextrin-based chiral stationary phases (CSP) were shown to be effective in separating the enantiomers of '-conazole' based compounds that are structurally similar to tetramisole. For that reason, a selection of Astec CYCLOBOND phases based on derivatized β-cyclodextrin was chosen for initial column screening to resolve the tetramisole enantiomers using an MS-compatible mobile phase system comprising 100 mM ammonium acetate and acetonitrile. Five Astec CYCLOBOND columns were screened (**Table 1**). Each column was 25 cm × 4.6 mm I.D. packed with 5 µm particles. Since levamisole is related to both the metabolic production of aminorex as well as a common cocaine adulterant, the developed separation conditions were investigated for their simultaneous separation.

Table 1. Astec CYCLOBOND Columns Screening Set

Column	Cyclodextrin type	Cyclodextrin derivative (2- and 3-position hydroxyls)
Astec CYCLOBOND I 2000 HP-RSP	Beta (β)*	R,S-Hydroxypropyl ether
Astec CYCLOBOND I 2000 DMP	Beta (β)	3,5-Dimethylphenylcarbamate
Astec CYCLOBOND I 2000 DNP	Beta (β)	2,6-Dinitro-4-trifluoromethyl phenyl ether
Astec CYCLOBOND I 2000 DM	Beta (β)	Dimethyl
Astec CYCLOBOND I 2000 AC	Beta (β)	Acetyl

* Cycloheptylamylose, glucose units: 7, stereogenic centers: 35, cavity size: 0.78 nm.

Results

Of the five β-cyclodextrin phases screened, only the dimethylphenylcarbamate derivative (Astec CYCLOBOND I 2000 DMP) showed significant selectivity toward the tetramisole enantiomers. (A detailed explanation of the separation mechanism behind the cyclodextrin-based CSPs can be found in Reference 5.) Method optimization resulted in the chromatographic separation shown in **Figure 2**. Confirmation of elution order was determined by injection of a standard levamisole preparation (**Figure 3**).

Under the initial mobile phase conditions (20% acetonitrile) aminorex eluted very early. Retention was increased by reducing the mobile phase to 10% acetonitrile which permitted the simultaneous resolution of all target analytes in less than 10 minutes (**Figure 4**).

Conclusions and Observations

Astec CYCLOBOND I 2000 DMP was shown to be effective for the chiral resolution of tetramisole enantiomers, levamisole and dexamisole. The conditions are also shown to separate a known metabolite of levamisole (aminorex) and thus may be used in certain studies aimed at understanding the origins the aminorex found in horse racing monitoring procedures. In addition, cocaine, which is often laced with levamisole, also separates within a reasonable time window, allowing the conditions to be useful in drug abuse investigations. Future work will focus on the ability of the method to resolve the aminorex enantiomers once standards of the individual enantiomers or an authenticated racemic mixture can be obtained.

Figure 2. Chiral Separation of Tetramisole Enantiomers on Astec CYCLOBOND I 2000 DMP

column: Astec CYCLOBOND I 2000 DMP, 25 cm × 4.6 mm I.D., 5 µm (20724AST)
mobile phase: [A] 100 mM ammonium acetate, pH 5 with acetic acid; [B] acetonitrile; (80:20, A:B)
flow rate: 1.0 mL/min
column temp.: 35 °C
detector: UV, 230 nm
injection: 10 µL
sample: 200 µg/mL tetramisole in mobile phase A:methanol (80:20, v/v)

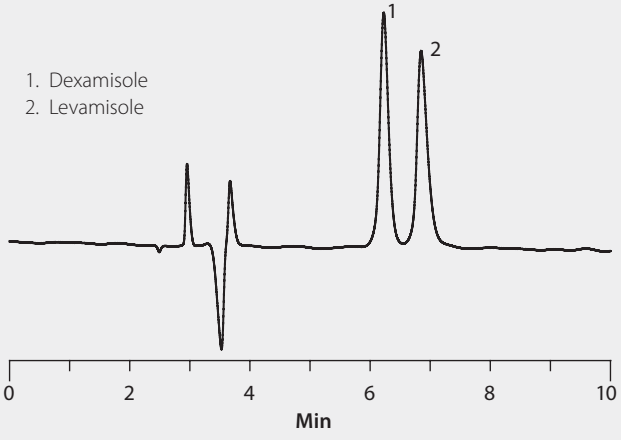


Figure 3. Identification of Elution Order of Tetramisole Enantiomers on Astec CYCLOBOND I 2000 DMP

Conditions as in Figure 2 except
sample: 100 µg/mL levamisole in 80:20 (A:B)

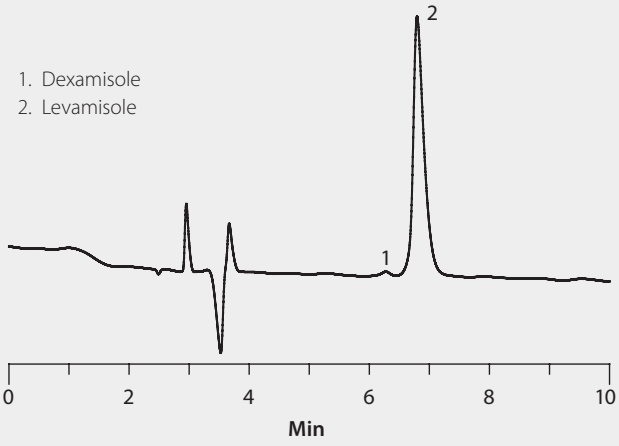
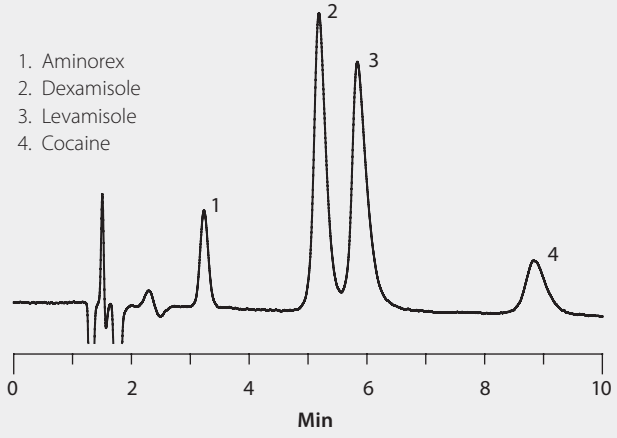


Figure 4. Separation of Tetramisole Enantiomers and Related Compounds on Astec CYCLOBOND I 2000 DMP

column: Astec CYCLOBOND I 2000 DMP, 10 cm × 2.1 mm I.D., 5 µm
mobile phase: [A] 100 mM ammonium acetate, pH 5 with acetic acid; [B] acetonitrile; (90:10, A:B)
flow rate: 0.2 mL/min
column temp.: 35 °C
detector: UV, 220 nm
injection: 2 µL
sample: 100 µg/mL tetramisole, 50 µg/mL each aminorex and cocaine in water:acetonitrile (20:80, v/v)



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References

1. Wong, J. K. Y.; Wan, T. S. M. Doping control analyses in Horse racing: A clinician's guide. *Veterinary Journal* **2014**, *200(1)*, 8-16.
2. Ho, E. N. M.; Leung, D. K. K.; Leung, G. N. W.; Wan, T. S. M.; Wong, A. S. Y.; Wong, C. H. F.; Soma, L. R.; Rudy, J. A.; Uboh, C.; Sams, R. Aminorex and rexamino as metabolites of levamisole in the horse. *Analytica Chimica Acta* **2009**, *638(1)*, 58-68.
3. Barker, S. A. The formation of aminorex in racehorses following levamisole administration. A quantitative and chiral analysis following synthetic aminorex or levamisole administration vs. aminorex-positive samples from the field: a preliminary report. *J. Vet. Pharmacol. Ther.* **2009**, *32(2)*, 160-166.
4. Hofmaier, T.; Luf, A.; Seddik, A.; Stockner, T.; Holy, M.; Freissmuth, M.; Ecker, G. F.; Schmid, R.; Sitte, H. H.; Kudlacek,O.. Aminorex, a metabolite of the cocaine adulterant levamisole, exerts amphetamine like actions at monoamine transporters. *Neurochemistry International* **2014**, *73*, 32-41.
5. Armstrong, D. W.; Chang, C. D.; Lee, S. H. (R)- and (S)-naphthylethylcarbamate-substituted β-cyclodextrin bonded stationary phases for the reversed-phase liquid chromatographic separation of enantiomers. *J. Chromatogr.* **1991**, *539(1)*, 83-90.

Featured Products

Description	Cat. No.
Chiral HPLC Columns	
Astec® CYCLOBOND® I 2000 DMP, 25 cm × 4.6 mm I.D., 5 µm	20724AST
Astec CYCLOBOND I 2000 DMP, 10 cm × 2.1 mm I.D., 5 µm	custom
Mobile Phase Components	
Water, tested for UHPLC-MS	14263
Acetonitrile, tested for UHPLC-MS	14261
Methanol, tested for UHPLC-MS	14262
Ammonium acetate, LC-MS Ultra; eluent additive for UHPLC-MS	14267
Acetic acid, eluent additive for LC-MS	49199
Standards and CRMs	
Tetramisole hydrochloride	T1512
(–)-Levamisole hydrochloride solution, 1.0 mg/mL in methanol (as free base), ampule of 1 mL, certified reference material	L-025
Cocaine solution, 1.0 mg/mL in acetonitrile, ampule of 1 mL, certified reference material	C-008
Aminorex solution, 1.0 mg/mL in acetonitrile, ampule of 1 mL, certified reference material	A-040

Benefits of Ascentis Express Phenyl-Hexyl over Biphenyl for the Separation of Pain Management Opiates

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hugh.cramer@sial.com

Pain management drugs are among the most prescribed medications, and they are also the most abused class of prescription drugs.[1-2] Therefore, many states have enacted laws governing the prescription of pain management drugs. Patients enrolled in pain management programs need to be monitored for compliance, which means appropriate use of prescribed drugs and abstinence from non-prescribed drugs. In a clinical setting, the presence of an illicit or non-prescribed drug does not necessarily negate the legitimacy of the patient's pain complaints, but it may suggest a concurrent disorder such as drug abuse or addiction.

High performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS) offers high resolving power, high selectivity, and wide dynamic range, all of which enable simultaneous quantification of a broad spectrum of drugs present in biological matrices.[3] In clinical laboratories, HPLC-MS/MS can be used as a confirmation method in conjunction with a prior immunoassay-based screening method, or it can be used as a stand-alone screening method offering quantitative results with high confidence.

Phenyl-hexyl Column Chemistry Compared to C18 and Biphenyl

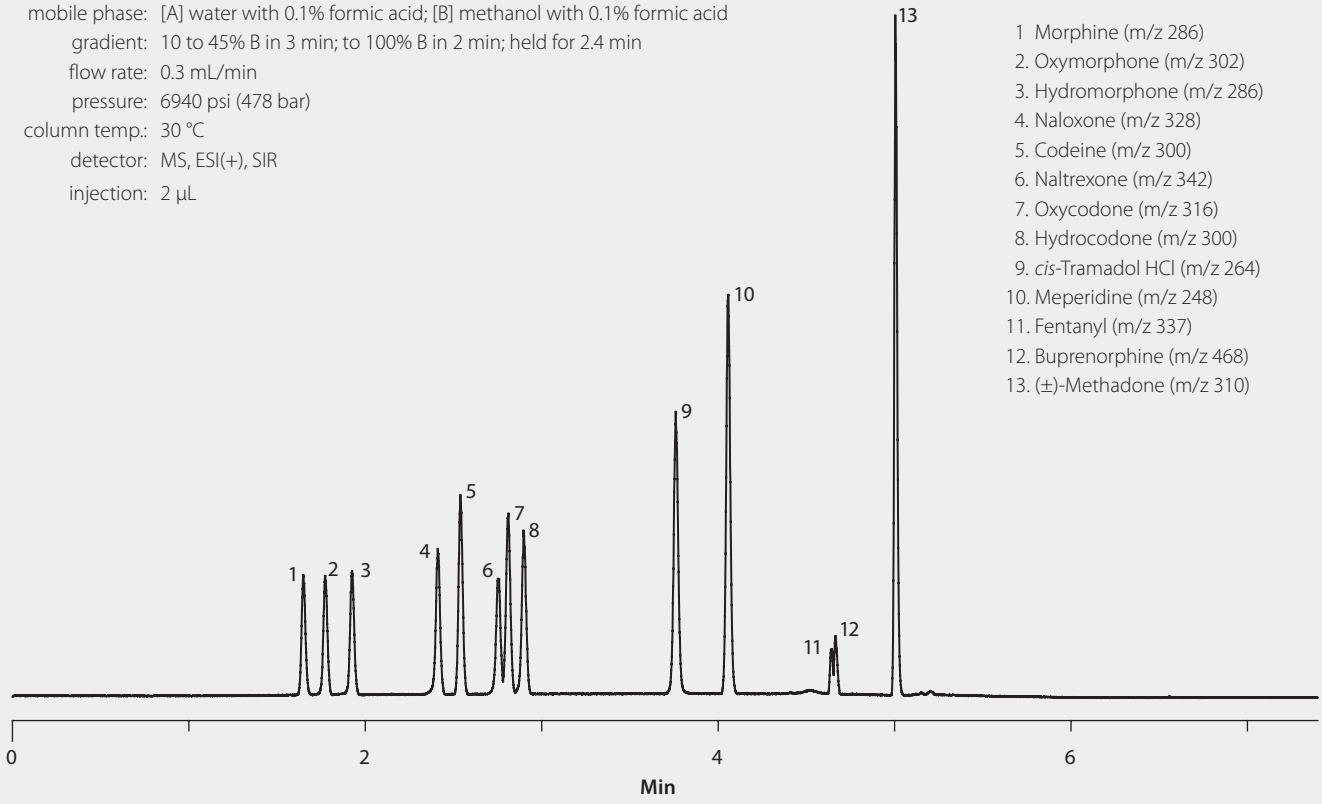
Phenyl-based HPLC phases have proven particularly suited for the separation of pain management compounds. A good example is the Ascentis® Express Phenyl-Hexyl which is based on the covalent modification of high-purity, spherical, Fused-Core® silica particles with alkyl-aromatic functional groups. The distinct selectivity of the phenyl-hexyl phase comes from analytes interacting with the aromatic ring and its delocalized electrons. In **Figure 1**, the phenyl-hexyl phase is shown separating the set of pain management compounds listed **Table 1**.

Table 1. Pain Management Compounds

- Morphine
- Oxymorphone
- Hydromorphone
- Naloxone
- Codeine
- Naltrexone
- Oxycodone
- Hydrocodone
- *cis*-Tramadol HCl
- Meperidine
- Fentanyl
- Buprenorphine
- (±)-Methadone

Figure 1. LC/MS Analysis of Pain Management Opioids on Ascentis Express Phenyl-Hexyl

column: Ascentis Express Phenyl-Hexyl, 10 cm × 2.1 mm, 2.7 µm particles (53336-U)
mobile phase: [A] water with 0.1% formic acid; [B] methanol with 0.1% formic acid
gradient: 10 to 45% B in 3 min; to 100% B in 2 min; held for 2.4 min
flow rate: 0.3 mL/min
pressure: 6940 psi (478 bar)
column temp.: 30 °C
detector: MS, ESI(+), SIR
injection: 2 µL



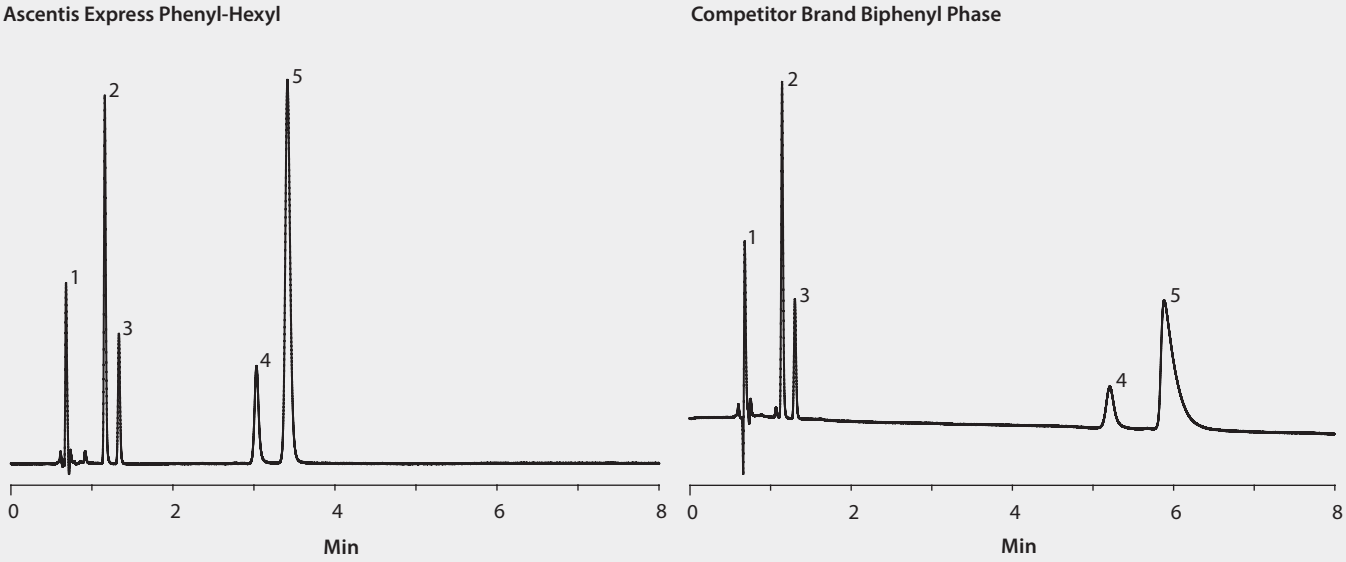
(continued on next page)

Phenyl-hexyl is complementary (orthogonal) to conventional reversed-phase C18 phases because of its aromatic character, and to biphenyl phases because of its aliphatic nature. Although both biphenyl and phenyl-hexyl phases can resolve the pain management compounds, the Ascentis Express Phenyl-Hexyl exhibits substantially less silanol-derived ion exchange activity. To measure this, both phases were run under conditions designed to test for silanol activity.[4] The longer retention and poor peak shape of amitriptyline in the resulting chromatograms in **Figure 2** indicate the strong presence of silanol activity on the competitive brand biphenyl phase that is not seen on Ascentis Express Phenyl-Hexyl. Although one could explain the excessive silanol activity to poor bonded phase coverage on the biphenyl column, it does point to the need to look beyond the bonded phase molecule alone when developing, optimizing, or troubleshooting a method, including when the objective is to resolve pain management compounds.

Figure 2. Probes for Silanol Activity on Phenyl-Hexyl and Biphenyl Phases

Retention of amitriptyline shows peak tailing due to higher silanol activity on the competitive brand biphenyl that is not seen on Ascentis Express Phenyl-Hexyl.

column:	Ascentis Express Phenyl-Hexyl, 10 cm × 2.1 mm I.D., 2.7 µm particles (53336-U) vs. biphenyl column of the same dimensions
mobile phase:	[A] 20 mM potassium phosphate dibasic, pH 7 (adjusted with phosphoric acid); [B] methanol; (20:80, A:B)
flow rate:	0.3 mL/min
column temp.:	35 °C
detector.:	UV, 250 nm
injection:	1 µL
sample:	uracil (5 µg/mL), toluene, ethylbenzene (each 500 µg/mL), quinizarin, amitriptyline (each 50 µg/mL) in 25:75, water:methanol



Conclusion

Ascentis Express Phenyl-Hexyl phase exhibits perfect balance of hydrophobicity, shape selectivity, and bonding density, while delivering excellent peak shape for polar analytes. While a biphenyl phase will also provide resolution, there are subtle differences that can cause poor peak shape due to its higher silanol activity. The combination of unique bonded phase chemistry and Fused-Core silica particles results in Ascentis Express Phenyl-Hexyl showing:

- High aromatic selectivity and high hydrophobic retention
- Excellent peak shape for silanol-sensitive analytes
- High efficiency from the reduced C-term (diffusion) of the Fused-Core particles
- Rugged, reliable, long-term operation, even with biological samples

These benefits make Ascentis Express Phenyl-Hexyl an ideal choice for testing labs running pain management panels.

References

1. Compton, P.; Athanasos, P. Chronic pain, substance abuse and addiction. *Nurs. Clin. North Am.* **2003**, *38*, 525-537.
2. Manchikanti, L.; Cash, K. A.; Damron, K. S.; Manchukonda, R.; Pampati, V.; McManus, C D. Controlled Substance Abuse and Illicit Drug Use in Chronic Pain Patients: An Evaluation of Multiple Variables. *Pain Physician.* **2006**, *9*, 215-226.
3. Porter, W. H. in *Tietz Textbook of Clinical Chemistry and Molecular Diagnostics*, 4th ed.; Burtis, C. A.; Ashwood, E. A.; Bruns, D. E., Eds.; Elsevier Saunders: St. Louis, MO, 2006, 1891.
4. Sander, L. C., Wise, S. A. A new standard reference material for column evaluation in reversed-phase liquid chromatography. *J. Sep. Sci.* **2003**, *26*, 283-294.

Featured Products

Description	Cat. No.
HPLC Column	
Ascentis Express Phenyl-Hexyl, 10 cm × 2.1 mm I.D., 2.7 mm particles	53336-U
Mobile Phase Components	
Water, tested for UHPLC/MS	39253
Methanol, tested for UHPLC/MS	34966
Formic acid, eluent additive for LC/MS	14265
Potassium phosphate, dibasic	60349
Standards	
Buprenorphine solution, 1.0 mg/mL in methanol, ampule of 1 mL, certified reference material	B-044
cis-Tramadol hydrochloride solution, 1.0 mg/mL in methanol (as free base), ampule of 1 mL, certified reference material	T-027
Codeine solution, 1.0 mg/mL in methanol, ampule of 1 mL, certified reference material	C-008
Fentanyl solution, 1.0 mg/mL in methanol, ampule of 1 mL, certified reference material	F-013
Hydrocodone solution, 1.0 mg/mL in methanol, ampule of 1 mL, certified reference material	H-003
Hydromorphone solution, 1.0 mg/mL in methanol, ampule of 1 mL, certified reference material	H-004
Meperidine solution, 1.0 mg/mL in methanol, ampule of 1 mL, certified reference material	M-035
(±)-Methadone solution, 1.0 mg/mL in methanol, ampule of 1 mL, certified reference material	M-007
Morphine solution, 1.0 mg/mL in methanol, ampule of 1 mL, certified reference material	M-005
Naloxone solution, 1.0 mg/mL in methanol, ampule of 1 mL, certified reference material	N-004
Naltrexone solution, 1.0 mg/mL in methanol, ampule of 1 mL, certified reference material	N-007
Oxycodone solution, 1.0 mg/mL in methanol, ampule of 1 mL, certified reference material	O-002
Oxymorphone solution, 1.0 mg/mL in methanol, ampule of 1 mL, certified reference material	O-004

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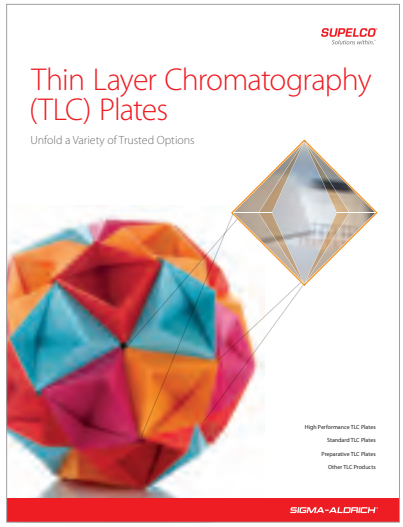

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Flow-Modulation Comprehensive 2D GC/MS for the Determination of Allergens in Fragrances

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Introduction

Perfumes are complex mixtures comprising essential oils, aroma compounds, fixatives, and solvents. Contact dermatitis, an allergic response, may develop following skin contact with a sufficient amount of these substances in susceptible individuals. Rashes are most common on the face, under the arms, and on the hands, areas most often in direct contact with perfumes and other cosmetics. While *per se* a latent condition without visible signs or symptoms, the sensitivity can persist lifelong. The condition can be severe and generalized, with a significant impairment to quality of life and potential consequences for ability to work.

In 1999, the European Commission's Scientific Committee on Cosmetic Products and Non-Food Products (SCCNFP) identified a set of 26 fragrance allergens with a well-recognized potential to cause contact allergy, for which information should be provided to consumers about their presence in cosmetic products.[1] Such a list has shown to be important in the clinical management of patients who are allergic to one or more of these 26 fragrance chemicals. In fact, these compounds must be reported in the ingredient list of cosmetic products sold in Europe so they can be avoided by patients with known allergic reactions. The aforementioned allergens are regulated by the European Directive 2003/15/EC fixing a maximum residue limit for "leave-on" and "rinse-off" products of 0.001% (10 ppm) and 0.01% (100 ppm), respectively.[2]

In 2012, the revision of the SCCNFP Opinion (SCCS/1459/11, June 2012) on the clinical and experimental data published in 1999, confirmed the previously regulated allergens and stressed attention should be paid to additional fragrance substances which have been shown to be sensitizers in humans. Consequently, an updated list of allergens was reported, with a systematic and critical review of the scientific literature to identify them, both as single chemicals and as natural extracts. Eighty-two compounds were finally classified as contact allergens in humans, of which 54 were single chemicals and 28 were natural extracts.[3]

The Scientific Committee on Consumer Safety (SCCS) examined available elicitation dose-response data to decide whether safe thresholds can be established for the fragrance allergens. The few studies available indicated that a general level of exposure of up to 0.8 µg/cm^2 (0.01% in cosmetic products) may be tolerated by most consumers. The SCCS opinion is that levels of exposure below this would be sufficient to prevent elicitation for the majority of allergic individuals, unless there is experimental or clinical substance-specific data allowing the derivation of individual thresholds. With regards to the natural extracts, it was not possible to provide a safe threshold as no specific investigations exist and the model providing the general threshold (<0.01%) was based on individual chemicals. However, the SCCS considers that the maximum use concentration applied to the identified chemicals, both if added as chemicals or as an identified constituent of a natural ingredient, will reduce the risk of sensitization and elicitation from natural extracts.

The aim of the present research was the development of a flow-modulation comprehensive 2D GC/MS (FM GCxGC/MS) method for the determination of the recently indicated 54 allergens in fragrances. The detector was a rapid-scanning single quadrupole MS (qMS).

Experimental

Fifty-three allergen standards with purity higher than 95% were used. A standard for santolol (mixture of α- and β-santalol) was not commercially available, thus it was isolated from sandalwood oil using a preparative MDGC system developed in our laboratory. Methanol (purity >99 %) was used to prepare the calibration solutions. Two fragrances were purchased in a local store. Calibration solutions and both samples were analyzed using the conditions listed in **Figure 1**. Mass spectral matching was carried out by using the FFNSC database.[4]

Results and Conclusion

The 54 standard allergens, plus two internal standards (50 ppm), were used for calibration purposes (5, 10, 20, 50, and 100 ppm). Quantification was performed by using extracted ion chromatograms (**Table 1**). There are more than 54 allergens reported in **Table 1** because some are considered to be mixtures (e.g. α- and β-santalol), and are here listed as single compounds. Method linearity was satisfactory, being in the range 0.9950-0.9988 (coefficients of determination). Method limits of quantification (LOQ) were all lower than 1 ppm, which is well below the European Commission's regulated limit. LOQ values were extrapolated by considering a signal-to-noise (s/n) value of 10.

Two samples (1 and 2) of perfume were subjected to analysis. The quantification values expressed in ppm are reported in **Table 1**. Altogether 14 allergens were quantified in each sample (n = 2). The full-scan GCxGC-qMS chromatograms of sample 1 and 2 are shown in **Figure 1**. As can be seen, the SLB-5ms/SLB-35ms combination produced a satisfactory distribution of the analytes across the 2D space. Both columns are characterized by high thermal stability as can be seen from the absence of column bleed in the chromatogram. Moreover, retention time precision was excellent over a prolonged period on time (45 days of applications), confirming the stability of the 5ms and 35ms phases in each dimension.

The proposed method appears to be a reliable and sensitive approach for the determination of allergens in perfumes following the SCCNFP Opinion (SCCS/1459/11, June 2012).

Figure 1. GCxGC-qMS Chromatograms of Perfume Samples 1 and 2

(See Table 1 for component identification.)

column: ([1]D) SLB-5ms, 20 m x 0.18 mm I.D., 0.18 µm (28564-U)

column: ([2]D) SLB-35ms, 5 m × 0.25 mm I.D., 0.25 µm; cut from a 30 m × 0.25 mm I.D., 0.25 µm column (29804-U)

oven: ([1]D) 45 °C, 3 °C/min to 230 °C

oven: ([2]D) 50 °C, 3 °C/min to 230 °C

oven: (flow modulator) stainless steel accumulation loop (20 cm x 0.71 mm O.D. x 0.52 mm I.D.), modulation period 5.4 sec (accumulation period 4.9 sec, injection period 0.5 sec)

inj. temp.: 310 °C

detector: qMS, 200 °C, m/z = 40-360

MSD interface: 250 °C

carrier gas: ([1]D) helium, 0.46 mL/min

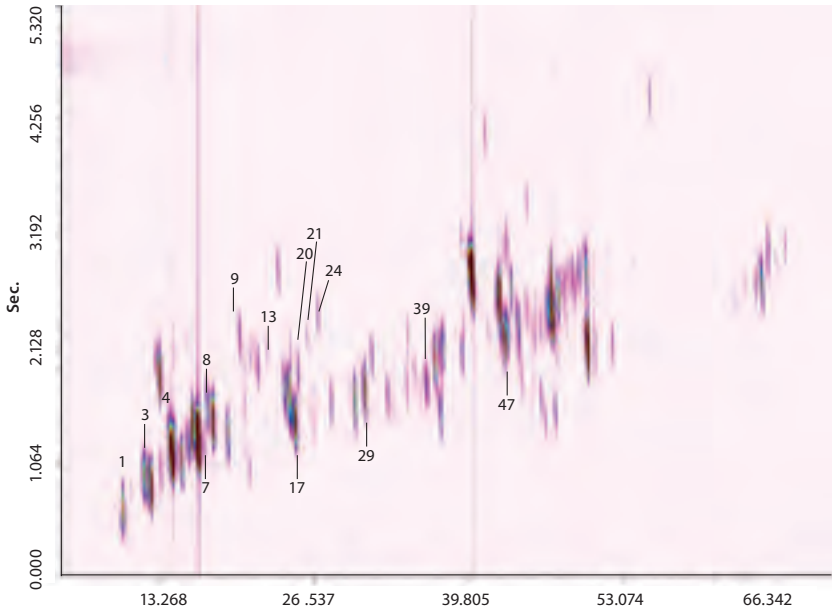
carrier gas: ([2]D) helium, 7 mL/min

injection: 1 µL, 10:1 split

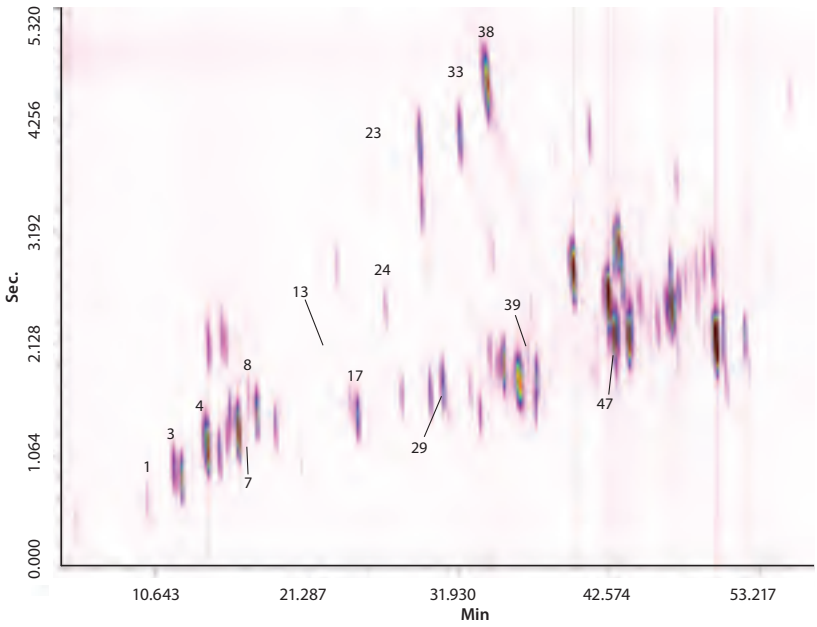
liner: 3.4 mm I.D., split/splitless type, wool packed straight FocusLiner™ design (2877601-U)

sample: perfume

Perfume Sample 1



Perfume Sample 2



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References

1. European Commission, Health and Food Safety "SCCS final opinion on Fragrance allergens in cosmetic products now available." http://ec.europa.eu/dgs/health_consumer/dyna/enews/enews.cfm?al_id=1283 (accessed June 2, 2015).
2. Directive 2003/15/EC of the European Parliament and of the Council of the European Union 27 February 2003 amending Council Directive 76/768/EEC on the approximation of the laws of the Member States relating to cosmetic products. http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2003:066:0026:0035:en:PDF (accessed June 2, 2015).
3. European Union Scientific Committee on Consumer Safety (SCCS) Opinion on fragrance allergens in cosmetic products. http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_102.pdf (accessed June 2, 2015).
4. Mondello, Luigi. *Flavors and Fragrances of Natural and Synthetic Compounds 2, 2nd Edition*. John Wiley & Sons, Inc.: Hoboken, NJ, 2011; ISBN 978-1-118-14583-8.

Featured Products

Description	Cat. No.
GC Capillary Columns	
SLB-5ms, 20 m × 0.18 mm I.D., 0.18 µm	28564-U
SLB-35ms, 30 m × 0.25 mm I.D., 0.25 µm (a 5 m portion was used in this study)	29804-U
Solvents	
Methanol, for GC/MS analysis of volatile organics, ≥99.9%	414816
Standards	
See Table 1 for part numbers	

Table 1. List of Allergens and Internal Standards, Linearity, Quantification Ions, and Concentrations in Two Perfume Samples

No.	Compound	R^2	Quantifier Ion	Sample 1 (ppm)	Sample 2 (ppm)	Cat. No.
1	α-Pinene	0.9969	93	76.8	27.4	80605
2	Benzaldehyde	0.9972	77	—	—	09143
3	β-pinene	0.9968	69	597.1	375.3	80607
4	Limonene	0.9981	93	353.6	322.4	62118, 62128
5	Benzyl Alcohol	0.9973	108	—	—	47509-U
6	Salicylaldehyde	0.9979	122	—	—	03273
7	Terpinolene	0.9960	93	4.9	5.3	86485
8	Linalool	0.9974	93	264.1	247.1	61706, 51782
9	Camphor	0.9968	95	23.3	—	21300
10	Menthol	0.9970	81	—	—	05174
ISTD	Benzene, 2-Bromoethenyl		152	—	—	157449
11	Methyl 2-Octynoate	0.9983	95	—	—	68982
12	Methyl Salicylate	0.9980	120	—	—	76631
13	α-Terpineol	0.9972	93	40	26.7	83073
14	β-Terpineol	0.9971	93	—	—	—
15	γ-Terpineol	0.9971	93	—	—	—
16	Citronellol	0.9952	69	—	—	303461, 303488, W230901
17	Linalyl Acetate	0.9977	93	81	168.4	49599
18	Neral (Citral component)	0.9976	69	—	—	43318
19	Carvone	0.9988	82	—	—	22060, 22070
20	Geraniol	0.9966	69	32.1	—	48798
21	Geranial (Citral component)	0.9958	69	268.2	—	43318
22	Farnesol	0.9958	69	—	—	43348
23	Cinnamal (Cinnamaldehyde)	0.9958	131	—	4.4	W228613
24	Hydroxycitronellal	0.9964	59	36.8	54.8	66010
25	Anise Alcohol (Anisyl alcohol)	0.9965	138	—	—	W209902, W209910
26	*trans*-Anethol	0.9951	148	—	—	10368
27	Dimethylbenzyl Carbinyl Acetate	0.9958	132	—	—	W363200
28	Amyl Cinnamyl Alcohol	0.9984	133	—	—	W206504
29	Eugenol	0.9978	164	50.5	36.1	35995
30	δ-Damascone	0.9972	69	—	—	55137
31	Rose Ketone-4 (Damascenone)	0.9965	69	—	—	W342017
32	α-Damascone	0.9969	69	—	—	59574
33	Vanillin	0.9950	152	—	259	30304
34	*cis*-β-Damascone	0.9969	177	—	—	W324300
35	Trimethylbenzene Propanol (Majantol)	0.9970	106	—	—	69791
36	Ebanol	0.9976	121	—	—	44064
37	Isoeugenol	0.9982	164	—	—	34038
38	Coumarin	0.9981	118	—	27.9	72609
39	α-Isomethyl Ionone	0.9979	135	5.6	3.7	W271410
40	Butylphenyl Methylpropional	0.9983	189	—	—	95338
41	Amyl Salicylate	0.9983	120	—	—	44041
42	6-Methylcoumarin	0.9952	160	—	—	69391
43	Propylidene Phthalide	0.9978	159	—	—	75012
44	β-Caryophyllene oxide	0.9979	93	—	—	91034
45	Amyl Cinnamal (α-Amylcinnamaldehyde)	0.9979	129	—	—	64397
46	Lyral	0.9982	136	—	—	55862
47	Tetramethyl Acetylocta-hydronaphthalenes	0.9986	109	551.1	480.4	—
48	Cinnamyl Alcohol	0.9974	92	—	—	93066
49	Hexyl Cinnamal (α-Hexylcinnamaldehyde)	0.9979	129	—	—	09178
50	Benzyl Benzoate	0.9978	105	—	—	68183, 55177
51	Acetyl Cedrene	0.9981	43	—	—	18017
52	Hexamethylindanopyran	0.9983	243	—	—	W520608
53	Benzyl Salicylate	0.9977	91	—	—	51031
54	Hexadecanolactone	0.9982	69	—	—	14643
ISTD	4,4′-Dibromobiphenyl		152	—	—	442398
55	Benzyl Cinnamate	0.9955	192	—	—	69139
56	Sclareol	0.9981	95	—	—	49944
57	β-Santalol	0.9950	94	—	—	75831
58	α-Santalol	0.9954	94	—	—	75831

Dual-Column GC Analysis of Organochlorine Pesticides on SLB-5ms and SLB-35ms

Lynne Perez-Blanco, 2015 R&D Intern (Pennsylvania State University); Katherine K. Stenerson, Principle R&D Scientist; and Michael D. Buchanan, Product Manager
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The analysis of low levels of 20 organochlorine pesticides and 2 surrogate compounds by gas chromatography-electron capture detector (GC-ECD) is routinely performed by analysts in environmental laboratories. Methods require the analysis of each sample extract on two columns with orthogonal selectivities for confirmation of identifications.

An ECD is highly sensitive, capable of detecting analytes at picogram levels. Its highly sensitive nature, in combination with the low detection limit requirements of promulgated methods (i.e. US EPA Method 608, 8081, and OLM04.2 PEST), make it necessary to use GC columns with low bleed. In addition, the susceptibility of several analytes to degradation and adsorption make inertness another extremely important characteristic when choosing analytical columns.

Dual-Column Analysis

This application requires two analytical columns for the analysis of samples. The first, often referred to as the "primary" column, is used to determine, by retention time comparison with a standard, if any of the target analytes could be present in the sample. If peaks are found within an analyte's retention time window on the primary column, the presence of the analyte must be "confirmed" on a secondary or "confirmation" column with a different selectivity in order to be considered positive.

Our SLB®ms column family is designed for GC and GC-MS analysts who require low bleed, inert, durable, and consistent capillary GC columns. Their use can help achieve low detection limits, easy mass spectral identification, less instrument downtime, good resolution, short analysis times, and long column life. The SLB-5ms is virtually equivalent in polarity to a poly (5% diphenyl/95% dimethyl siloxane) polymer stationary phase. The SLB-35ms is virtually equivalent in polarity to a poly (35% diphenyl/65% dimethyl siloxane) polymer stationary phase. A complete list of column specifications can be found in **Table 1**. Together, these two columns offer the orthogonal selectivity and stringent performance characteristics that are necessary to successfully perform the analysis of organochlorine pesticides using the dual-column approach previously described.

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A low-level mixture of twenty organochlorine pesticides and two surrogate compounds, each analyte at 50 ppb, was prepared in hexane. This mixture was first injected on the SLB-5ms column using conditions which maximized resolution. The mixture was then injected on the more polar SLB-35ms column using the same run conditions. The resulting chromatograms are shown in **Figure 1** (SLB-5ms) and **Figure 2** (SLB-35ms).

On both columns, peaks were easily integrated and baselines were exceptionally stable, with minimal rise observed, even when running to a final oven temperature of 340 °C. The ability of both columns to be used at a high final oven temperature allowed for elution of the last peak, decachlorobiphenyl, in less than 20 minutes. These run conditions were chosen to reduce analysis time, but a slower oven ramp rate can be used to increased resolution if desired.

Table 1. Column Specifications

SLB-5ms

- **Application:** The 5% phenyl equivalent phase provides a boiling point elution order with a slight increase in selectivity, especially for aromatic compounds. The low bleed characteristics, inertness, and durable nature make it the column of choice for environmental analytes (such as semivolatiles, pesticides, PCBs, and herbicides) or anywhere a low bleed non-polar column is required.
- **USP Code:** This column meets USP G27 and G36 requirements.
- **Phase:** Bonded and highly crosslinked; silphenylene polymer virtually equivalent in polarity to poly(5% diphenyl/95% dimethyl siloxane)
- **Temp. Limits:** ≤0.32 mm I.D.: -60 °C to 340 °C (isothermal) or 360 °C (programmed)
- **Temp. Limits:** ≥0.53 mm I.D.: -60 °C to 330 °C (isothermal) or 340 °C (programmed)

SLB-35ms

- **Application:** The 35% phenyl equivalent phase provides a higher polarity option compared to columns containing a lower phenyl content, resulting in a greater retention of polar analytes relative to non-polar compounds. Its selectivity is complementary when paired with SLB-5ms for applications that require dual-column confirmatory analysis, such as for environmental analytes (such as pesticides, PCBs, and herbicides). The low bleed characteristics, inertness, and durable nature make it a great column anywhere a low bleed intermediate polar column is required.
- **USP Code:** This column meets USP G42 requirements.
- **Phase:** Bonded and highly crosslinked; proprietary polymer virtually equivalent in polarity to poly(35% diphenyl/65% dimethyl siloxane)
- **Temp. Limits:** ≤0.32 mm I.D.: ambient to 350 °C (isothermal) or 360 °C (programmed)
- **Temp. Limits:** ≥0.53 mm I.D.: ambient to 330 °C (isothermal) or 340 °C (programmed)

(continued on next page)

Figure 1. Organochlorine Pesticides on SLB-5ms

column : SLB-5ms, 30 m × 0.25 mm I.D., 0.25 µm (28471-U)
oven : 100 °C (2 min), 15 °C/min to 340 °C (5 min)
inj. temp. : 250 °C
carrier gas : helium, 0.9 mL/min
detector : µECD, 340 °C
injection : 1.0 µL, splitless (0.75 min)
liner : 4 mm I.D., split/splitless type, wool packed single taper FocusLiner™ design
sample : 200 ppm organochlorine pesticide standard mix (CRM46845) containing 22 analytes, diluted to 50 ppb with hexane

1. 2,4,5,6-Tetrachloro-*m*-xylene (surr.)
2. α-BHC
3. β-BHC
4. γ-BHC (Lindane)
5. δ-BHC
6. Heptachlor
7. Aldrin
8. Heptachlor epoxide
9. γ-Chlordane
10. Endosulfan I
11. α-Chlordane
12. 4,4′-DDE
13. Dieldrin
14. Endrin
15. 4,4′-DDD
16. Endosulfan II
17. Endrin aldehyde
18. 4,4′-DDT
19. Endosulfan sulfate
20. Methoxychlor
21. Endrin ketone
22. Decachlorobiphenyl (surr.)

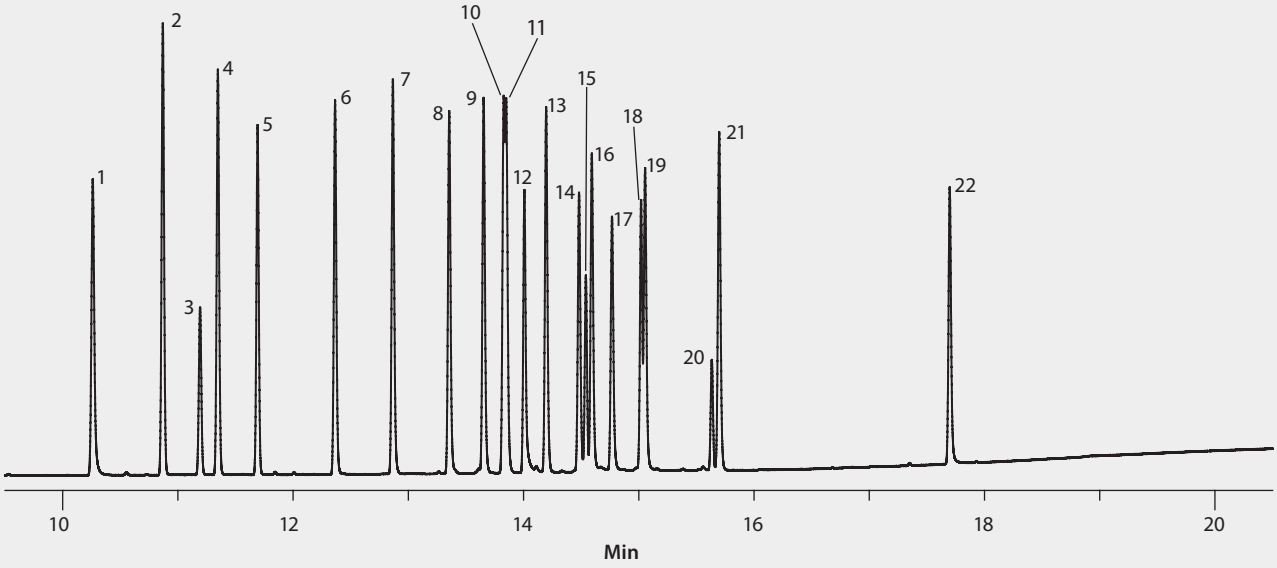
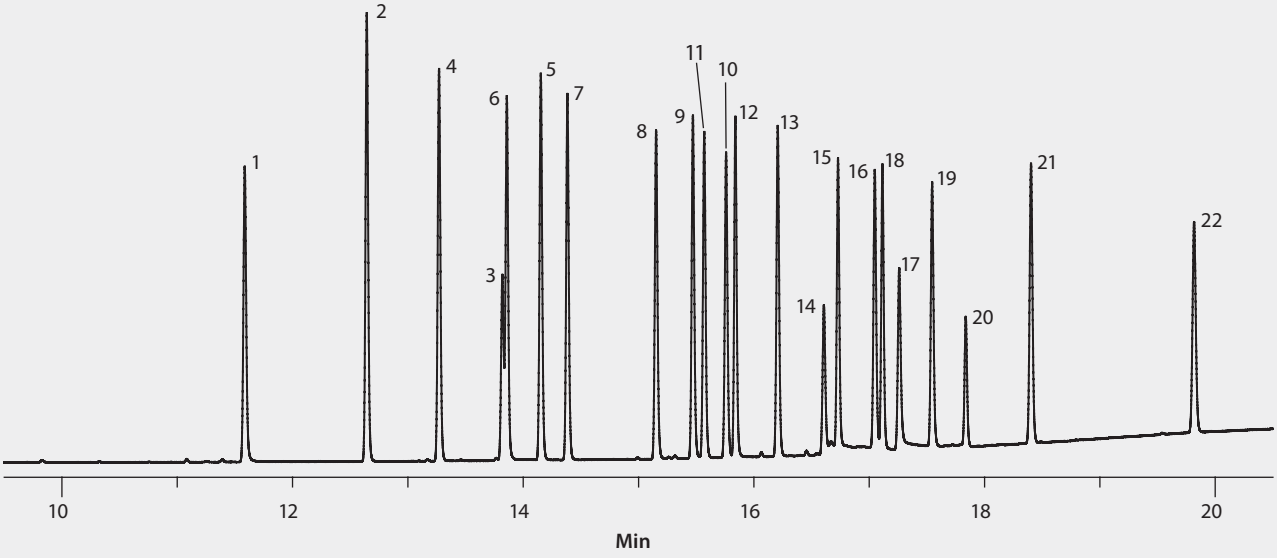


Figure 2. Organochlorine Pesticides on SLB-35ms

column: SLB-35ms, 30 m x 0.25 mm I.D., 0.25 µm (29804-U)
All other conditions and peak IDs are the same as Figure 1



Discussion

SLBms columns were developed for use in applications using a mass selective detector (MSD). Their extremely low bleed characteristics also make them useful for applications requiring the use of other highly sensitive detectors, such as an ECD.

The proprietary surface deactivation of SLBms columns provides the inertness necessary for the analysis of compounds susceptible to degradation and adsorption, such as organochlorine pesticides. For example, endrin and 4,4′-DDT are susceptible to degradation, and must be monitored. Both of these analytes exhibited good response on both columns.

Conclusion

As shown, the SLB-5ms/SLB-35ms pair is an effective column set for the dual-column GC analysis of low levels of organochlorine pesticides.

Featured Products

Description	Cat. No.
SLB-5ms, 30 m × 0.25 mm I.D., 0.25 µm	28471-U
SLB-35ms, 30 m × 0.25 mm I.D., 0.25 µm	29804-U

Related Products

Description	Cat. No.
SLB-5ms Capillary GC Columns	
15 m × 0.10 mm I.D., 0.10 µm	28466-U
20 m × 0.18 mm I.D., 0.18 µm	28564-U
30 m × 0.25 mm I.D., 0.10 µm	28467-U
15 m × 0.25 mm I.D., 0.25 µm	28469-U
60 m × 0.25 mm I.D., 0.25 µm	28472-U
30 m × 0.25 mm I.D., 0.50 µm	28473-U
60 m × 0.25 mm I.D., 0.50 µm	28474-U
30 m × 0.32 mm I.D., 0.25 µm	28482-U
30 m × 0.53 mm I.D., 0.50 µm	28541-U
30 m × 0.53 mm I.D., 1.00 µm	28559-U
SLB-35ms Capillary GC Columns	
15 m × 0.10 mm I.D., 0.10 µm	29808-U
20 m × 0.18 mm I.D., 0.18 µm	29809-U
30 m × 0.25 mm I.D., 0.10 µm	29802-U
15 m × 0.25 mm I.D., 0.25 µm	29803-U
60 m × 0.25 mm I.D., 0.25 µm	29805-U
30 m × 0.25 mm I.D., 0.50 µm	29806-U
60 m × 0.25 mm I.D., 0.50 µm	29807-U
30 m × 0.32 mm I.D., 0.25 µm	29810-U
60 m × 0.32 mm I.D., 0.25 µm	29811-U
30 m × 0.53 mm I.D., 0.50 µm	29812-U
30 m × 0.53 mm I.D., 1.00 µm	29814-U
Analytical Standard	
EPA 8081 Pesticide Standard Mix	CRM46845

Related Information

For additional chromatograms, product information, real-time availability, pricing, and ordering, visit our SLBms page:
sigma-aldrich.com/slb

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Passive Air Sampling Tubes for US EPA Method 325

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Supelco's FLM™ Carbopack™ X Thermal Desorption Tubes are designed for the new US EPA Method 325. This new method is specific for monitoring the air concentration of volatile organic compounds emitted from petroleum refineries. There are approximately 140 active petroleum refineries operating in the United States. The target compounds for the method include a number of Hazardous Air Pollutants (HAPs) including benzene, toluene, and xylenes just to name a few. This method uses thermal desorption sampling tubes to passively collect air samples along the perimeter of the property. The air concentration is determined by analyzing the tubes using thermal desorption and gas chromatography. The method requires the sampling tubes be placed in a non-emitting shelter to protect the tubes from the weather elements as shown in **Figure 1**. The shelters are permanently installed along the fence line (property line) of the refinery, and the sampling tubes are replaced from the shelters every 14 days. The required quantity and placement of the air sampling tubes is based on the size of the property, and prevailing winds. The specifics of the tube placement is detailed in the proposed method.

Figure 1. FLM Carbopack X Tubes Placed in a Shelter

Shelter supplied courtesy of Enthalpy Analytical Inc. Durham NC.

The FLM Carbopack X passive sampling tube is made of stainless steel and has the dimensions of 6.35 mm O.D. × 5 mm I.D. × 89 mm long (about the size of ball-point pen as shown in **Figure 2**. The tube is packed with Carbopack™ X. The physical design of the stainless steel tube has been in used for well over 25 years, and is well accepted in the market place for collecting both passive and active air samples. This particular size tube fits into a number of thermal desorbers made by different manufacturers, such as PerkinElmer®, Markes International®, DANI™, and Shimadzu™.

Figure 2. Size Comparison of the FLM Carbopack X Tube

These passive sampling tubes work by collecting the air sample without the need of an air sampling pump to physically pull the air sample through the adsorbent. The FLM Carbopack X tube relies on the natural movement (of the HAP's) across a concentration gradient inside the inlet of the tube called the "air gap". The volatile HAPs travel through the air gap and are adsorbed by the Carbopack X contained inside the tube.

Carbopack X is a granular graphitized carbon black (GCB) adsorbent with a surface area of 240 m^2/g. This particular Carbopack contains pores in the meso range that allows it to retain a broader range of volatile analytes than other GCB's, or porous polymers like Tenax® TA. Carbopack X is hydrophobic, so no appreciable amount of water vapor is retained when sampling in humid conditions. The sampling tubes are packed with 40/60 mesh material. This mesh size packs uniformly in the tube and prevents channeling along the walls of the tube from occurring during sampling. The pre-conditioned FLM Carbopack X tube comes supplied with brass endcaps on each end of the tube that creates an impermeable seal for the Carbopack X before and after sampling. To collect an air sample, the brass endcap from the inlet side is removed and is replaced with a diffusive sampling cap as shown in **Figure 3** (Cat. No. 28017-U). The brass endcap on the outlet of the tube remains attached during the 14-day sampling period.

Figure 3. Diffusive Sampling Caps

This diffusive sampling cap serves two purposes; first it defines the starting point of the "air gap" between the sampling cap and the Carbopack X adsorbent. Second it prevents abnormal air movement within the diffusive air gap when the tubes are deployed in windy locations.

The rate at which the volatile HAPs are retained by the Carbopack X is called the uptake rate, or passive sampling rate. Each compound has its own uptake rate because of its unique chemical properties, and how it interacts with the particular adsorbent. The US EPA has determined the uptake rate for a number of the volatile (HAPs) specific for Carbopack X and are listed in the method.

The concept of passive sampling is based on Fick's law, which states a gas molecule will diffuse from an area of higher concentration to an area of lower concentration according the equation below:

Fick's First Law of Diffusion:

$$Q = D (A/L) C T$$

Where:
Q = amount collected (ng)
D = diffusive uptake rate (cm^2/min)
A = cross-sectional area of the air gap (cm^2)
L = length of the air gap (cm)
C = airborne concentration (mg/m^3)
T = sampling time (min)

During manufacturing we have several in-process inspections to assure you're receiving the highest quality sampling tubes. We batch test every lot of tubes for back pressure, which assures a consistently packed tube. The benzene emissions from the refineries are of specific concern because of its toxic properties. We perform a final QC test for each pack of tubes to assure the benzene background is below 2 nanograms per tube. The actual QC testing report is included with each box of tubes as shown in **Figure 4**.

Figure 4. Example of the QC Testing Report

Because the quantity of sampling tubes required for Method 325 can be significant, an efficient way to identify the tubes is essential. So each tube includes a barcode along with its corresponding unique ID number permanently etched on the outside of each tube (**Figure 5**). This assures traceability of the samples and helps maintain the chain of custody throughout the sampling process. A barcode reader can automate portions of the data collection process, but reading the barcode on shinny curved surface can be challenging for common laser based readers. By using an image based reader such as, a DataMan® 8600 from Cognex or a PowerScan™ PD9530-DPM from Datalogic, assures the barcode reader can detect the barcode on the tube quickly and efficiently. In addition we also etch the FLM Carbopack X name on the opposite side of the tube so you're assured the correct tube is being used to collect the sample (**Figure 6**).

Figure 5. An FLM Carbopack X Tube with a Barcode and a Unique ID Number

Figure 6. FLM Carbopack X Etching (shown with a diffusive sampling cap sold separately)

We source high quality stainless steel (grade 316) to manufacture our tubes, and also provide an additional level of protection for the FLM Carbopack X tubes by deactivating the stainless steel prior to packaging. Our Supelcoat™ deactivation process produces a ceramic like protective coating on the stainless steel surface. The coating covalently bonds to the steel surface and protects the surface from oxidation. The Supelcoat treatment is stable to (>400 °C), and creates a reproducible surface on the inner diameter of the tubes that withstands repeated use.

We have used the Supelcoat deactivation for more than 10 years on passive sampling tubes supplied for various US EPA air sampling projects like the Detroit Exposure Aerosol Research Study (DEARS). DEARS was a large air monitoring effort which used several different air sampling devices/techniques to collect a broad range of different pollutants. Other studies included the Dallas Traffic Related Exposures to Air Toxics (DTREAX), Detroit Children's Health (DCHS), and Beaumont-EPA Region 6 Air Toxics Monitoring Study.

The new FLM Carbopack X tube is designed for the US EPA Method 325 method. We have incorporated several important features that make the tubes ready-to-use for most applications. The tubes are pre-conditioned, and each box of tubes is QC tested to assure low background levels. Each tube includes a unique barcode, and an ID number permanently etched on the tubes, to aid in tracking the tubes. These tubes have a long proven track record for a number of US EPA air sampling projects.

Featured Products

Description	Qty.	Cat. No.
Pre-Conditioned FLM SS TD Tube packed w/Carbopack X	10	28686-U
Diffusive Endcaps (standard)	10	28017-U

Improved Resolution of Benzene (and Other Aromatics) and Oxygenates in Reformulated Gasoline Using a One-Column Approach

Leonard M. Sidisky, R&D Manager; Gustavo Serrano Izaguirre, R&D Scientist; and Michael D. Buchanan, Product Manager
mike.buchanan@sial.com

The amount of benzene in gasoline is a concern because it is a known human carcinogen, and exposure to it has been linked to detrimental health effects. The challenge with the analysis lies in the complex composition of gasoline, which consists of hundreds of different compounds. Reformulated gasoline also contains additives to produce more complete combustion and subsequent lower emissions of harmful compounds. These additives accomplish this by boosting the oxygen content, and are commonly referred to as "oxygenates." Ethanol is a commonly used oxygenate. Therefore, to measure benzene in reformulated gasoline, it must be resolved from the aliphatic hydrocarbons, other aromatics, ethanol, plus any other oxygenates. This typically requires the use of a two-column switching procedure.[1]

SLB-ILD3606 Chemistry

We developed SLB-ILD3606, a capillary gas chromatography (GC) column engineered for the determination of benzene (and other aromatics) and oxygenates in gasoline. **Table 1** lists its specifications. It is a modified (inert) version of SLB-IL111, a GC column that employs an imidazolium dicationic ionic liquid stationary phase. This new column provides the unique selectivity of the extremely polar SLB-IL111, but with improved peak shapes for oxygenates, resulting in improved resolution for all analytes.

Table 1. Column Specifications

Application: Modified (deactivated) version of SLB-IL111 provides better inertness. Each column is tested to ensure resolution and sharp peak shapes of aromatics and alcohols. Excellent at separations involving benzene (and other aromatics) and oxygenates in petroleum products, such as gasoline. Also a good GCxGC column choice. Launched in 2015.

USP Code: None

Phase: Non-bonded; 1,5-di(2,3-dimethylimidazolium)pentane bis(trifluoromethylsulfonyl)imide

Temp. Limits: 50 °C to 260 °C (isothermal or programmed)

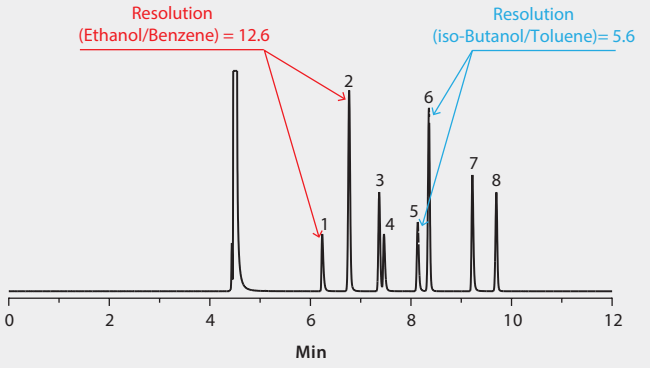
Aromatic and Alcohol Mix

To show selectivity and inertness capabilities, a mixture containing two aromatics, five alcohols, and one ketone was prepared in isooctane following the guidelines for a control standard to be used with ASTM® D3606. This mix was analyzed using temperature programming, and the resulting chromatogram is shown in **Figure 1**. The inertness of the column resulted in sharp peak shapes for all alcohols, which in turn resulted in great resolution between ethanol and benzene (R_S=12.6), and also between *iso*-butanol and toluene (R_S = 5.6).

Figure 1. Aromatic and Alcohol Standard

column: SLB-ILD3606, 60 m × 0.25 mm I.D., 0.20 µm (29691-U)
oven: 50 °C (6 min), 15 °C/min to 265 °C (10 min)
inj. temp.: 250 °C
carrier gas: helium, 21 cm/sec
detector: FID, 250 °C
injection: 1 µL, 100:1 split
liner: 4 mm I.D., split type, cup design
sample: 8 analytes at various concentrations in isooctane

1. Ethanol
2. Benzene
3. *sec*-Butanol
4. *n*-Propanol
5. *iso*-Butanol
6. Toluene
7. *n*-Butanol
8. Methyl isobutyl ketone (MIBK)



Reformulated Gasoline Sample

Figure 2 shows the chromatogram resulting from the analysis of a reformulated gasoline sample on SLB-ILD3606. As shown:

- The extremely polar selectivity of this column resulted in the elution of benzene after the aliphatic portion, and also great resolution between benzene and ethanol
- Several other aromatic and oxygenate compounds are also fully resolved
- A few aromatics (e.g. *p*-/*m*-xylene) and oxygenates (e.g. MTBE, TAME) are partially resolved
- This column can be used up to 260 °C, so allows the timely elution of the heavy polycyclic aromatic hydrocarbon (PAH) constituents in gasoline
- The phase stability of the SLB-ILD3606 column exhibits a stable baseline when subjected to a temperature ramp

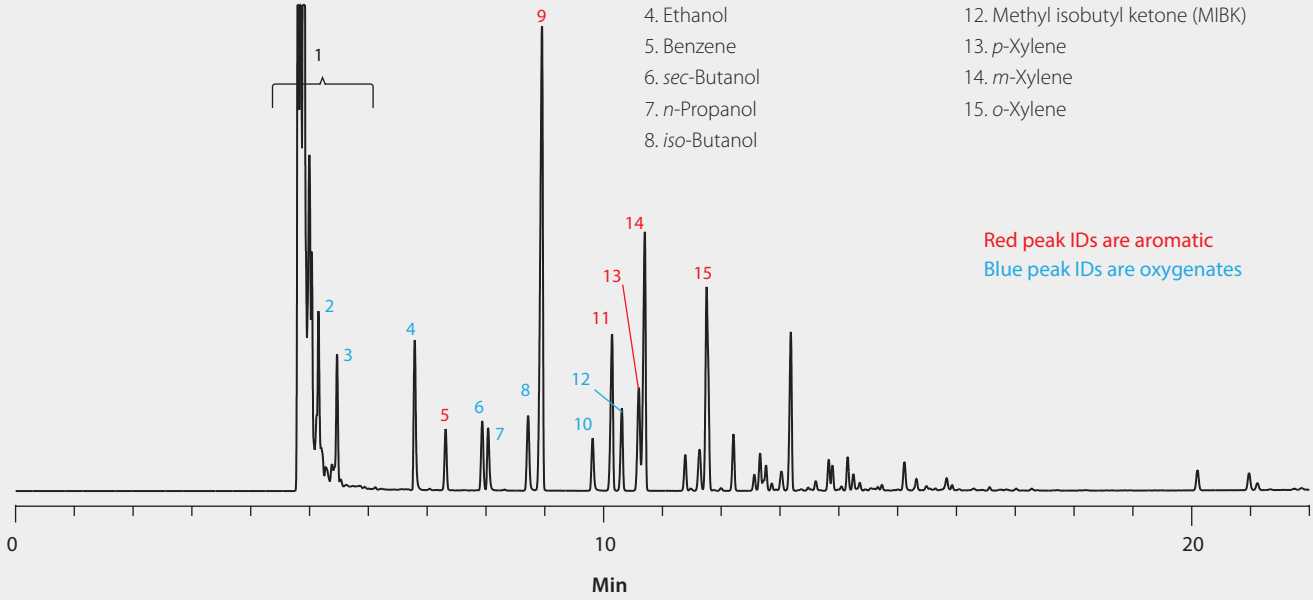
These observations indicate the SLB-ILD3606 is an effective alternative to the two-column switching procedure currently required for the determination of benzene and other aromatics in reformulated gasoline.

Figure 2. Reformulated Gasoline

sample: reformulated gasoline (contains 10% ethanol) with 7 other oxygenates added (at 2.5-5%)
All other conditions the same as Figure 1

1. C5-C11 aliphatic hydrocarbons
2. Methyl tert-butyl ether (MTBE)
3. *tert*-Amyl butyl ether (TAME)
4. Ethanol
5. Benzene
6. *sec*-Butanol
7. *n*-Propanol
8. *iso*-Butanol
9. Toluene
10. *n*-Butanol
11. Ethylbenzene
12. Methyl isobutyl ketone (MIBK)
13. *p*-Xylene
14. *m*-Xylene
15. *o*-Xylene

Red peak IDs are aromatic
Blue peak IDs are oxygenates



Conclusions

The measurement of benzene and oxygenate compounds in reformulated gasoline is a common application performed worldwide, both in on-site labs at industrial facilities and also in third-party testing labs. As shown, the SLB-ILD3606 column is able to resolve both benzene and toluene from oxygenates (such as alcohols, ketones, and ethers) and also the aliphatic portion of gasoline in a one-column set-up.

Reference

1. ASTM® D3606, *Benzene and Toluene in Unleaded Gasoline and Aviation Fuel.*

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Featured Product

Description	Cat. No.
SLB-ILD3606, 60 m × 0.25 mm I.D., 0.20 µm	29691-U

Related Product

Description	Cat. No.
SLB-ILD3606, 30 m × 0.25 mm I.D., 0.20 µm	29687-U

Related Information

For additional chromatograms, product information, real-time availability, pricing, and ordering, visit our GC page for petroleum analyses:
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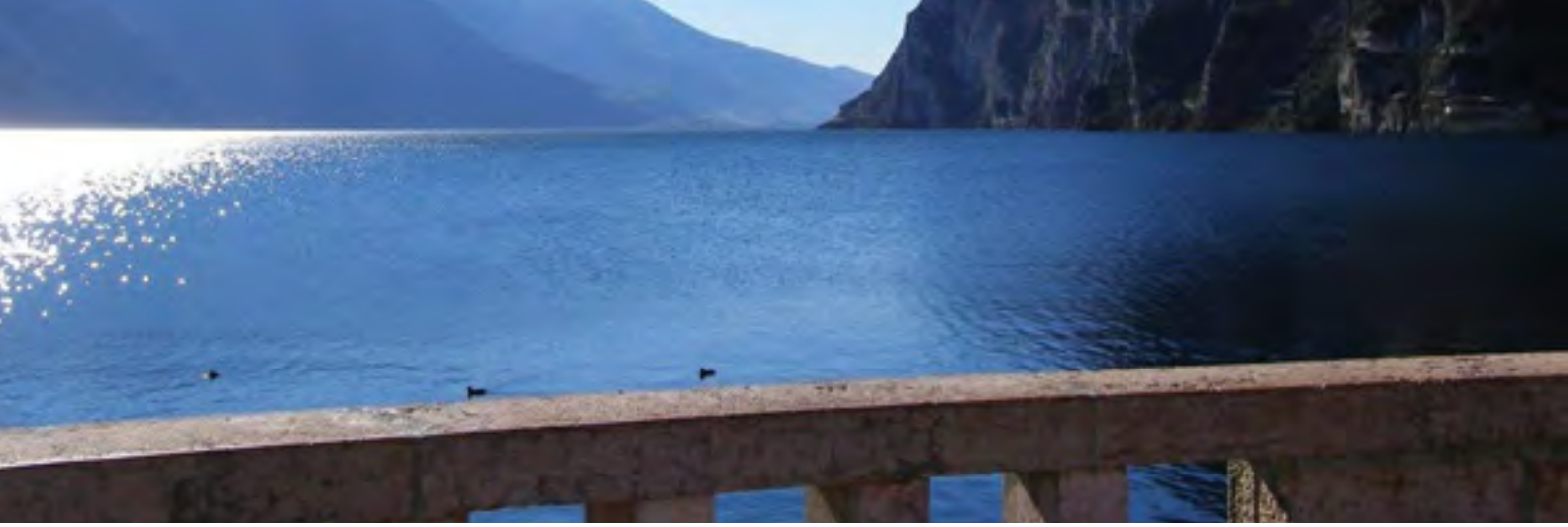

40th International Symposium on Capillary Chromatography and 13th GCxGC Symposium

...with particular emphasis on MS hyphenation

The 40th International Symposium on Capillary Chromatography (ISCC) and the 13th GCxGC Symposium is a "hyphenated" meeting which will be held at the Palazzo dei Congressi in wonderful Riva del Garda (Italy) from May 29 to June 3, 2016.

Apart from the most recent advances in the fields of pressure and electrodriven microcolumn separations, and comprehensive 2D GC, particular emphasis will be directed to combinations of capillary chromatography with various forms of MS… from unit-mass to high resolution, and from single to hybrid analyzers. Consequently, both the importance and complementary nature of chromatographic and MS processes will be emphasized. Within the wider context of separation science, attention will be given to sample preparation processes, in both oral and poster sessions.

The ISCC/GCxGC scientific program will be a rich one, characterized by:

- Invited contributions from leading scientists reporting the latest and most exciting developments
- Keynote lectures from promising young researchers
- Informative poster sessions
- Discussion sessions
- Workshop seminars presenting the most recent novelties in scientific instrumentation
- A world-class GCxGC course

Researchers in all areas relevant to the subjects of the symposia are invited to submit abstracts.

As is traditional for the Riva meetings, the majority of presentations will be in a poster format and the Scientific Committee will select contributions for oral presentations. As always, many awards will be assigned in both the ISCC and GCxGC events, recognizing excellence in both established and young scientists, in oral and poster presentations.

Exhibitors and sponsors are a fundamental part of the meeting (without them ... Riva wouldn't be Riva), and are encouraged to participate by submitting abstracts, to reserve booth space, and to promote the ISCC and GCxGC events.

Last, but not least, the traditional "Riva" social program will be entirely maintained, with one or two events each day: cocktails, the welcome reception, the concert, the wine and cheese evening, and of course, the disco night!

To check for new information as it becomes available, please visit www.chromaleont.it/iscc

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