

A Technical Newsletter for Analytical & Chromatography

# The Reporter

volume 24.1

 **SUPELCO**

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Riedel-deHaën

## Highly Reproducible Polar Columns

Suitable for HPLC Method Development

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# Elevated Performance

*“One Step” at a time*

RP-Amide



**Ascentis™**  
HPLC Columns from Supelco

Supelco has taken another step in performance and value with Ascentis RP-Amide columns. The amide functionality is the most popular of all Embedded Polar Group (EPG) phases and has resulted in the first EPG phase recognized with a USP code designation (L60). As an innovator in EPG bonding chemistry, Supelco has moved ahead of the competition again.

## Supelco's most recent advances in bonding chemistry have resulted in:

- Better reproducibility for easy method validation
- Lower bleed for LC-MS applications providing better sensitivity
- Excellent selectivity for polar compounds increasing resolution and reducing coelution

## Service before and after the sale

Our Technical Service Department will gladly share our knowledge and experience with you in the selection of HPLC columns and accessories, and provide detailed technical assistance for your chromatographic techniques.

*For more information* on Supelco's Ascentis HPLC columns, contact our technical experts at **800-359-3041 / 814-359-3041**, email us at [techservice@sial.com](mailto:techservice@sial.com), or visit our website at [sigma-aldrich.com/ascentis](http://sigma-aldrich.com/ascentis)

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LABORATORY ESSENTIALS

DRUG DISCOVERY

# Are Embedded Polar Group (EPG) Stationary Phases Suitable for HPLC Method Validation?

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Ascentis RP-Amide columns are fourth generation amide-based EPG phases available from Supelco. These phases are a culmination of 20 years of research in EPG phase chemistry at

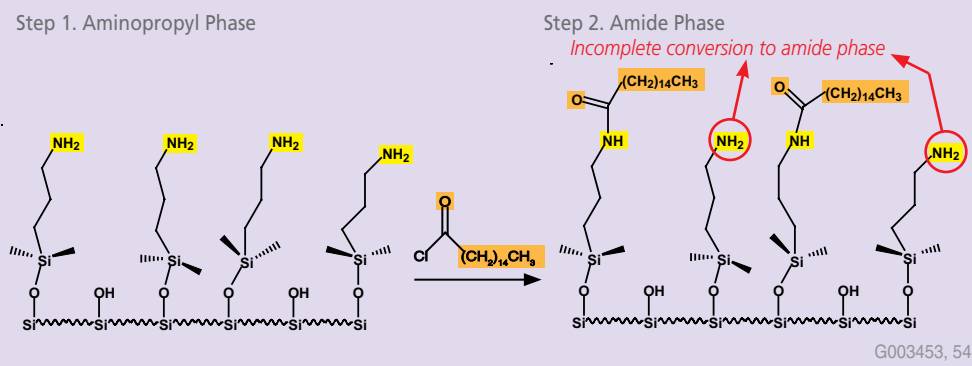
Although early generation EPG amide phases were a commercial success and are still used in many HPLC systems throughout the world, they can suffer from reproducibility and symmetry issues, especially with acidic compounds such as carboxylic acids. This performance issue arises from the slight

variation in the ratio of unreacted amines and amide groups. The amine moiety can introduce additional interactions with analytes as a 1) nucleophile, 2) strong base, 3) ion exchanger (pH dependent), and 4) a strong hydrogen bond.

In 1997, the Discovery® RP-Amide was introduced to the market. Through innovative research, a one-step process for the production of the amide

phase was realized. (Figure 2) In the single step process, no free amino ligands occur since the amide is introduced as a whole unit. As a last step, a final end-cap was performed

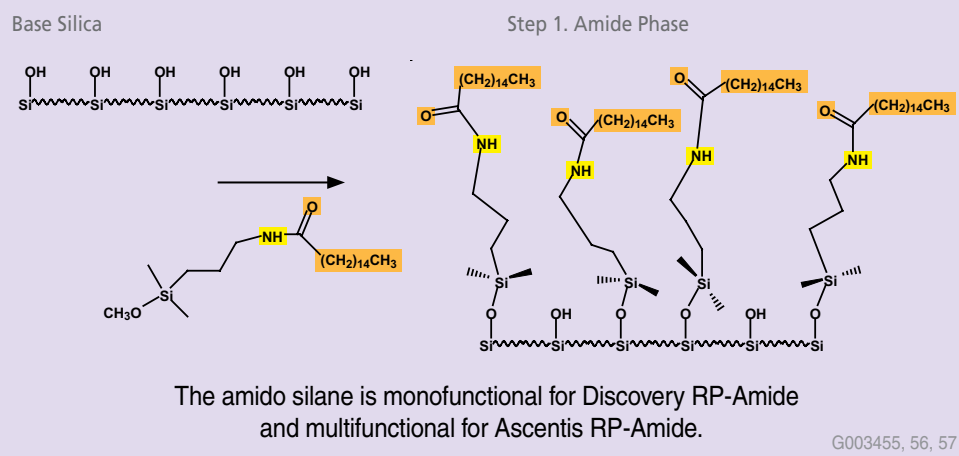
Figure 1. Early Generation Two-step Bonding Process for EPG Amide Phases



Supelco and represent the current state-of-the-art available today in terms of reproducibility, phase stability, and orthogonal selectivity as compared to C18 stationary phases.

The EPG phase was first commercially introduced in 1988 by Supelco as the Suplex pkb-100. Subsequent improvements were released in 1990 and 1993 as SUPELCOSIL® ABZ and SUPELCOSIL ABZ+. These early generation EPG amide phases were based on a two-step bonding process to create the amide phase. (Figure 1). The first step was the bonding of an aminopropylsilane to the base silica surface creating a surface with amine functionality. This surface was then treated with palmitoyl chloride that would react with the amine to create a long chain amide. Unfortunately, not all amines would be converted in the process, leaving a mixed system. Acetyl chloride was then utilized in an attempt to convert remaining amines to amides. Although this worked to some extent, residual amines still remained on the surface. As a last step, a final silanol end-cap was performed with a traditional trimethyl silane.

Figure 2. Next Generation One-step Bonding Process for EPG Amide Phases



with a traditional trimethyl silane reagent. This revolutionary process produced a much more reproducible phase since secondary interactions from exposed amino groups were no longer present. Although reproducibility was greatly improved, Discovery RP-Amide suffered from stability issues that were most notable in mass spectrometric (MS) detection. While all HPLC phases suffer from some level of phase

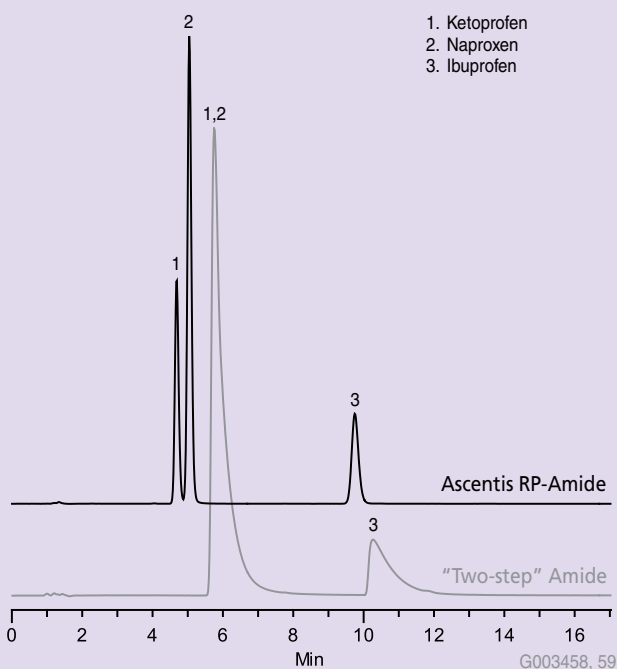
(continued on page 4)

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### Figure 3. Comparison of "One-step" Ascentis RP-Amide and a Commercial "Two-step" Amide

column : Ascentis RP-Amide, 15 cm x 4.6 mm I.D., 5  $\mu$ m particles (565324-U)  
 mobile phase: 45:55, 10 mM monobasic potassium phosphate, pH 3 with phosphoric acid:acetonitrile  
 flow rate: 1.0 mL/min.  
 temp.: 35  $^{\circ}$ C  
 det.: UV, 230 nm  
 injection: 5  $\mu$ L  
 sample: as indicated in 10 mM monobasic potassium phosphate, pH 3.0

This test mix demonstrates that differences in phase preparation can show up with samples, especially acidic compounds



stability often referred to as column bleed, EPG phases are notorious in MS systems because the ligands are easily ionized and thus easily detected in MS system. Traditional C18 ligands also suffer from column bleed, but the ligands are not easily ionized, and hence not easily detected.

In November 2004, Ascentis RP-Amide was launched after extensive R&D efforts to improve upon the shortcomings of previous EPG phases. The Ascentis RP-Amide builds upon the

one step bonding process utilized in Discovery RP-Amide, but utilizes a multifunctional amide ligand instead of the traditional monofunctional amide ligand. (Figure 2) This multifunctional ligand, with a proprietary bonding scheme, allows for a uniform "phase network" to be created which truly enhances phase stability while preserving efficiency and reproducibility. This is realized in lower column bleed as well as the ability to work at low pH ranges for an extended time as compared to previous generation EPG phases. Furthermore, instead of a traditional end-cap process, a proprietary end-capping process has been developed and utilized in the Ascentis RP-Amide to achieve a highly uniform silica surface. Figure 3 compares the advanced surface chemistry of Ascentis RP-Amide with other commercial amide phases using a carboxylic acid test mixture to detect evidence of residual amine groups.

Supelco's Ascentis RP-Amide columns have set a new level of performance and value among Embedded Polar Group (EPG) HPLC columns. The amide functionality is the most popular of all EPG phases and has resulted in the first EPG phase recognized with a USP code designation (L60). The achievements of the Ascentis RP-Amide phase have been demonstrated through the successful validation of many HPLC methods by a host of companies.

#### + Related Products

Description	Cat. No.
<b>Ascentis RP-Amide HPLC Columns</b>	
5 cm x 2.1 mm I.D., 3 $\mu$ m particles	565300-U
15 cm x 4.6 mm I.D., 3 $\mu$ m particles	565322-U
5 cm x 2.1 mm I.D., 5 $\mu$ m particles	565303-U
10 cm x 2.1 mm I.D., 5 $\mu$ m particles	565304-U
15 cm x 4.6 mm I.D., 5 $\mu$ m particles	565324-U
25 cm x 4.6 mm I.D., 5 $\mu$ m particles	565325-U

#### ! Related Information

For more information on the Ascentis HPLC columns talk to your technical sales specialists at 877-787-4437 or request the Ascentis Brochure, T404114 (HLV).



**For information on the complete Ascentis product line featuring the RP-Amide, C18, and C8 phases, request the Ascentis Brochure.**

# New Regulation Requires trans Fat Content to be Listed on Food Labels

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## New US FDA Regulation

On July 9, 2003, the United States Food and Drug Administration (FDA) issued a regulation requiring manufacturers to list trans fatty acids, or trans fat, on the Nutrition Facts panel of foods and some dietary supplements. With this rule, consumers will have more information to make healthier food choices that could lower their consumption of trans fat as part of a heart-healthy diet. Scientific reports have confirmed the relationship between trans fat and an increased risk of coronary heart disease. As of January 1, 2006, food manufacturers are required to list trans fat on the nutrition label. FDA estimates that by 2009, trans fat labeling will have prevented from 600 to 1,200 cases of coronary heart disease and 250 to 500 deaths each year. (1)

## Current Technology

Food analysts currently use the 100 m SP-2560 column for detailed analyses of fatty acid isomers. Unfortunately, this procedure may take 38 minutes or more to perform. Due to the issuing of the new regulation, more analyses need to be performed now than in the past. Therefore, analysts need a faster method than the current 100 m SP-2560 provides.

## New Fast GC Column for FAME Analyses

Supelco has recently developed a 75 m Fast GC version of the SP-2560 that allows the same analyses to be completed in a significantly shorter time. Figure 1 shows a detailed separation of cis and trans C18 FAMES in less than 21

minutes. This same analysis on the traditionally used 100 m SP-2560 would require 38 minutes.

## Conclusion

Using hydrogen carrier gas, a 45% reduction in analysis time can be realized compared to the traditionally used 100 m SP-2560 column. These shorter analysis times will allow analysts to keep up with the demand for higher throughput caused by the new trans fat labeling regulation.

## Reference

1. Information obtained from US FDA web site [www.fda.gov/oc/initiatives/transfat/](http://www.fda.gov/oc/initiatives/transfat/)

Description	Cat. No.
SP-2560, 75 m x 0.18 mm I.D., 0.14 $\mu$ m	23348-U

## Related Products

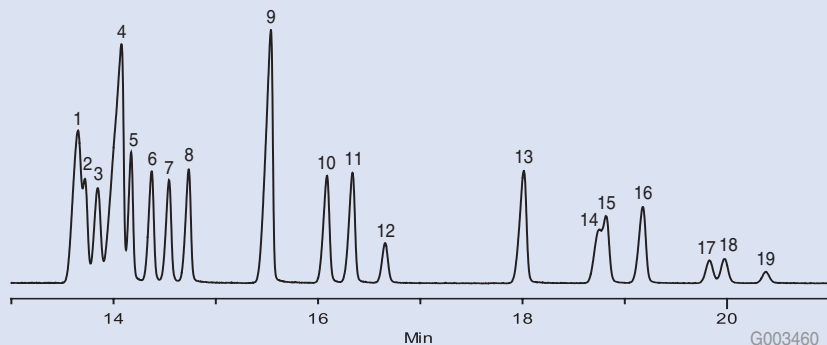
Description	Cat. No.
SP-2560, 100 m x 0.25 mm I.D., 0.20 $\mu$ m	24056
Linoleic Acid Methyl Ester Mix, cis/trans 10 mg/mL (total wt.) in methylene chloride, 1 mL	47791
C18:2 $\Delta$ 9c, 12c (10% w/w)	C18:2 $\Delta$ 9t, 12c (20% w/w)
C18:2 $\Delta$ 9c, 12t (20% w/w)	C18:2 $\Delta$ 9t, 12t (50% w/w)
Linolenic Acid Methyl Ester Isomer Mix 10 mg/mL (total wt.) in methylene chloride, 1 mL	47792
C18:3 $\Delta$ 9c, 12c, 15c (~3% w/w)	C18:3 $\Delta$ 9t, 12c, 15c (~7% w/w)
C18:3 $\Delta$ 9c, 12c, 15t (~7% w/w)	C18:3 $\Delta$ 9t, 12c, 15t (~15% w/w)
C18:3 $\Delta$ 9c, 12t, 15c (~7% w/w)	C18:3 $\Delta$ 9t, 12t, 15c (~15% w/w)
C18:3 $\Delta$ 9c, 12t, 15t (~15% w/w)	C18:3 $\Delta$ 9t, 12t, 15t (~30% w/w)

## Related Information

For more information on the analyses of fatty acids, check out our web site: [sigma-aldrich.com/fame](http://sigma-aldrich.com/fame)

Figure 1. Fast Analysis of cis and trans FAME isomers on 75 m SP-2560

column: SP-2560, 75 m x 0.18 mm I.D., 0.14  $\mu$ m (23348-U)  
oven: 180 °C, isothermal  
inj.: 220 °C  
det.: FID, 220 °C  
carrier gas: hydrogen, 25 cm/sec. @ 180 °C  
injection: 0.5  $\mu$ L, 100:1 split  
liner: 4 mm I.D. split, cup design  
sample: mixture of C18:1, C18:2, and C18:3 FAMES in methylene chloride



- C18:1 FAMES
1. 18:1  $\Delta$  7t and 18:1  $\Delta$  6t
  2. 18:1  $\Delta$  9t
  3. 18:1  $\Delta$  11t
  4. 18:1  $\Delta$  12t, 18:1  $\Delta$  6c, 18:1  $\Delta$  7c and 18:1  $\Delta$  13t
  5. 18:1  $\Delta$  9c
  6. 18:1  $\Delta$  11c
  7. 18:1  $\Delta$  12c
  8. 18:1  $\Delta$  13c
- C18:2 FAMES
9. 18:2  $\Delta$  9t, 12t
  10. 18:2  $\Delta$  9c, 12t
  11. 18:2  $\Delta$  9t, 12c
  12. 18:2  $\Delta$  9c, 12c
- C18:3 FAMES
13. 18:3  $\Delta$  9t, 12t, 15t
  14. 18:3  $\Delta$  9t, 12t, 15c
  15. 18:3  $\Delta$  9t, 12c, 15t
  16. 18:3  $\Delta$  9c, 12t, 15t and 18:3  $\Delta$  9c, 12c, 15t
  17. 18:3  $\Delta$  9c, 12t, 15c
  18. 18:3  $\Delta$  9t, 12c, 15c
  19. 18:3  $\Delta$  9c, 12c, 15c

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ordering: 800-247-6628 (US only) / 814-359-3441

Gas Chromatography

SUPELCO

# Exactly Why is Low Bleed Important for Capillary Columns?

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## Abstract

Capillary column manufacturers often use the term low bleed to describe their columns. How exactly does low bleed relate to the needs of end-users? Low detection limits, positive mass spectral identifications, and instruments that are not down for maintenance are all important daily goals for a GC analyst. Using a capillary column, such as Supelco SLB-5ms, that has a low bleed characteristic is an important step in achieving these goals.

## Introduction

Capillary column manufacturers often use the term low bleed to describe their columns. How exactly does low bleed relate to the needs of end-users? Let's find out.

## Low Bleed = Better Signal-to-Noise Ratio = Lower Detection Limits

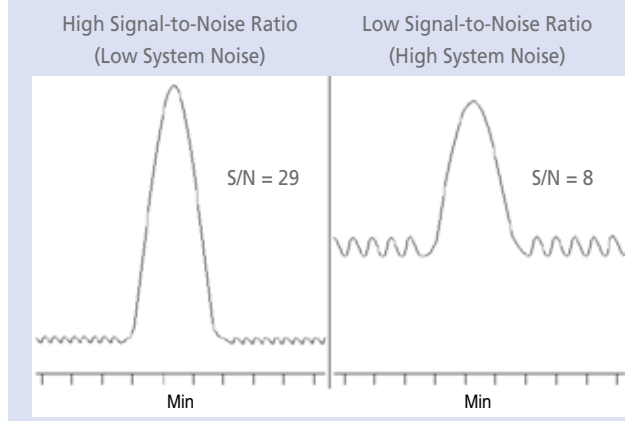
Signal refers to the response from an analyte passing through a detector. It is the peak you see when looking at a chromatogram. System noise refers to everything else, other than the analyte, producing a response in the detector. It is the baseline on a chromatogram. The ratio of the peak height to the baseline variability is termed the signal-to-noise ratio.

High noise level is undesirable since it makes it difficult for the integration software to adequately measure all of the peak area. The better the signal-to-noise ratio, the more area counts are obtained, resulting in the ability to achieve lower detection limits. Figure 1 shows a graphic illustration of a peak when column bleed (a significant source of system noise) is low vs. high.

*Simply stated, the greater the area counts, the lower the detection limits*

Analysts rely on GC-MS and other GC methods to provide highly sensitive, low-level detection. When measurement is required at the ppb or even ppt level, extreme care must be taken to ensure that nothing interferes with the analysis. For this reason, today's chemists require capillary columns, such as Supelco SLB-5ms, that exhibit a very low level of bleed in

Figure 1. Illustration of Signal-to-Noise Ratio



addition to being inert towards the various analytes in the method.

## Low Bleed = Cleaner Mass Spectra = Easier Mass Spectral Identifications

Analysts using MS detection have an additional concern; high column bleed may interfere with proper mass spectral identification of analytes. In addition to the primary, or quant ion in a mass spectrum, MS programs are set to measure the abundance of other ions that are characteristic to the analyte of interest. For the software to assign a high probability of positive identification, these so-called secondary, or qualifier ions must be within specific ratio ranges relative to the primary ion when the mass spectrum from a peak in a real-world sample extract is compared to a mass spectral library entry. If extraneous ions, such as from column bleed, are present in the mass spectrum, the software will assign a lower probability of positive identification.

Additionally, many US EPA Methodologies require GC-MS analysts to assign tentative identification to non-target "unknown" compounds which may also be present in the sample extract. These are called Tentatively Identified Compounds, or TICs. In order for the software to assign a high probability of positive identification, the mass spectrum from the sample extract must compare well with the mass spectral library entry. High column bleed levels can interfere with this comparison, resulting in the reporting of TICs that are either poorly identified or misidentified.

Figures 2 and 3 show mass spectra of benzo(g,h,i)perylene at a 5 ng on-column level and an oven temperature of 325 °C. The major column bleed ion,  $m/z = 207$ , resulting from the

formation of hexamethylcyclotrisiloxane (D3), should be present at a level lower than  $m/z = 138$  and  $m/z = 277$ , two secondary ions used to confirm the identity of the peak. Figure 2 is from a Supelco SLB-5ms column. The major bleed ion,  $m/z = 207$ , is lower than both of the secondary ions. Figure 3 is from a Competitor "A" column. The major bleed ion is actually larger in size than the two secondary ions.

What does all this mean? The MS software would assign a low probability of positive identification for benzo(g,h,i)perylene for analyses using the Competitor "A" column. For TICs, the situation would be less desirable since retention time data would not be available to assist with identification.

### Low Bleed = Reduced Detector Contamination = Less Instrument Downtime

High column bleed can foul the GC detector, reducing detector sensitivity. When the fouling becomes severe enough to warrant action, the detector must be dismantled and cleaned, a procedure that may remove an instrument from service for one or more days. MSD lenses can become coated and require polishing. The active foil in an ECD can

become fouled to the point the entire detector needs to be sent out for refurbishing. Partially plugged FID jets need to be replaced. PID windows can become layered with contamination and require cleaning.

The more instrument downtime you experience, the fewer billable samples you will be able to run in a given period of time. When you have a backlog of analyses to perform, you simply cannot afford unnecessary instrument downtime.

### Conclusion

Low detection limits, positive mass spectral identifications, and instruments that are not down for maintenance are all important daily goals for a GC analyst. Using a capillary column, such as Supelco SLB-5ms, that has a low bleed characteristic is an important step in achieving these goals.

### ! Related Information

For a complete listing of Supelco Low Bleed SLB-5ms capillary columns, request T405130 (IKA) or visit our web site [sigma-aldrich.com/capillary-ms](http://sigma-aldrich.com/capillary-ms)

Figure 2. Mass Spectrum From Supelco SLB-5ms Column

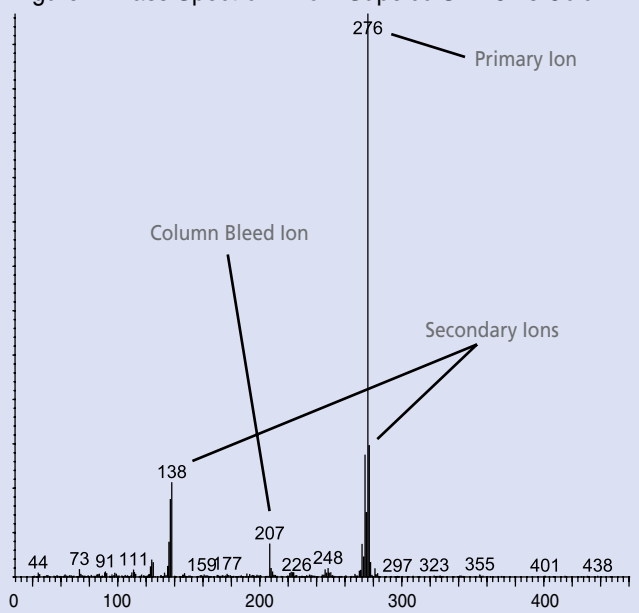
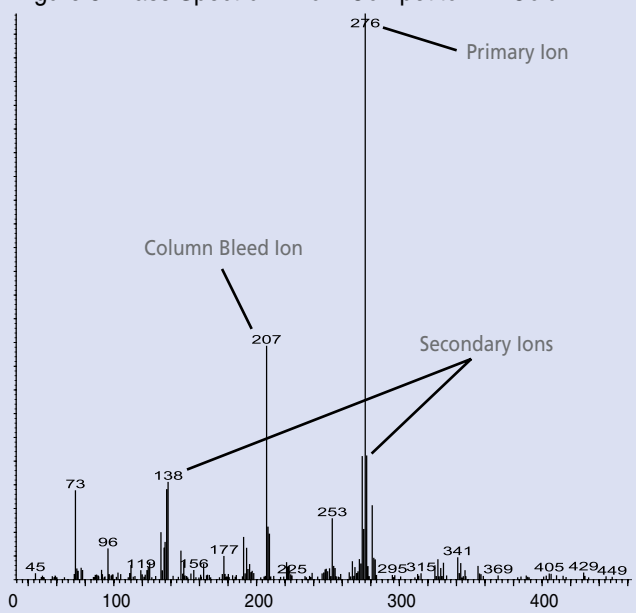


Figure 3. Mass Spectrum From Competitor "A" Column



**Low bleed, inert, durable and consistent capillary GC columns for trace analyses.**

[sigma-aldrich.com/capillary-ms](http://sigma-aldrich.com/capillary-ms)

# Controlling SPE Selectivity through pH and Organic Modifier Manipulation

An Trinh, Laura Marlatt, David S. Bell  
 atrinh@sial.com

## Introduction

Solid phase extraction (SPE) methods are frequently developed by copying/modifying an existing application or choosing a generic method. Although these approaches are less time-consuming, it can often be very difficult to determine and troubleshoot the root cause(s) of problems associated with an SPE method when they arise. For example, if poor recovery is observed, is it due to: 1) poor analyte retention during sample load; 2) pre-mature analyte elution during the wash step (b/w sample load and elution); or 3) analyte over retention during elution?

Like HPLC, SPE is a form of chromatography, and as such, basic chromatographic principles should be used when developing, optimizing, and troubleshooting a given SPE method. In this report, we demonstrate the use of pH and organic modifier manipulation during SPE wash/elution to control the retention and elution of three different compounds (neutral, basic, and acidic) on three different reversed-phase SPE chemistries of decreasing hydrophobicity (C18, C8, and cyanopropyl-CN).

## The Role of pH and Organic Modifiers in SPE

Most reversed-phase SPE protocols follow the general procedure in which the phase is first conditioned and equilibrated with aqueous miscible solvent (e.g., methanol or acetonitrile) followed by sample load. The sample must be aqueous because a polar mobile phase environment is necessary to drive reversed-phase retention. To elute compounds of interest, reversed-phase interactions are disrupted by decreasing polarity of the mobile phase environment. Common elution solvents include methanol and acetonitrile. Prior to elution, a wash step of intermediate solvent strength is typically employed to remove any endogenous interferences that may have co-retained with the analytes of interest (e.g., 5-20% methanol).

Most analytes contain ionizable functional groups, and a compounds ionization state can drastically change its retention and elution characteristics on a given SPE sorbent. When an analyte is in its neutral form, it becomes more hydrophobic and retention strengthens under reversed-phase conditions. This may allow for stronger wash solvents to remove co-retained interferences prior to elution. In contrast, in the ionized form, compounds become more

polar weakening the interaction strength between analytes of interest and reversed-phase functional groups. As a result, one may be able to elute with weaker solvent conditions (e.g. 50% methanol as opposed to 100% methanol) which could possibly eliminate the evaporation/reconstitution step common in SPE protocols. Figure 1 describes the role of pH in SPE.

Figure 1. The Role of pH in Reversed-Phase SPE

Acids (e.g. Carboxylic Acids):  $R-COOH \leftrightarrow R-COO^-$

HA (neutral)	$\leftrightarrow$	H <sup>+</sup> + A <sup>-</sup> (ionized)
50%	@ pKa	50%
100%	2 pH units below pKa	0%
0%	2 pH units above pKa	100%

Bases (e.g. Amines):  $R-NH_3^+ \leftrightarrow R-NH_2$

BH <sup>+</sup> + OH <sup>-</sup> (ionized)	$\leftrightarrow$	B (neutral)
50%	@ pKa	50%
0%	2 pH units below pKa	100%
100%	2 pH units above pKa	0%

Neutral State (Blue) = Strengthens reversed-phase interaction  
 Ionized State (Red) = Weakens reversed-phase interaction

## Method

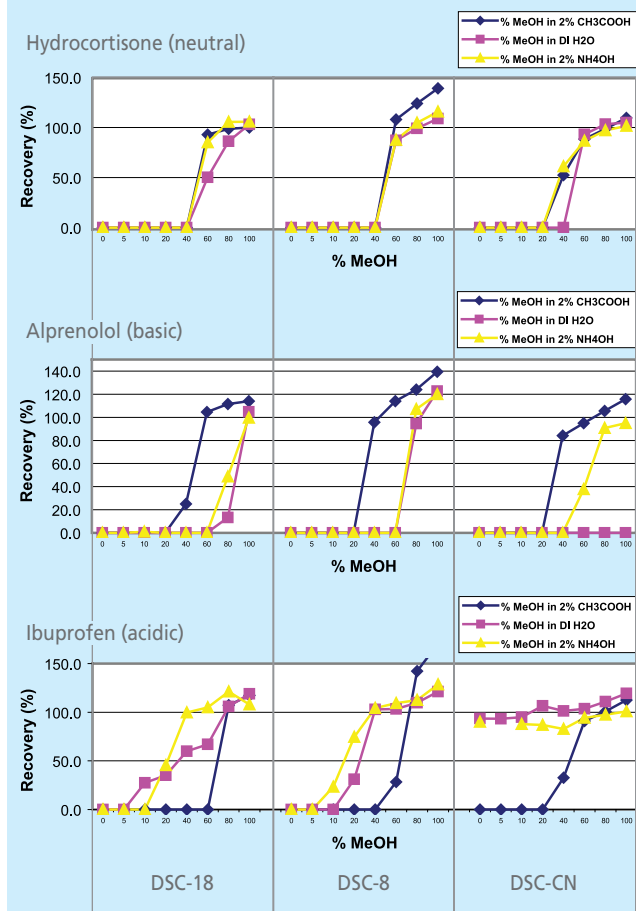
1 mL standards of 20 µg/mL ibuprofen (acidic), hydrocortisone (neutral), and alprenolol (basic) in 20 mM potassium phosphate, pH 7 were loaded on to three different 96-well SPE plates conditioned and equilibrated with 1 mL methanol and DI water per well. The SPE phase chemistries tested were Discovery DSC-18 (C18), DSC-8 (C8), and DSC-CN (cyanopropyl), 100 mg/well.

Respective wells were washed/eluted with 1 mL test solvents ranging from 0-100% methanol in 2% NH<sub>4</sub>OH, pH 11 (high pH), DI H<sub>2</sub>O (neutral pH), and 2% CH<sub>3</sub>COOH, pH 3 (low pH). The wash/elute eluate was collected for each well, and analyzed for compound breakthrough via HPLC-UV.

## Retention-Elution Profile for Hydrocortisone (neutral)

Figure 2 represents a retention-elution profile of the three compounds tested in which % recovery was measured against changing extraction conditions (pH vs. % organic modifier vs. phase chemistry). Hydrocortisone is a neutral compound that contains no ionizable functional groups. In Figure 2, we see that changes in pH across all three SPE chemistries had very little effect in manipulating elution

Figure 2. Retention-Elution Profile Hydrocortisone, Alprenolol, and Ibuprofen on DSC-18, DSC-8, and DSC-CN SPE



selectivity. Up to 40% methanol can be used as a possible wash solvent for both DSC-18 and DSC-8. DSC-CN is much more polar reversed-phase SPE chemistry. As a result, analyte breakthrough occurs between 20-40% methanol. 100% methanol is required to completely recover this moderately polar to non-polar compound.

#### Retention-Elution Profile for Alprenolol (basic)

Alprenolol is a basic compound with a pKa of ~9.5. At higher pH levels, it deprotonates into its neutral form. At low pH levels, it is in its ionized form. In contrast with hydrocortisone, pH modification has a great affect in controlling selectivity. At neutral and high pH conditions, alprenolol can withstand up to 60% methanol on DSC-18 and DSC-8 SPE before compound breakthrough occurs. At low pH conditions, compound breakthrough initiates at greater than 20% methanol. On DSC-CN, the short alkyl functional groups allow greater compound access to silanol groups which act as a secondary weak cation exchanger. As a result, at neutral pH conditions compounds are retained by both cation exchange and reversed-phase, and compounds remain retained from 0-

100% methanol. At high pH conditions, alprenolol is in its neutral form disrupting secondary ionic interactions allowing for elution from 40-100% methanol. At low pH, alprenolol is ionized but silanol groups are protonated and neutral resulting in elution between 20-100% methanol.

#### Retention-Elution Profile for Ibuprofen (Acidics)

Ibuprofen is an acidic compound with a pKa of ~4.2. In contrast to alprenolol, the compound is neutralized at low pH and ionized at high pH environments. On DSC-18 and DSC-8, up to 60 and 40% methanol in 2% acetic acid can be used as a possible wash solvent. At neutral and high pH levels where the compounds are ionic and thereby more polar, the retention limit is 5-10% methanol before compound breakthrough occurs. On DSC-CN, retention is very weak at high and neutral pH, and buffer alone will elute the compound. At low pH levels, wash solvents of up to 20% methanol can be employed.

#### Note on High Recoveries

Note that >100% recovery was often observed. When injecting a sample of greater solvent strength than the HPLC mobile phase, fluctuations in retention time and peak shape are often observed (data not shown) which can result in erroneously high signals. We observed this trend in our data because the SPE eluate was directly analyzed, and a high level of % methanol was used during SPE elution in part of the study. Although recovery data was not accurate, the purpose of the data was to describe general recovery trends observed by systematically changing elution conditions.

#### Conclusion

Both pH and % organic modifier play a critical role in determining retention and elution of ionizable compounds in reversed-phase SPE. By controlling the pH of the SPE mobile phase, one can control the relative hydrophobicity of an ionizable compound allowing for stronger wash solvents resulting in improved sample cleanup. pH manipulation may also allow for weaker elution solvents possibly minimizing processing time by negating the eluate evaporation/reconstitution step common in most reversed-phase procedures. By understanding how a compound interacts with the SPE phase under changing extraction conditions, one can manipulate the conditions to offer the most selective procedure.

#### ! Related Information

For more information, please request T404049 (GUX). This information is available in electronic form only. Be sure to include your email address on the request form.

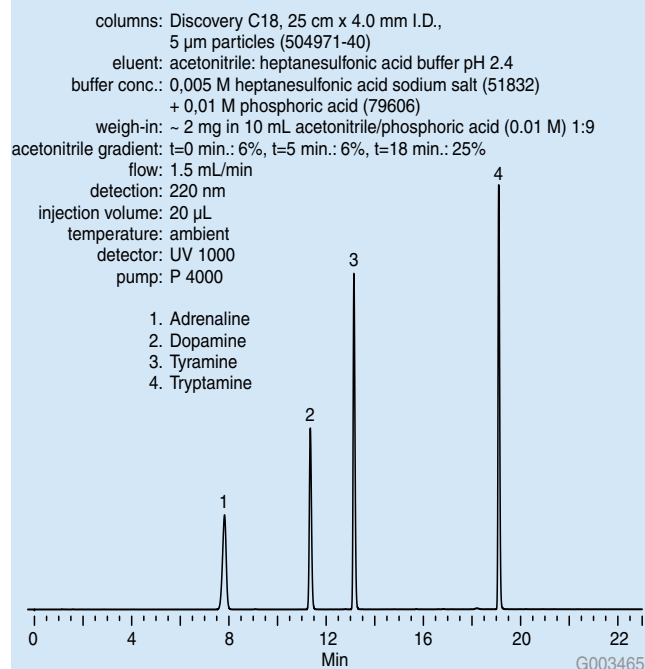
# Two Challenging Mixtures Resolved with Fluka IPC Reagents

Rainer Walz  
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## Separation of Biogenic Amines

Biogenic amines such as adrenaline, dopamine, tyramine and tryptamine play important roles as monoamine neurotransmitters. The analysis of these cations by RP-HPLC is not easy: their retention times are similar and interferences are often observed. The addition of an IPC reagent can be of help to resolve such mixtures. The successful separation of cations by IPC is often obtained by using alkylsulfonic acid sodium salts. For these monoamines, the popular sodium-1-heptanesulfonate (51832) was chosen. Figure 1 shows how the mixture containing adrenaline, dopamine, tyramine and tryptamine was efficiently resolved.

Figure 1. Mixture of Biogenic Amines Resolved by IPC



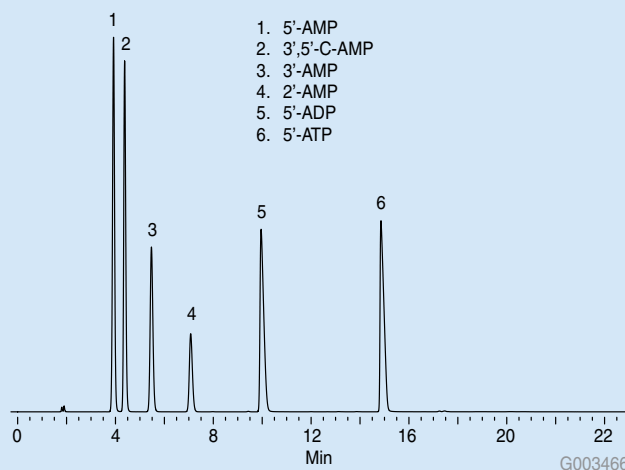
## Separation of Nucleotides

It can be a challenge to separate mixtures containing nucleotides by RP-HPLC, as they cover a wide range of polarities and functionalities. IPC can be the key to resolve them, as the separation of anions becomes possible using quarternary ammonium salts.

In this case, the IPC reagent is butylammonium bisulfate (86853). The chromatogram obtained for a sample containing nucleotides is shown in Figure 2. The mixture was separated with excellent resolution and highly reproducible results.

Figure 2. Mixture of Nucleotides Resolved by IPC

column: Discovery C18, 25 cm x 4.0 mm I.D.,  
5 µm particles (504971-40)  
eluent: acetonitrile:tetrabutylammonium buffer pH 7.0  
buffer conc.: 0,005 M tetrabutylammonium hydrogensulfate (86853)  
+ 0,01 M Na<sub>2</sub>HPO<sub>4</sub>·12 H<sub>2</sub>O (71649)  
weigh-in: ~ 4 mg in 10 mL acetonitrile / water 1:9  
acetonitrile gradient: t=0: 10%; t=4 min. 10%; t=14 min.: 25%  
flow: 1.5 mL/min  
detection: 254 nm  
injection volume: 20 µL  
temperature: ambient  
detector: UV 1000  
pump: P 4000



## How to Select the Right IPC Reagent

If you have a mixture of ionic and non-ionic analytes, we recommend that you start by optimizing the method for the non-ionic components. Then select the appropriate IPC reagent to provide the necessary counter ion. Alkylsulfonates are a good first choice for basic solutes, whereas quarternary amines are useful for the acidic ones. Halogenated IPC reagents are only suitable for isocratic applications and should not be used in gradient systems. Tables 1 and 2 on page 11 give you an overview of IPC reagents for the separation of cations and Table 3 shows IPC reagents suitable for the separation of anions.

After selecting the appropriate IPC reagent, the method can be further optimized by adjusting the pH and concentration. For short or medium chain length IPC reagents, a 0.005 M solution is suitable for most separations. The optimum concentration of long chain IPC reagents varies from 0.0005 M to 0.002 M. In Table 4 you'll find a selection of buffers and buffer concentrates for exact pH adjustment. All buffers are tested for suitability for chromatography.

Table 1. Selection of solid IPC reagents suitable for cation separation sorted by carbon chain length.

Compound Carbon Length	Cat. No.
1,2-Ethanedisulfonic acid disodium salt C2	02374
2-Propanesulfonic acid sodium salt monohydrate C3	81808
1-Butanesulfonic acid sodium salt C4	19022
1-Pentanesulfonic acid sodium salt monohydrate C5	76952
1-Hexanesulfonic acid sodium salt monohydrate C6	52862
1-Heptanesulfonic acid sodium salt monohydrate C7	51832
1-Octanesulfonic acid sodium salt monohydrate C8	74882
Octyl sulfate sodium salt C8	75073
1-Nonanesulfonic acid sodium salt C9	74316
1-Decanesulfonic acid sodium salt C10	30631
Sodium decyl sulfate C10	71443
1-Undecanesulfonic acid sodium salt C11	94133
1-Dodecane sulfonic acid sodium salt C12	44123
Sodium dodecyl sulfate C12	71726
1-Hexadecanesulfonic acid sodium salt C16	52263
Sodium 1-octadecanesulfonate C18	74734

Table 3. Overview of solid IPC reagents suitable for anionic separation sorted by carbon chain length (longest chain is shown).

Compound Carbon Length	Cat. No.
Tetramethylammonium sulfate C1	02799
Tetramethylammonium bromide C1	87708
Tetramethylammonium hydrogensulfate C1	87724
Tetramethylammonium hydrogensulfate concentrate (~0.33 M) C1	87727
Tetramethylammonium hydroxide concentrate C1	87728
Tetraethylammonium bromide C2	86608
Tetraethylammonium hydrogensulfate C2	86626
Tetraethylammonium hydroxide concentrate C2	86635
Tetrapropylammonium bromide C3	88103
<b>Tetrapropylammonium hydrogensulfate C3</b>	<b>88106</b>
Tetrapropylammonium hydroxide concentrate C3	88109
Tetrapropylammonium hydroxide solution, 1.0 M in water C4	86832
Tetrabutylammonium bromide concentrate C4	86846
<b>Tetrabutylammonium hydrogensulfate concentrate C4</b>	<b>86847</b>
Tetrabutylammonium hydroxide concentrate C4	86851
Tetrabutylammonium chloride C4	86852
<b>Tetrabutylammonium hydrogensulfate C4</b>	<b>86853</b>
Tetrabutylammonium hydroxide solution, ~40% in water C4	86854
Tetrabutylammonium bromide C4	86857
Tetrabutylammonium chloride concentrate C4	86862
Tetrabutylammonium dihydrogenphosphate concentrate C4	86899
Tetrabutylammonium iodide C4	86903
Tetrabutylphosphonium bromide C4	86915
Tetrabutylphosphonium hydrogensulfate C4	86925
Tetrapentylammonium bromide C5	87997
Tetrahexylammonium bromide C6	87297
<b>Tetrahexylammonium hydrogen sulfate C6</b>	<b>87299</b>
Tetrahexylammonium dihydrogenphosphate concentrate C6	87313
Tetraheptylammonium bromide C7	87296
Tetraoctylammonium bromide C8	87996
Decamethonium bromide C10	30518
Tetrakis(decyl)ammonium bromide C10	87578
Dodecyltrimethylammonium bromide C12	44239
Dodecyltrimethylammonium hydrogensulfate C12	44243
Tetradecyltrimethylammonium bromide C14	87208
Tetradecyltrimethylammonium hydrogensulfate C14	87215
Hexadecyltrimethylammonium dihydrogenphosphate C16	52363
Hexadecyltrimethylammonium bromide C16	52367
Hexadecyltrimethylammonium hydrogensulfate C16	52371

Products in Red: recommended for initial trials.

Table 2. Selection of IPC reagent concentrates suitable for cation separation sorted by carbon chain length.

Compound Carbon Length	Cat. No.
1-Butanesulfonic acid sodium salt concentrate (~0.33 M) C4	19029
1-Hexanesulfonic acid sodium salt concentrate (~0.33 M) C6	52864
1-Heptanesulfonic acid sodium salt concentrate (~0.33 M) C7	51834
1-Octanesulfonic acid sodium salt monohydrate, concentrate (~0.33 M) C8	74886
Sodium dodecyl sulfate concentrate (~0.33 M) C12	71735

Concentrates available in packages with 6 ampuls.

Dilute to 1-liter with HPLC grade water (95304) to obtain a 0.005 M eluent solution.

Table 4. Selection of Buffers tested for chromatography applications.

Compound Carbon Length	Cat. No.
CAPS buffer solution, 20 mM, pH 10.0	82606
CAPS buffer solution, 20 mM, pH 10.5	82607
CAPS buffer solution, 20 mM, pH 11.0	82608
Citric acid/Sodium hydroxide buffer solution, 20 mM, pH 2.5	82581
Citric acid/Sodium hydroxide buffer solution, 20 mM, pH 3.0	82582
ortho-Phosphoric acid 50%	79607
ortho-Phosphoric acid 85%	79606
Phosphoric acid concentrate, (~0.66 M)	79626
Phosphoric acid/di-Sodium hydrogenphosphate concentrate, (~0.33 M)	79629
Phosphoric acid/Potassium dihydrogenphosphate, concentrate, (~0.33 M)	79628
Potassium phosphate buffer solution, 150 mM, pH 3.0	82622
Potassium dihydrogenphosphate	60221
Potassium dihydrogenphosphate concentrate (~0.66 M)	60232
Sodium citrate buffer solution, 20 mM, pH 3.5	82583
Sodium citrate buffer solution, 20 mM, pH 4.0	82584
Sodium citrate buffer solution, 20 mM, pH 4.5	82585
Sodium citrate buffer solution, 20 mM, pH 5.0	82586
Sodium citrate buffer solution, 20 mM, pH 5.5	82587
Sodium citrate buffer solution, 20 mM, pH 6.0	82588
Sodium phosphate buffer solution, 100 mM, pH 2.5	82578
Sodium phosphate buffer solution, 100 mM, pH 3.0	82599
Sodium phosphate buffer solution, 100 mM, pH 7.0	82637
Sodium phosphate buffer solution, 100 mM, pH 8.0	82634
Sodium phosphate buffer solution, 50 mM, pH 2.5	82635
Sodium phosphate buffer solution, 50 mM, pH 7.0	82636
Sodium phosphate buffer solution, 50 mM, pH 8.0	82633
Sodium phosphate buffer solution, 20 mM, pH 6.5	82589
Sodium phosphate buffer solution, 20 mM, pH 7.0	82591
Sodium phosphate buffer solution, 20 mM, pH 7.5	82592
Sodium phosphate buffer solution, 20 mM, pH 8.0	82593
Sodium phosphate buffer solution, 20 mM, pH 8.5	82601
Sodium phosphate buffer solution, 20 mM, pH 9.0	82603
Sodium phosphate buffer solution, 20 mM, pH 9.5	82605
Sodium phosphate dibasic concentrate I (~0.33 M)	71648
Sodium phosphate dibasic concentrate II (~0.50 M)	71651
Sodium phosphate dibasic/Potassium phosphate monohydrate, concentrate (~0.33 M)	71653
Sodium tetraborate buffer solution, 20 mM, pH 8.0	82594
Sodium tetraborate buffer solution, 20 mM, pH 8.5	82602
Sodium tetraborate buffer solution, 20 mM, pH 9.0	82604

Concentrates available in packages with 6 ampuls.

Dilute to 1-liter with HPLC grade water (95304) to obtain a 0.005 M eluent solution

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Standards & Reagents



# Improved Sampling and Analysis of the “New” Smell

**Jamie Brown, Jamie Desorcie**  
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## Introduction

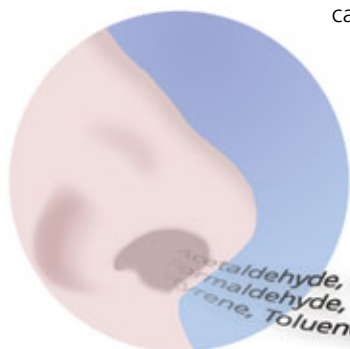
You buy a new house, car, furniture or carpeting and you get that “smell”. The odors emanating from new products are typically volatile organic compounds (VOC) off-gassing from adhesives or polymeric materials used in their construction. The EPA and CDC both agree that these VOC’s are not good for you. Due to ongoing pressure, manufacturers of some of these materials are making a conscious effort to monitor and reduce the emissions of potentially hazardous VOC’s from their products.

## VOC’s from Carpet by Solvent and Thermal Desorption

For example the Carpet and Rug Institute (CRI) has created a new program called the “Green Label Plus” program which assures the consumer is buying a carpet “certified” with the lowest emitting VOC’s in the market. Carpet manufacturers that participate in the program submit samples of the carpet to an independent certified laboratory where the carpet samples are analyzed to determine the concentration of certain VOC’s (Acetaldehyde, Benzene, Caprolactam, 2-Ethylhexanoic Acid, Formaldehyde, 1-Methyl-2-Pyrrolidinone, Naphthalene, Nonanal, Octanal, Styrene, Toluene, Vinyl Acetate, and 4-Phenylcyclohexene [the compound attributed to the “new carpet smell”]). If the carpet sample meets the criteria set forth by CRI, the carpet manufacturer can use the Green Label Certification on their product.

Supelco offers products for the sampling and analysis of carpet “odors” including LpDNPH sampling cartridges specifically designed for sampling carbonyls. The LpDNPH cartridges are made of high purity silica that is coated with 2,4-dinitrophenylhydrazine. During sampling the collected formaldehyde and acetaldehyde are derivatized to form

hydrazones. For the analysis, the cartridge is extracted with acetonitrile and analyzed via HPLC.



aldehyde, Benzene, Caprolactam, 2-Ethylhexanoic Acid, Formaldehyde, 1-Methyl-2-Pyrrolidinone, Naphthalene, Nonanal, Octanal, Styrene, Toluene, Vinyl Acetate, and 4-Phenylcyclohexene

The remaining odor compounds are collected using thermal desorption tubes packed with Tenax®-TA. After sampling, the tube is placed in a thermal desorber where it’s heated and the VOC’s are released from the adsorbent and swept onto a capillary column where they’re separated and analyzed by either a flame ionization or mass spec detector. Supelco offers thermal desorption tubes for a variety of thermal desorbers in the market.

Supelco also offers a variety of columns that are suited for this analysis and can also supply custom calibration standards for these test compounds. We are also working on developing a thermal desorption tube with the calibration standard pre-spiked onto the tubes so labs can use the tubes to develop calibration curves for the instrumentation or to validate that their own calibration is valid.

## Increased Sensitivity for Carbonyls in Air Through Background Improvements

The need to measure trace concentrations of airborne carbonyl contaminants requires the use of DNPH sampling cartridges possessing low background levels of formaldehyde and similar compounds. In recent years, Supelco has responded by developing processes that reduce background levels in LpDNPH packing. These efforts have been two-fold.

1. Purification procedures have been implemented that remove carbonyl contaminants from the 2,4-dinitrophenylhydrazine (DNPH) coating and silica support.
2. Packing procedures have been modified to prevent contamination of the media during manufacturing.

The result has been the ability to reduce the background specifications for LpDNPH samplers to levels that meet or exceed those of similar products on the market. The success of this work is found in the data from well over 100 batches of LpDNPH packing material that were manufactured during 2005 (Table 1).

Table 1. Background Data for LpDNPH Batches Manufactured in 2005\*

Background Component	Range (ng/Cartridge)	Mean (ng/Cartridge)	Competitive Product Specification
Formaldehyde	25 – 50	34	<60
Acetaldehyde	14 – 43	23	<150
Acetone	32 – 204	60	<380

\* S10 Sampler containing 350mg LpDNPH

## Chromatographic Separations

Following sampling and extraction of the sampler, a C18 column is typically used to analyze mixtures of carbonyl-DNPH derivatives. In general, this provides good separations. Acetone and acrolein are exceptions and are poorly resolved with the C18 stationary phase. When acetone and acrolein are both present, baseline separation of the derivatives can be achieved using the Ascentis RP-Amide column. This is shown in the chromatogram in Figure 1 (peaks 4 and 5).



## Related Information

For more information on products for the analysis and testing of carpet samples or DNPH products request *Monitoring Carbonyls in Air Using the LpDNPH S10 Cartridge with Analysis by HPLC*, T396092 (ARZ) and the *LpDNPH Air Monitoring Cartridges* data sheet, T795040, or contact Supelco Technical Service 800-359-3041/814-359-3041. This information is available in electronic form only. Be sure to include your email address on the request form.

## Did you know...?

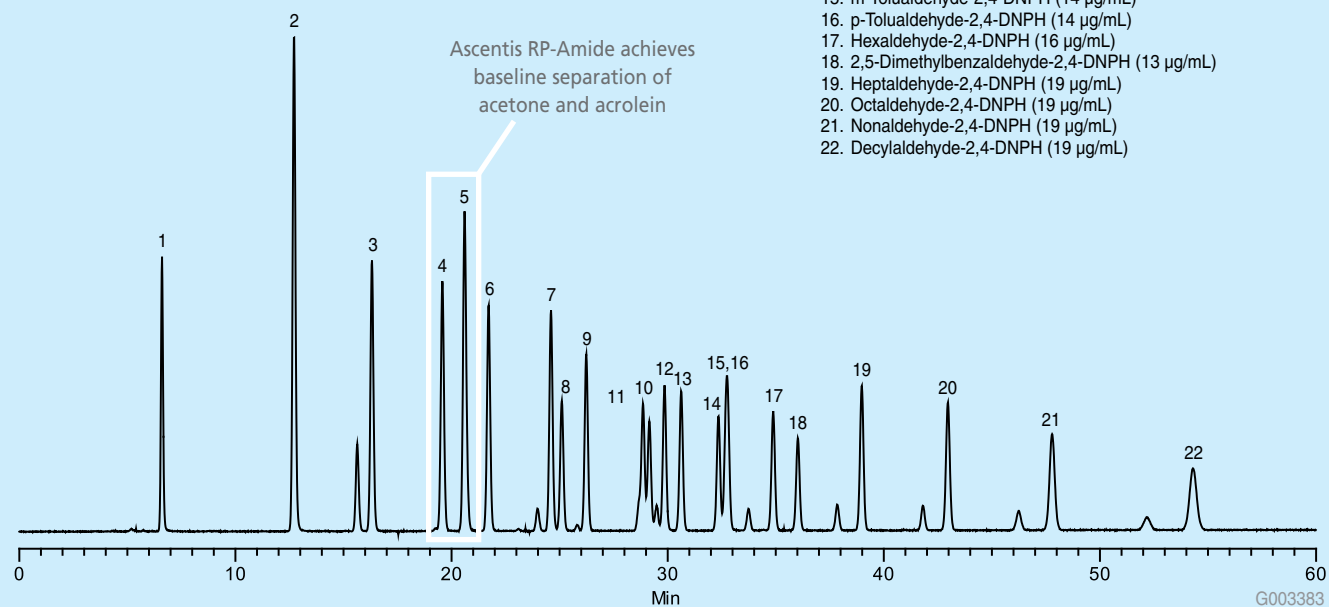
Supelco offers a range of products such as thermal desorption, solid phase microextraction and LpDNPH cartridges that have long been used in the sampling and analysis of unpleasant and/or hazardous odors.

Figure 1. Carbonyl-DNPH Derivatives Using an Ascentis RP-Amide Column

column: Ascentis RP-Amide, 25 cm x 4.6 mm I.D., 5 µm particles (565325-U)  
 mobile phase A: 60:40, water:acetonitrile  
 mobile phase B: 25:75, water:acetonitrile  
 flow rate: 1.5 mL/min.  
 temp.: 40 °C  
 det.: UV at 360 nm  
 injection: 10 µL  
 sample: as listed in mobile phase  
 gradient:

Min	%A	%B
0	100	0
5	100	0
25	40	60
40	0	100
60	0	100

- Dinitrophenylhydrazine (100 µg/mL)
- Formaldehyde-2,4-DNPH (40 µg/mL)
- Acetaldehyde-2,4-DNPH (29 µg/mL)
- Acetone-2,4-DNPH (23 µg/mL)
- Acrolein-2,4-DNPH (24 µg/mL)
- Propionaldehyde-2,4-DNPH (23 µg/mL)
- Crotonaldehyde-2,4-DNPH (20 µg/mL)
- 2-Butanone-2,4-DNPH (10 µg/mL)
- Butyraldehyde-2,4-DNPH (20 µg/mL)
- Benzaldehyde-2,4-DNPH (15 µg/mL)
- Cyclohexanone-2,4-DNPH (10 µg/mL)
- Isovaleraldehyde-2,4-DNPH (18 µg/mL)
- Valeraldehyde-2,4-DNPH (18 µg/mL)
- o-Tolualdehyde-2,4-DNPH (14 µg/mL)
- m-Tolualdehyde-2,4-DNPH (14 µg/mL)
- p-Tolualdehyde-2,4-DNPH (14 µg/mL)
- Hexaldehyde-2,4-DNPH (16 µg/mL)
- 2,5-Dimethylbenzaldehyde-2,4-DNPH (13 µg/mL)
- Heptaldehyde-2,4-DNPH (19 µg/mL)
- Octaldehyde-2,4-DNPH (19 µg/mL)
- Nonaldehyde-2,4-DNPH (19 µg/mL)
- Decylaldehyde-2,4-DNPH (19 µg/mL)



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# SGT Super Clean™ Gas Purifiers

**Bob Wallace**  
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## Introduction

Brief exposure to contaminated carrier gas can cause serious damage to GC capillary columns. Exposure to contaminants such as oxygen, moisture, and hydrocarbons can reduce performance and decrease column life. To prevent the irreversible degradation of the column, the GC system must include a gas management system designed to eliminate contaminants.

Supelco now offers the unique Click-On Inline Super Clean Gas Purifiers and Super Clean Gas Filters with Fast-Connect technology by SGT. When used as part of a comprehensive gas management system, these state-of-the-art purifiers eliminate costly instrument downtime.

## Click-On Inline Super Clean Gas Purifiers

Changing out a traditional inline purifier allows the open gas line to be exposed to unpurified room air, requiring a lengthy and unproductive flushing process. Getting dirt in the threads or over-tightening of gas line fittings can prevent proper sealing, providing a source for gas contamination. The Click-On Inline Super Clean Gas Purifiers have none of those disadvantages. They are 100% diffusion-proof and are made from glass and metal. They can be used for purification of carrier gas and fuel gases for your GC or GC-MS system. Reduction of hydrocarbons, oxygen, and moisture to produce better than 6.0 gas quality (99.9999% purity) can be met independent of the input quality of the gas. Click-On Inline Super Clean Gas Purifiers are available with or without visual indicators.



E000937

In sharp contrast to traditional inline purifier replacement, change out of the SGT Click-On Inline Super Clean Gas Purifiers is simple and eliminates the risk of damaging compression fittings. Once the Click-On connectors are installed into the gas line, those connections never need to be broken. Subsequent

replacement of purifiers can be done in seconds. The new purifier is snapped in place and the fittings are hand tightened. Each Click-On connector contains a needle valve that snaps shut the instant the purifier is removed. This prevents introduction of room air into the gas line and allows the change out without shutting down the GC system. Click-On connectors are available in either brass or stainless steel with either 1/8 " or 1/4 " compression fittings.

## SGT Super Clean Gas Purifiers

SGT Super Clean Gas Purifiers with Fast-Connect technology use a base plate design. Replacement purifiers can be installed in seconds without exposing the gas lines to room air. The specially designed base plate rests on the bench top and has needle valves that instantly close to provide a diffusion-proof seal when the gas purifier is removed. Replacement gas purifiers need only be held in place on the base plate while the retaining ring is hand tightened.

The unique point-of-use glass/metal, Super Clean Gas Purifiers purify carrier, fuel and other gases for your GC or GC-MS system. Hydrocarbons, oxygen (color indicating), and moisture (color indicating) are removed to produce gas that is better than 6.0 (99.9999%) gas quality. This purification is independent of the original gas quality.



E000938

## SGT Super Clean LC-MS Gas Purifiers

Super Clean Gas LC-MS Purifiers, a new revolution in clean gas purifiers, purify nitrogen from a nitrogen generator for LC-MS systems. These point-of-use glass/metal, diffusion-proof purifiers can remove hydrocarbons to produce nitrogen that is better than 6.0 gas quality, independent of the original gas quality.

The Super Clean Fast-Connect design avoids MS source damage and helps to eliminate LC-MS downtime. The two cartridge purifier systems, used in parallel (Figure 1), is the only fully glass/metal cartridge purifier capable of purifying

## SGT Super Clean Gas Purifier Specifications

Type of Purifier	H <sub>2</sub> O (g)	O <sub>2</sub> (mL)	Hydrocarbon (g)	Estim. Lifetime*
GC – Moisture	7.2	-	-	> 2 years
GC – Charcoal	-	1000	12 (as n-butane)	> 2 years
GC – Combi (moisture/charcoal)	-	-	6 (as n-butane)	> 1.5 years
GC-MS – Triple (moisture/oxygen/charcoal)	1.8	500	4 (as n-butane)	> 1 years
GC-MS – Triple : gas specific helium (moisture/oxygen/charcoal)	2.0	600	4 (as n-butane)	> 1 years

\* The specified lifetimes are strongly dependent on the quality of the incoming gas.

LC-MS N<sub>2</sub> carrier gas for all major hydrocarbon contaminants. The activated charcoal bed of the indicating hydrocarbon filter



adsorbs organic compounds larger than ethane. The indicating material changes from bright yellow to dark green when the trap has reached saturation and needs to be replaced.

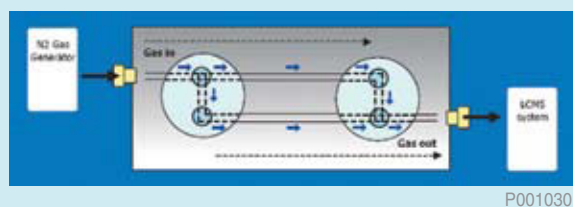
### LC-MS Filter Adsorbents & Indicator Specifications

Compounds Removed: Hydrocarbons (> C<sub>2</sub>)  
 Capacity: 1.64 grams of Hexane  
 Efficiency: < 1ppb for the adsorbents;  
 < 3ppb for the indicator  
 Indicator Color: from Yellow to Green  
 Pressure Limit: 100 psig  
 Temperature Limit: 100 °C

### Pressure Drop Specifications

At a flow of max. 20 L/min, the pressure drop is max. 2%

Figure 1. LC-MS Gas Purifier Flow Diagram



### ! Related Information

For more information, request *SGT Super Clean LC-MS Gas Purifiers*, T405137 (ILD), *SGT Click-On Inline Super Clean Gas Purifiers*, T405138 (ILE), and *SGT Super Clean Gas Filters*, T405139 (ILF). This information is available in electronic form only. Be sure to include your email address on the request form.

## SGT Super Clean Gas Purifiers

Description	Cat. No.
<b>SGT Click-On Inline Super Clean Purifiers</b>	
Click-On Moisture Inline Super Clean Trap	28861-U
Click-On Oxygen Inline Super Clean Trap	28862-U
Click-On Hydrocarbon Inline Super Clean Trap	28863-U
Click-On Triple O <sub>2</sub> /Moisture/Hydrocarbon Inline Super Clean Trap for Carrier Gas	28864-U
Click-On Gas Specific (He) Triple O <sub>2</sub> /Moisture/Hydrocarbon Inline Super Clean Trap	28865-U
Click-On Combi Moisture/Hydrocarbon Inline Super Clean Trap for Fuel Gas	28866-U
Click-On Indicating Triple O <sub>2</sub> /Moisture/Hydrocarbon Inline Super Clean Trap	28867-U
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Click-On Connector 1/4" Brass, Pk 2	28868-U
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Click-On Connector 1/4" Stainless Steel, Pk 2	28872-U
Click-On Connector 1/8" Stainless Steel, Pk 2	28873-U
Click-On Double Version Connector 1/8", Pk 2	28874-U
Click-On Replacement O-ring, Pk 10	28875-U
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Combi GC Replacement (Charcoal/Moisture) Fuel Gas Super Clean Purifier w/o Indicator	SU861025
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High Flow Special Moisture Super Clean Purifier, Pk of 2	SU861028
Charcoal Cartridges for LC, N <sub>2</sub> Purification Super Clean Purifier, Pk of 2	SU861029
High Flow Charcoal Super Clean Purifier for LC-MS	28877-U
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High Flow Special Moisture Super Clean Purifier Kit (2 Purifiers/Baseplate)	SU861045
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Replacement O-rings for Super Clean Cartridges (10 Large/10 Small), Pk 20	SU861050
2-Position Baseplate for High Flow LC-MS	28879-U

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