

Reporter

Volume 27.3

Colors of the World: Fast Separation of Dyes with Ascentis Express



Popular imported spices include ground turmeric, cumin, and chili powder varieties; cinnamon sticks, cardamom pods, star anise, and cloves. Spices are tested for the presence of harmful dyes that might be used to enhance their visual appeal.

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



Ingo Haag

Marketing Manager

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Dear Colleague,

Although we routinely use print-based methods to communicate with our customers (this newsletter is a good example) there is no substitute for the spoken word. Whether delivered face-to-face or over the Internet, seminars and webinars permit valuable dialog between the speaker and the audience. They also are able to communicate the speaker's expertise and passion for the subject matter and capture the audience's attention in ways that most print media cannot.

Seminars are an essential way we demonstrate innovative new products and how they can be applied to solve current analytical challenges. And because a new analytical chemist is born every day, we offer training on basic chromatographic and analytical techniques, like HPLC, GC, SPE, spectroscopy, titration and many other topics.

The seminar approaches we offer fall into these general categories:

- On-site, delivered at individual facilities
- Open, held at convenient venues close to our customer base
- Webinars, both live and archived for viewing anytime
- Tradeshows

Delivered by our technical experts, the seminar topics we cover are just as varied as our products and customer base. The topics include HPLC (e.g. Fused-Core™ Technology), chiral chromatography, GC, ionic liquids, sample prep for LC-MS, SPE, SPME and Karl Fischer titration, to name just a few. Our up-to-date seminar and tradeshow schedule can be found on our web site: sigma-aldrich.com/analytical-events

If you are interested in having a webinar or seminar at your facility, contact your local Sigma-Aldrich representative or send an email to ac_seminars@sial.com. Let us know what you are interested in and we will make sure the right people get in touch with you to discuss your seminar or training needs.

We look forward to sharing our analytical expertise with you!

Kind Regards,

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Colors of the World: Fast Separation of Dyes with Ascentis Express

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Dyes surround us everywhere every day. They can be found in common places like the printing ink in magazines or books and in plastics, textiles, and leather, but also in unusual places like diesel fuel and tattoo color. Most of these synthetic colors are based on aromatic ring structures containing heteroatoms and tend to have a high potential for causing cancer; as a result, they are not intended for use in food coloring. But since 2003, there have been several incidents of Sudan I contamination in chili powder. This situation necessitates the analysis of spice mixtures to determine if they have been adulterated (1, 2).

Further, a sensitive HPLC method is needed for quality control testing of dyes and the identification of byproducts. Supelco's Ascentis® Express HPLC columns provide outstanding sensitivity and resolution for such applications.

Method Development for Dyes

Table 1 contains a list of dyes added to one sample and dissolved in a mixture of methanol and acetonitrile. The sample was injected on an Ascentis Express C8 HPLC column under varying mobile phase conditions to determine the best separation parameters. Temperature, injection volume, detector settings, and flow rate were kept constant.

The chemical and physical properties of the dyes differ strongly, so the first step in developing a suitable HPLC method was the use of a gradient run ranging from 25% acetonitrile to 100% acetonitrile (B) and 0.1% formic acid in water as an aqueous counterpart (A). The UV chromatogram of the combined wavelengths 360, 550, and 620 nm showed good chromatography of all compounds except for the poor peak shape of Sudan 410 at 17.35 minutes (Figure 1A).

To optimize that peak shape, methanol was added to the organic mobile phase (acetonitrile:methanol, 90:10); the gradient run was repeated, resulting in better peak shape for Sudan 410 (Figure 1B). In a final experiment, the gradient profile was changed and optimum conditions were attained (see Table 2). Figure 1C shows the step-by-step improvements in the chromatography.

Only three runs were needed to get the final method, showing how easy and fast it is to develop methods with Ascentis Express columns. Further, Ascentis Express columns contain Fused-Core™ particles that allow for faster run times; even separations performed on standard HPLC systems can be sped up by up to 30% with Ascentis Express.

(continued on page 4)

Table 1. Structure and Mass of the Dyes in the Sample Mixture. Most of the Compounds are Detected as [M+H]⁺ Ions except (5), which gives [M]⁺ Ions

Peak No.	Structure	Name / Exact Mass	Peak No.	Structure	Name / Exact Mass
1	G004779 	Parafuchsin C ₁₉ H ₁₇ N ₃ 287.142247	5	G004783 	Malachite Green C ₂₃ H ₂₅ N ₂ 329.201773
2	G004780 	Basic Fuchsin C ₂₀ H ₁₉ N ₃ 301.157897	6	G004784 	Sudan III C ₂₂ H ₁₆ N ₄ O 352.132411
3	G004781 	Methylfuchsin C ₂₁ H ₂₁ N ₃ 315.173547	7	G004785 	Sudan 410 C ₂₆ H ₂₄ N ₄ O 408.195011
4	G004782 	Newfuchsin C ₂₂ H ₂₃ N ₃ 329.189197			

ordering: 800-247-6628 (US only) / 814-359-3441 technical service: 800-359-3041 (US and Canada only) / 814-359-3041

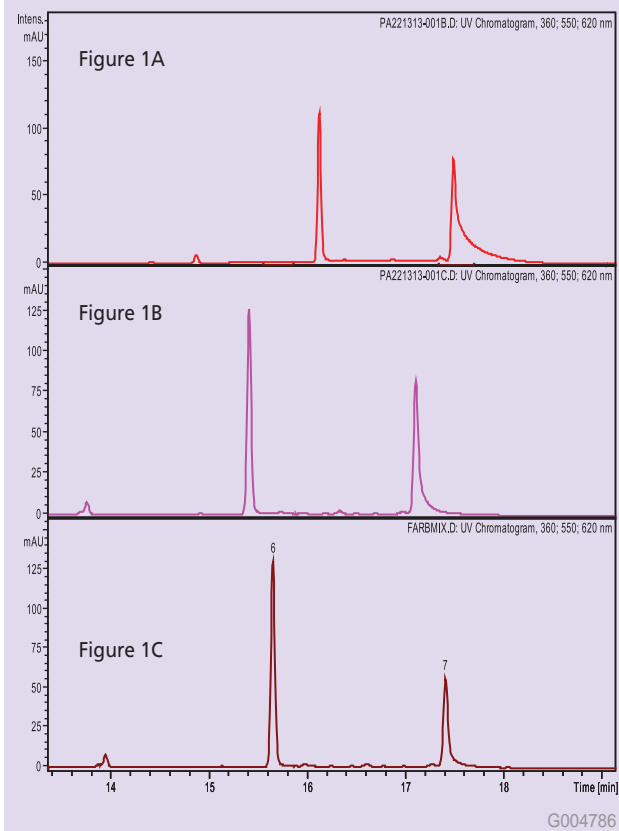
Liquid Chromatography

SUPELCO
Analytical

Table 2. Initial and Final HPLC Method Settings for Separation of the Seven Dyes Listed in Table 1, After Optimization

Fixed Parameters						
column:	Ascentis Express C8, 10 cm x 4.6 mm I.D., 2.7 μ m particles					
flow rate:	0.8 mL/min					
temp:	55 $^{\circ}$ C					
UV DAD:	200–950 nm					
MS:	ESI(+), SPS target 500 m/z, stability 100%, trap vl. 100%, optimize normal, range 100–1500 m/z, nebulizer 50 psi, dry gas 12 L/min, dry temp. 365 $^{\circ}$ C.					
injection volume:	3 μ L					
run time:	25 min (5 min posttime)					
Variable Parameters						
Initial Conditions				Final Conditions		
solvents:	(A) water with 0.1% formic acid (B) acetonitrile			(A) water with 0.1% formic acid (B) acetonitrile:methanol (90:10)		
gradients:	Time	%A	%B	Time	%A	%B
	0.0	75	25%	0.0	75	25%
	1.5	75	25%	1.5	75	25%
	15.0	0	100%	15.0	2	98%
	22.0	0	100%	22.0	2	98%
	25.0	75	25%	25.0	75	25%

Figure 1. UV Chromatograms of Sudan III and Sudan 410. (A) Initial Conditions, (B) Addition of Methanol to Mobile Phase, (C) Final Conditions after Adjusting Gradient

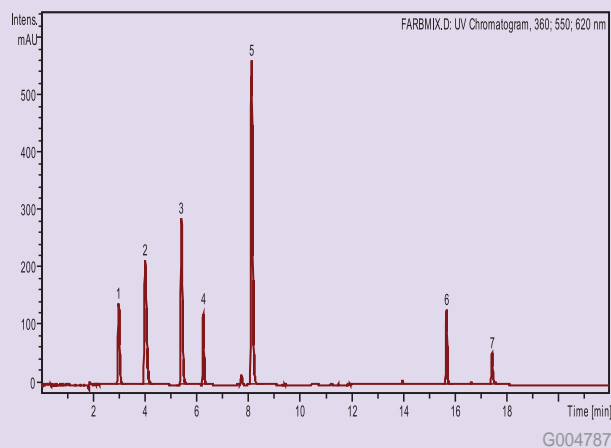


(continued from page 3)

Figure 1 shows UV chromatograms of Sudan III (Peak 6) and Sudan 410 (Peak 7) after three optimization steps. An organic phase mixture of methanol:acetonitrile (10:90) and a final gradient composition of 98% organic mobile phase resulted in the best overall peak shapes with a minimum of tailing of compounds (Peak 7). Figure 3 shows the final chromatogram with very good separation of all analytes.

Figure 2 is a UV chromatogram of the final HPLC method. Resolution, sensitivity, and peak symmetry were optimal for all analytes. The total run time on a standard HPLC instrument (Agilent 1100) was 25 minutes, but the separation could easily be performed faster on ultra-performance instruments.

Figure 2. UV Chromatogram of the Final HPLC Method

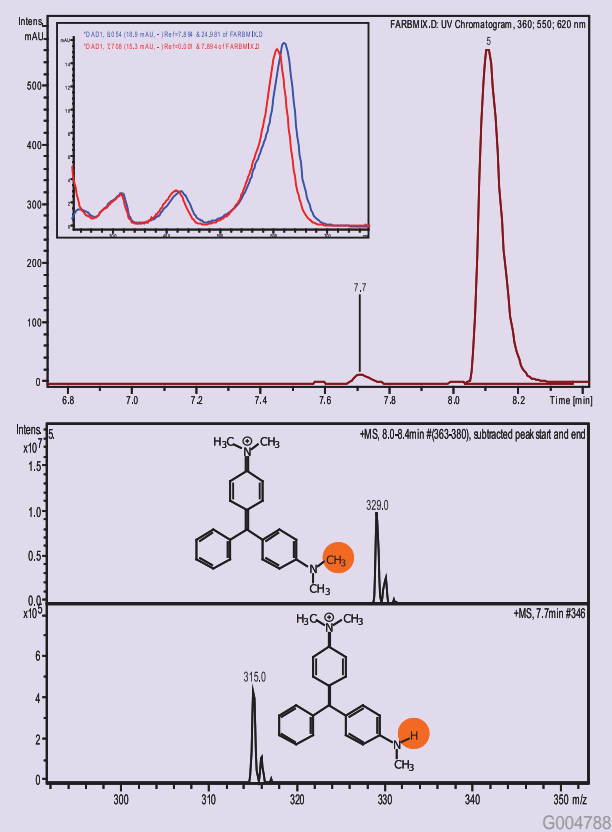


Ascentis Express HPLC Columns: High Efficiency and LC-MS Compatibility

Using Ascentis Express columns on standard HPLC, fast LC, and ultra-performance instruments can yield heightened sensitivity (Figure 2). Mass detectors in LC-MS systems are very sensitive to contaminants in solvents and column bleed, both of which are very low with Ascentis Express columns combined with the right set of Fluka® LC-MS solvents and additives. Both aspects, high efficiency and low column bleed, are basic requirements in trace analysis of small target analyte concentrations or in identification of byproducts which may influence dramatically the quality and application of dyes. Figure 3 shows an example of the identification of low concentrations of byproducts even in very complex mixtures. The unknown substance at the retention time of 7.68 minutes shows a nearly identical UV spectrum to Malachite Green (Peak 5 at 8.10 minutes), but the mass is 14 Da lower. This may correspond to the exchange of a methyl residue with a proton at a position in the Malachite Green molecule that has no influence on the chromophore. Only the displayed molecular structures fit the UV and mass spectroscopic results.

Figure 3. Expanded View of UV Chromatogram Showing Unknown Impurity at 7.7 min. and Malachite Green (5)

The inset shows the UV spectra of malachite green (blue) and the unknown impurity (red). The mass spectra are of malachite green (top) and unknown impurity (bottom).



To get optimal results from your LC-MS system and accurate UV and mass spectra of impurities with a high signal-to-noise level, it is best to use high purity LC-MS solvents from Fluka and high performance HPLC columns such as Ascentis Express from Supelco.

References

1. Commission Decision. Official Journal of the European Union. L154/114. June 10, 2003.
2. Rapid Alert System for Food and Feed (RASFF). 2004. Annual Report. European Commission of Health & Consumer Protection Directorate General.

Featured Products

Description	Cat. No.
Ascentis Express C8, 10 cm x 4.6 mm I.D.	53837-U

Related Products

I.D. (mm)	Length (cm)	Ascentis Express C18	Ascentis Express C8
Ascentis Express Columns			
2.1	3.0	53802-U	53839-U
2.1	5.0	53822-U	53831-U
2.1	7.5	53804-U	53843-U
2.1	10.0	53823-U	53832-U
2.1	15.0	53825-U	53834-U
3.0	3.0	53805-U	53844-U
3.0	5.0	53811-U	53848-U
3.0	7.5	53812-U	53849-U
3.0	10.0	53814-U	53852-U
3.0	15.0	53816-U	53853-U
4.6	3.0	53818-U	53857-U
4.6	5.0	53826-U	53836-U
4.6	7.5	53819-U	53858-U
4.6	10.0	53827-U	53837-U
4.6	15.0	53829-U	53838-U

Related Information

For more information on Ascentis Express columns, request T407044 (JHD) or visit sigma-aldrich.com/express

ordering: 800-247-6628 (us only) / 814-359-3441 technical service: 800-359-3041 (us and Canada only) / 814-359-3041

Liquid Chromatography

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Separation of Proteins and Peptides on Discovery BIO Wide Pore (300Å) C18, C8 and C5 Phases

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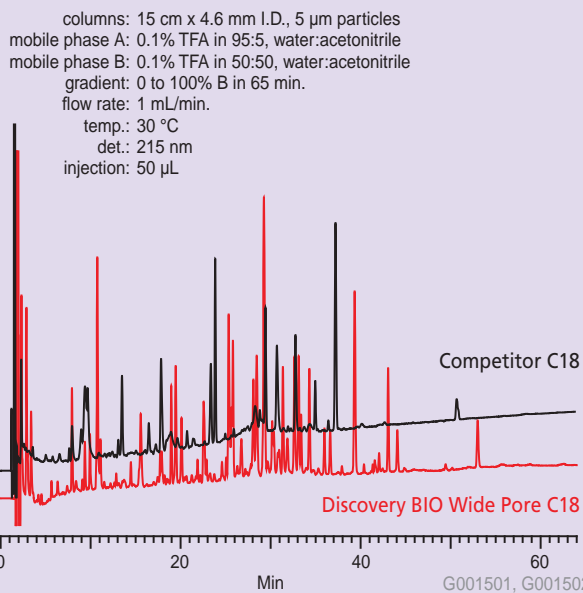
Abstract

When performing protein and peptide separations, scientists prefer HPLC bonded phases to be stable and reproducible while exhibiting high resolution. Discovery® BIO Wide Pore C18, C8 and C5 were specifically developed for those needs. The C18 and C8 phases demonstrate high resolution peptide mapping and purification. The C5 phase exhibits enhanced stability and is excellent for protein separation. All of the phases show high lot-to-lot reproducibility.

Peptide Mapping and Purification with Discovery BIO Wide Pore C18 and C8 phases

Peptide and protein chemists use C18 or C8 phases for peptide mapping and purification. However, problems of peak tailing or low resolution may be encountered due to secondary chromatographic effects. Some unnecessary active sites, such as active silanol groups and metal impurities, may still remain on the surface. This is especially critical for peptide mapping because it often requires resolving more than a hundred peaks in one hour. Figure 1 presents a comparison of a peptide map with well resolved peaks on Discovery BIO Wide Pore C18 phase vs. the same application on a prominent competitor's column.

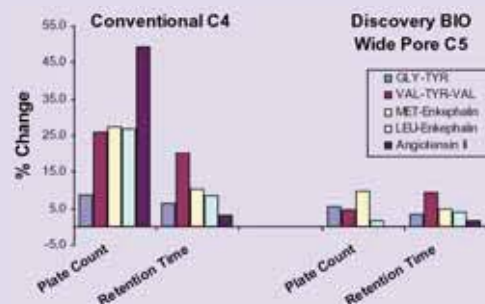
Figure 1. Efficiency of Discovery BIO Wide Pore C18 Gives Higher Resolution Tryptic Maps than Competitive C18 Phase



Protein Analysis and Purification with C5 Phase

In protein analysis and purification, possible sample denaturation is a consideration. For this reason, we would choose to use a short alkyl chain phase, such as C4, for the application. This may reduce the length of time the sample is retained on the column and lessen the amount of the stronger organic solvent needed. However, C4 is very susceptible to acid hydrolysis and may not remain stable over time. This problem is overcome on the Discovery BIO Wide Pore C5. It demonstrates almost identical selectivity to C4, but is substantially more stable, translating into longer column life. Figure 2 compares the stabilities between Discovery BIO Wide Pore C5 and a conventional C4 phase.

Figure 2. Stability Comparison of Discovery BIO Wide Pore C5 and a Conventional C4 HPLC Column



Reproducibility of the Phase

Reproducibility is another key factor to consider when selecting a column, and is generally discussed in terms of run-to-run, column-to-column, and lot-to-lot (or batch-to-batch). Variation in run-to-run reproducibility is generally very low, provided the HPLC instrumentation is well maintained and the bonded phase is stable. Differences in column-to-column reproducibility within the same lot are also negligible with today's standardized column packing and manufacturing processes. Rather, most reproducibility problems arise from lot-to-lot variances. Factors influencing lot-to-lot reproducibility include differences in silica lots, reagent lots, other processing materials, and operators. However, when strictly controlled, those effects can be greatly minimized. Figure 3 presents the results of a peptide test mix performed on three lots of Discovery BIO Wide Pore C5. The RSD of the retention time of last peak is less than 2%.

NEW Supel-Select HLB SPE for Pharmaceutical Analysis

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Introduction

Within the last 5-10 years, hydrophilic polymer SPE phases have become the phase of choice when developing new extraction methods that recover, concentrate, and cleanup small molecules (e.g., pharmaceutical compounds) from complex aqueous sample matrixes (e.g., biological



P001336

fluids, water, etc.). Unlike traditional reversed-phase chemistries such as C18, hydrophilic polymers are typically comprised of a hydrophobic component (e.g., polystyrene and/or divinyl benzene) and a hydrophilic component (e.g., methacrylate, vinylamidazol, N-vinyl pyrillidone, and/or hydroxyl). Because hydrophilic polymer SPE phases contain both non-polar and polar functional groups, they offer distinct advantages over traditional silica-based reversed-phase SPE chemistries. Unlike silica-based reversed-phase SPE chemistries that can only retain moderately-polar to non-polar compounds, hydrophilic polymer phases can retain a much broader range of polar to non-polar acidic to basic analytes. Once such compounds are retained, they are easily eluted with an aqueous miscible solvent such as methanol and/or acetonitrile. As a result, hydrophilic polymer phases are highly amenable to generic methodology. In addition, because hydrophilic polymer phases contain polar functionalities, they are very resistant to over-drying, often making associated extraction methods more reproducible/robust. In contrast, alkyl chain collapse can occur for silica-based reversed-phase chemistries if the stationary phase over-dries during conditioning.

In this report, we demonstrate the utility of a new hydrophilic polymer phase called Supel™-Select HLB SPE (Select HLB) in which we use the phase to extract a range of pharmaceutical compounds and personal care products from both plasma and drinking water. The plasma application describes a generic method used to extract two Statin drugs, potent cholesterol-lowering agents. The drinking water method is a modified version of EPA Method 1694: *Pharmaceuticals and Personal Care Products in Water, Soil,*

Sediment, and Biosolids by HPLC/MS/MS. In recent years, there has been increasing evidence to suggest the detrimental effects of pharmaceutical and personal care compounds finding their way into the public's drinking water supplies. For example, such effects can possibly lead to the disruption of human hormone metabolic pathways, the emergence of antibiotic-resistant bacterial strains, and increased incidence rates of certain cancer types (1). In 2002, the US Geological Survey tested water samples downstream of urban areas, and found pharmaceutical compounds in 80% of the streams sampled (2).

What is Supel-Select HLB SPE?

Select HLB SPE is a hydrophilic modified styrene-based polymer developed for the solid phase extraction of a highly broad range of compounds from aqueous samples. The retention mechanism is predominately based on reversed-phase interaction. However, because the phase is hydrophilic modified, it is also selective for more polar

Table 1. Specifications of Select HLB SPE

Phase Chemistry:	Hydrophilic modified styrene polymer
pH Compatibility:	0-14
Particle Size:	55-60 µm
MS Suitable:	Yes
Surface Area:	400-410 m ² /g
Pore Volume:	0.88 mL/g
Pore Size:	87 Å

compounds (HLB: hydrophilic lipophilic balance). Examples of more polar compounds that are retained and recovered on Select HLB include (but are not limited to): pyridoxine (logPo/w -0.56), riboflavin (logPo/w -2.02), and biotin (logPo/w 0.11). Tables 1 and 2 describe the specifications and features/benefits of the technology, respectively.

Extraction of Statins from Plasma

In this study, pravastatin and atorvastatin were spiked in rat plasma at the levels of 100 and 5 ng/mL. The spiked rat plasma samples (n=3) were extracted using Select HLB SPE, 30 mg/1 mL (54181-U) and two leading competitor polymer SPE phases using the method described in Table 3 and analyzed via LC-MS/MS. From the results described in Figure 1, Select HLB SPE offered good sample clean-up and high recoveries for both analytes at the spike concentrations tested, similar to the leading competitors.

Table 2. Features & Benefits of Select HLB SPE

Feature	Benefit
Hydrophilic modified styrene resin	Extract and recover a broad range of analytes (polar to non-polar, acidic to basic) using a single sorbent
Generic methodology	Save time, money, and headache during method development
Greater capacity	Allows for smaller bed weights = smaller elution volumes = time savings in sample processing
Resistant to overdrying	More robust methodology
Low UV and MS extractables	Lower background for greater sensitivity
Stringent production & QC	Greater lot-to-lot, tube-to-tube, and well-to-well consistency for improved accuracy and precision

Extraction of Pharmaceutical & Personal Care Products in Water

In this study, Select HLB SPE was tested against a leading competitor's hydrophilic polymer SPE using EPA Method 1694 - Pharmaceuticals and Personal Care Products in Water, Soil, Sediment, and Biosolids by HPLC/MS/MS (3). A calibration solution containing 48 x Group 1 pharmaceutical compounds were prepared at the concentration levels (25 – 25,000 ng/mL) described in EPA Method 1694, Table 11a, column CS-5; and the test sample was prepared by

spiking 1 L drinking water with 4 mL of CS-5 calibration solution. The 1 L spiked sample was extracted using the procedure described in Table 4 (page 10), and analyzed using Ascentis Express C18, 10 cm x 2.1 mm (52823-U) in conjunction with LC-MS/MS conditions

Table 3. Generic Select HLB SPE Procedure for the Extraction of Statins from Plasma

Condition & Equilibrate with methanol (or elution solvent) followed by DI water or buffer

Load Sample

Wash off or elute interferences buffer and/or 5-10% methanol or acetonitrile

Elute with methanol:acetonitrile (1:1, v/v)

Note: 0.5 mL volumes were used for each Select HLB SPE step.

Did you know...?

Did you know that Supel-Select HLB SPE was benchmarked against the leading competitor's polymer SPE phases during development and is priced ~30% less than the competition?

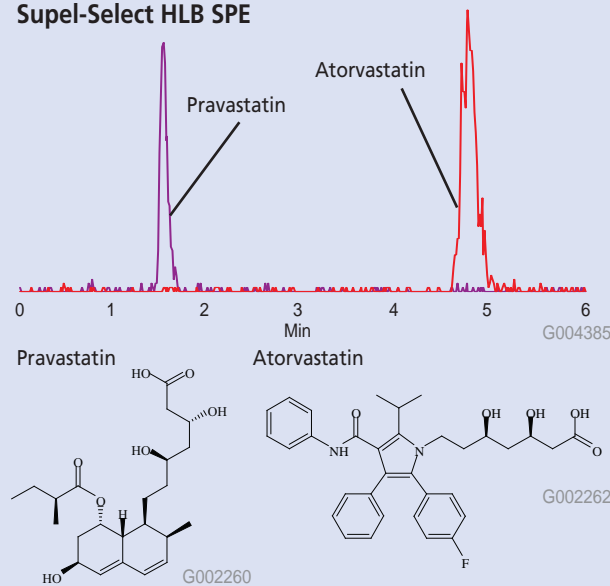
Did you know...?

Did you know that Supel-Select HLB SPE is slightly more hydrophobic than most other commercially available hydrophilic polymer SPE phases? As a result, Select HLB will often require a mixture of MeOH:MeCN (50:50) to completely elute retained analytes.

Figure 1. Total Ion Chromatogram of Rat Plasma Spiked with 5 ng/mL Statins and Extracted by Select HLB SPE

column: Ascentis Express C18, 5.0 cm x 2.1 mm I.D., 2.7 μ m (53822-U)
mobile phase: methanol:0.1% acetic acid (60:40)
flow rate: 0.2 mL/min.
temp.: 35 °C
detection: MS/MS, Applied Bio System 3200 Q TRAP
inj.: 5 μ L

Supel-Select HLB SPE



	Absolute Recovery \pm RSD (n=3)			
	5 ng/mL spike		100 ng/mL spike	
	Pravastatin	Atorvastatin	Pravastatin	Atorvastatin
Supel-Select HLB	84 \pm 8%	92 \pm 5%	103 \pm 4.2%	89 \pm 3.9%
Competitor W	83 \pm 17%	92 \pm 2%	104 \pm 2.2%	87 \pm 1.1%
Competitor P	77 \pm 5%	93 \pm 2%	102 \pm 3.0%	91 \pm 1.3%

described in EPA Method 1694, Tables 2 & 3. Absolute recovery was compared for both Select HLB and Competitor W using two different elution solvents: methanol and methanol:acetonitrile (50:50), see Table 5 (page 10). For the majority of the compounds tested, recovery values were similar between Select HLB and Competitor. Both products yielded an average absolute recovery of 61-65% recovery for the 48 analytes tested. Note that Select HLB SPE is slightly more hydrophobic than equivalent hydrophilic polymers that are commercially available. As a result, an elution solvent of methanol:acetonitrile (50:50) was tested. A CH₃OH:CH₃CN elution solvent improved recovery (by >10%) on Select HLB for at least 20 of the 48 compounds

(continued on page 10)

Table 4. EPA Method 1694 SPE Procedure for Group 1 Compounds

SPE: Supel-Select HLB SPE Tube, 200 mg/6 mL (54183-U) & Equivalent Competitor W SPE Product

1. Condition and equilibrate each SPE cartridge with 20 mL methanol followed by 6 mL DI water and 6 mL DI water, pH 4 (adjusted with 6 M HCl).
2. Load 1 L spiked water sample, pH 4 (adjusted with 6 M HCl).
3. Dry cartridge under negative pressure (using vacuum manifold) for 5 minutes.
4. Elute with 12 mL methanol OR 12 mL methanol:acetonitrile (50:50).
5. Evaporate SPE eluent to 4 mL under nitrogen at 40 °C and add 4 mL LC mobile phase.

Table 5. Absolute Recovery of Group 1 EPA Method 1694 Pharmaceutical Compounds

Absolute Recovery (%)	Select HLB		Competitor W	
	CH ₃ OH:CH ₃ CN (50:50) Elution	CH ₃ OH Elution	CH ₃ OH:CH ₃ CN (50:50) Elution	CH ₃ OH Elution
Analyte				
1,7-Dimethylxanthine	14.41	56.15	83.59	68.65
Acetaminophen	119.50	112.30	136.48	141.18
Ampicillin	0.00	14.10	0.00	12.50
Azithromycin	101.00	52.99	58.90	50.34
Caffeine	70.71	86.23	81.43	85.51
Carbadox	80.77	73.10	59.76	69.46
Carbamazepine	81.47	79.63	83.38	92.86
Cefotaxime	45.49	74.14	20.44	61.79
Ciprofloxacin	21.73	57.14	50.58	94.74
Clinafloxacin	69.46	59.29	74.29	70.18
Cloxacillin	42.83	0.00	28.48	15.00
Codeine	3.75	8.72	66.02	64.18
Cotinine	9.48	8.12	65.50	34.20
Dehydronifedipine	63.84	77.11	87.74	74.40
Digoxigenin	69.96	80.12	76.86	83.28
Digoxin	80.05	NA	50.33	NA
Diltiazem	114.46	124.52	123.47	138.04
Diphenhydramine	100.00	119.02	99.38	82.48
Enrofloxacin	55.53	58.37	40.91	80.42
Erythromycin hydrate	95.65	63.87	59.68	57.10
Flumequine	83.10	56.27	44.15	69.57
Fluoxetine	61.50	55.35	77.46	55.76
Lincomycin	2.62	7.98	44.74	31.98
Lomefloxacin	23.37	67.09	54.74	97.58
Miconazole	84.02	52.03	71.13	81.71
Norfloxacin	18.15	41.29	60.49	89.35
Norgestimate	392.24	39.36	215.30	41.35
Ofloxacin	23.10	59.14	56.58	75.96
Ormetoprim	27.54	86.80	66.27	103.55
Oxacillin	0.00	19.12	0.00	6.24
Oxolinic acid	64.97	44.26	50.27	43.82
Penicillin G	0.00	6.08	0.00	6.06
Penicillin V	27.89	55.12	81.87	38.77
Roxithromycin	32.64	39.30	19.73	31.33
Sarafloxacin	53.21	56.24	58.18	67.63
Sulfachloropyridazine	78.05	67.09	49.19	62.39
Sulfadiazine	104.79	70.16	77.84	76.70
Sulfadimethoxine	73.65	51.34	45.27	63.09
Sulfamerazine	93.64	76.88	57.73	89.25
Sulfamethazine	94.59	74.00	78.11	73.40
Sulfamethizole	85.61	74.48	48.99	76.72
Sulfamethoxazole	72.46	56.14	49.35	71.43
Sulfanilamide	10.11	7.49	24.80	32.84
Sulfathiazole	104.27	73.91	66.82	68.12
Thiabendazole	95.54	77.95	86.99	83.65
Trimethoprim	25.68	65.20	83.91	100.84
Tylosin	97.70	44.26	46.36	26.11
Virginiamycin	71.92	56.16	68.08	63.36

(continued from page 9)

tested. However, a neat CH₃CN elution provided greater recovery for 10 Group 1 compounds. For optimal recovery using Select HLB, we recommend to first elute with neat methanol followed by methanol:acetonitrile.

Conclusion

Select HLB SPE is a hydrophilic polymer SPE phase designed for the extraction of a highly broad range of compounds from complex aqueous sample matrices. In this report, we demonstrated the utility of this SPE phase for two applications: 1) statins in plasma and 2) 48 pharmaceutical compounds in drinking water. Select HLB SPE offers similar recovery and selectivity to the leading competitors and high recoveries using generic methodology.

References

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2. USGS, 2007 Pharmaceuticals, Hormones, and Other Organic Wastewater Contaminants in US Streams. USGS Fact Sheet FS-027-02 <http://toxics.usgs.gov/pubs/FS-027-02/>
3. US EPA Method 1694: Pharmaceuticals and Personal Care Products in Water, Soil, Sediment, and Biosolids by HPLC/MS/MS <http://www.epa.gov/waterscience/methods/method/files/1694.pdf>

Featured Products

Description	Qty/Pk.	Cat. No.
Supel-Select HLB SPE		
30 mg/1 mL	100	54181-U
60 mg/3 mL	50	54182-U
200 mg/6 mL	30	54183-U
500 mg/12 mL	20	54184-U
1 g/20 mL	20	54186-U
Supel-Select HLB 96-well SPE		
10 mg/ well	1	Inquire
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NEW! Radiello Diffusive Sampler for Monitoring 1,3-Butadiene and Isoprene in Workplace Air



Contributed Article

The following was generated by an outside source using Sigma-Aldrich products. Technical content provided by:

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Introduction

The manufacturing of elastomers makes wide use of 1,3-butadiene (CAS No. 106-99-0) and isoprene (2-methyl-1,3-butadiene, CAS No. 78-79-5). Both compounds are classified as carcinogenic by the International Agency for Research on Cancer (IARC). 1,3-Butadiene is currently listed in group 1, carcinogenic for humans, and isoprene is in group 2B, possibly carcinogenic for humans. These compounds may also be present in lower concentration in the environment (lower ppb levels).

The measurement of these compounds in workplace air requires high sensitivity and also the control of explosive hazards, which are frequent in chemical plants. Diffusive sampling followed by thermal desorption and GC-MS analysis represents a simple and affordable tool for this measurement, while satisfying the above requirements.

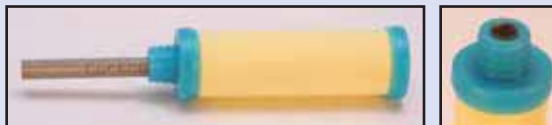
In general, the measurement of volatile organic compounds (VOC's) suspected of carcinogenic properties in workplace air requires high sensitivity, down to ppb level. The existing methods for the measurement of 1,3-butadiene involve the pumping of air through activated charcoal or molecular sieves, followed by solvent or thermal desorption and gas chromatography (1,2). Methods based on solvent desorption are inherently less sensitive because the sample is diluted by a factor of about 1,000 during extraction. Thermal desorption, when performed by two-stage thermodesorbers (desorption and concentration/trapping step), have a typical "dilution" factor of 50-100, arising from the combination of secondary and primary split, which are adjustable depending on the expected amount of analytes.

More recently, methods based on diffusive sampling onto molecular sieves or graphitized carbon blacks, were proposed (3,4). However, the sampling rates of such methods, based on axial diffusion tubes, are lower than 1 mL/min and do not allow ppb level sensitivities. The use of radial symmetry diffusive samplers like Radiello®, provide commonly higher sampling rate values (5) in combination with thermal desorption and allow the measurement of 1,3-butadiene and isoprene in workplace air with a sensitivity in the ppb range.

Sampling

The sampling of 1,3-butadiene and isoprene with Radiello diffusive samplers is performed using an adsorbing cartridge (4.8 mm diameter stainless steel net), packed with Carbopack™ X, the strongest adsorbent in the range of Supelco graphitized carbons, with a specific surface area of 240 m²/g (6).

Figure 1. Radiello Cartridge in Yellow Diffusive Body



The sampler is easily assembled by placing the cartridge into a yellow diffusive body RAD1202 (Figure 1), and attaching it to the triangular support plate (RAD121); once assembled, the sampler is typically exposed during the entire work shift (8 hours). After sampling, the adsorbing cartridge is placed again in the glass storage/transport container and the barcode label is placed on the container. Prior to analysis, the samples should be kept cold and stored in the freezer. Time until analysis should not exceed 24 hours.

Analysis

The analysis of diffusive samples includes two-stage thermal desorption (TD), followed by HRGC-MS. The sample loaded adsorbing cartridge is transferred into an empty SS thermal desorption tube (¼ inch O.D., min. 4.9 mm I.D.), which is then placed into the TD instrument. The collected sample is desorbed by heat and transferred onto a GC column.

Typical Thermal Desorption Conditions:

primary desorption temperature:	320 °C for 6 min.
primary desorption flow:	90 mL/min
primary split ratio:	3:1
cold trap:	Tenax® TA 60/80 mesh
cold trap cryofocusing temperature:	-20 °C
cold trap heating temperature:	290 °C
cold trap heating time:	1 min.
secondary desorption flow:	21 mL/min
secondary split ratio:	26:1

When choosing the proper GC column, the potential interference from other C4 alkenes, which may occur in real samples, like 1-butene, 2-butene (trans- and cis-) and isobutylene must be taken into account.

Calibration

The calibration standards for 1,3-butadiene can be prepared by the gas syringe spiking procedure, using a gastight microsyringe (10 µL or more) and measured volumes of either pure or 1:50 diluted gas that is loaded onto the

(continued on page 12)

(continued from page 11)

Carbopack X cartridges fitted into an empty SS thermal desorption tube that is connected to a GC injector (40 mL/min nitrogen purge stream). Cartridges used for preparing calibration cartridges should have a tight fit in the TD tube (slight resistance when pushing in tube) to ensure a reliable adsorption of standard volume. The isoprene calibration standards are prepared by injecting measured volumes of a methanol solution of the analyte.

The analysis is best performed within 24 hours after the exposure. If the analysis is performed later, the storage requirement of the EN 838 standard (7) is satisfied within 14 days of storage. In such a case, the maximum analyte loss is 7% for 1,3-butadiene and 8% for isoprene, which is less than the maximum loss (10%) allowed by the standard (7).

Calculation

The concentration C of 1,3-butadiene and isoprene determined by diffusive sampling are calculated by the general equation:

$$C_{(\mu\text{g}/\text{m}^3)} = \frac{(m_{(ng)} - mb_{(ng)}) \cdot 1000}{Q_{(\text{cm}^3/\text{min})} \cdot t_{(\text{min})}} \quad [1]$$

where

m = sampled mass of the analyte

mb = mass of the blank (usually negligible)

Q = diffusive uptake rate

t = exposure time

The diffusive uptake rate values for both compounds (Table 1) were determined according to EN 838, which gives guidance for using diffusive samplers in workplace air monitoring (7).

Table 1. Diffusive Uptake Rate for 8 Hour Exposure at 20 °C, 50% RH

Analyte	Concentration Range ($\mu\text{g}/\text{m}^3$)	Diffusive Uptake Rate Q (cm^3/min)
1,3-butadiene	34 – 2,960	30.5 ± 0.3
isoprene	2 – 6,680	41.2 ± 4.9

The combined effect of temperature and humidity may affect the sampling rate, with a significant decrease at high temperature and high humidity (40 °C and 80% RH); therefore it is recommended to avoid taking measurements when such extreme conditions are expected. At lower temperatures (5 °C), the effects are comparable and small.

A field validation on 40 workers (18 °C temperature and 75% relative humidity, on average) demonstrated that the measured concentrations agree with the results obtained by the OSHA method 56 (solvent desorption from TBC-treated charcoal and GC). The results obtained with Radiello samplers, plotted against the results of OSHA 56 method, show a regression line with slope very close to 1 and no significant intercept (data not shown).

Measurement of Uncertainty

Table 2 shows the values of the uncertainty in the measurements of 1,3-butadiene in the workplace, assessed by two different approaches. The uncertainties were first determined under laboratory conditions, following the methods of the ISO GUM (Guide to Expression of Uncertainty in Measurement, International Organization for Standardization, Geneva, 1993) and ISO 5725:1994 (Accuracy, trueness and precision of measurement methods and results). In this case, the uncertainty accounts for all contributions involved in the measurement process (effect of time, T, RH on uptake rate, uncertainty of measured mass and so on), which were determined according to EN 838:1995. Afterwards, the uncertainty was determined by a field comparison with the method OSHA 56 (as the reference method), following the standard ISO 13752.

Table 2. Measurement Uncertainty for 8 Hour Diffusive Sampling of 1,3-Butadiene in Workplace Air

Relative combined expanded uncertainty (2·u _c)	200 $\mu\text{g}/\text{m}^3$	442 $\mu\text{g}/\text{m}^3$ (0.1 TLV)	2,210 $\mu\text{g}/\text{m}^3$ (0.5 TLV)	4,420 $\mu\text{g}/\text{m}^3$ (TLV-TWA ACGIH)
Laboratory tests at 20 °C, 50% RH (EN 838, calculations by ISO GUM)	48.4%			
Field comparison (ISO 13752)	37.0%	25.0%	11.1%	7.9%

Conclusion

The thermal desorption Radiello sampler filled with Carbopack X represents a new and simple tool for assessing the workplace exposure to two carcinogenic chemicals: 1,3-butadiene and isoprene at ppb concentration levels. It is convenient in use and can be applied for any workplace where explosive hazards exist.

References

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- EN 838:1995 Workplace atmospheres – Diffusive samplers for the determination of gases and vapours – requirements and test methods.

Featured Products

Description	Pk.	Cat. No.
Radiello Cartridges 1,3-Butadiene/Isoprene w/Carbopack X	20	RAD141
Radiello Triangular Support Plate	20	RAD121
Radiello Diffusive Body, Yellow	20	RAD1202
Radiello Vertical Adapter for Personal Sampling	20	RAD122
Starter Kit 1,3-Butadiene/Isoprene Sampler Includes: 1 support plate, 1 yellow diffusive body, 1 vertical adapter, 2 adsorbing cartridges w/Carbopack X	1	RAD141S

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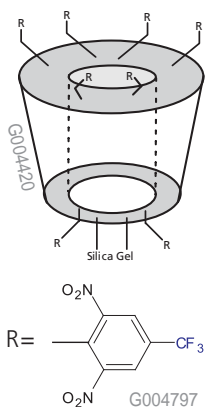
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Prevent GC Inlet Problems BEFORE They Cost You Time and Money!

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

Introduction

Our Technical Service chemists help GC users solve problems that are often the direct result of overused septa or dirty injection port items. Many of these costly problems could be avoided. This article discusses how simple, routine preventative maintenance of the injection port can reduce the risk of chromatographic problems and instrument downtime.

Getting Started

Every laboratory should have a preventative maintenance schedule that is adhered to, allowing their GC systems to produce optimal chromatography, day-after-day, month-after-month, and year-after-year. An example of a preventative maintenance schedule is shown in Table 1. A separate maintenance log book should be kept for each instrument, where all preventative maintenance is recorded.

Table 1. Example of a Preventative Maintenance Schedule

Item	Frequency
GC Septa, GC-MS	Daily
GC Septa, GC (non-MS)	Every 100 injections or every 3 days
Inlet Liners	Inspect daily
Liner O-Rings	Every 5 liners
Inlet Seals	Every column change or every 6 months
Injection Syringes, Manual	Every 2 months
Injection Syringes, Autosampler	Every 6 months
Split Lines	When needed
Carrier Gas Purifiers	As specified by purifier manufacturer

Prior to and during installation, always handle GC septa, inlet liners, liner o-rings, and inlet seals with clean, solvent-rinsed tweezers. Do not touch these items with bare hands (finger oil is a contaminant) or gloved hands (phthalate esters are contaminants). Additionally, be sure to follow your GC manufacturer's installation instructions.

GC Septa

The GC septum should be routinely replaced to reduce the risk of leaks and septum particle contamination. In fact, if the instrument is in heavy use, change the septum daily. Repeated use of the same septum will result in increased coring, which may introduce a leak and/or result

in septum fragments being deposited in the inlet liner.

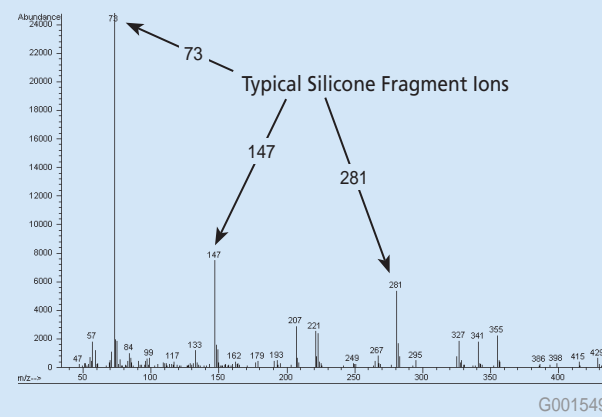
Septum fragments in the inlet liner may result in extra



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peaks being observed in the chromatogram. If using MS detection, the mass spectrum of an analyte could be corrupted with extra m/z, as shown in Figure 1.

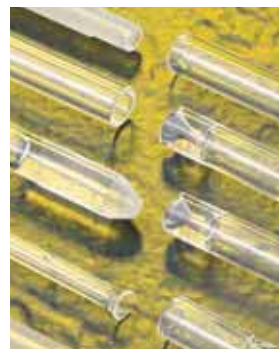
Figure 1. Mass Spectrum: Septa Fragments in Inlet Liner



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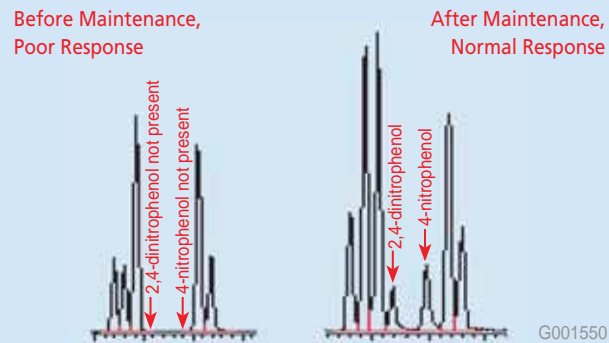
Inlet Liners

GC users should routinely inspect and replace the inlet liner to remove contaminants and active sites from the sample path. Samples passing through the liner can contain components that do not volatilize, leaving behind residue. As residue accumulates on the liner, chromatography is affected by the adsorption of subsequent analytes. Adsorption results in poor peak shapes, and sometimes loss of peaks in the chromatogram. The inlet liner should be inspected regularly, if not daily, and replaced with a new, deactivated liner when necessary. Figure 2 shows how active sites in a dirty liner can affect results.



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Figure 2. Chromatography Problems with a Dirty Liner



G001550

Liner O-Rings

If your instrument uses an o-ring that seals near the top of the inlet liner, it should be replaced frequently, at least once for every five liner changes. An o-ring is deformed and flattened slightly when sealed into the GC inlet. If the flattening is too severe, it can affect the o-ring's ability to form a proper seal. If the o-ring begins to crack, a leak may develop, compromising the split/splitless flow dynamics in the injection port.



Inlet Seals

Many instruments have an inlet seal, often gold-plated, that the inlet liner rests on. Similar to inlet liners, sample residue can also contaminate the inlet seal, forming active sites that result in analyte adsorption. Access to the inlet seal is not as easy as access to septa, inlet liners, or liner o-rings. From inside the GC oven, the column and a



reducing nut must first be removed from the injection port. A thin washer that rests below the inlet seal should also be replaced. The inlet seal and washer should always be replaced when a new column is installed.

Injection Syringes

An often forgotten component that needs to be replaced periodically is the injection syringe. Over time, contamination may reside in the syringe barrel or in the needle, observed as extraneous peaks in the chromatography. Additionally, when performing manual injections, finger oil can be transferred to the plunger, causing it to stick inside the syringe barrel.



Split Lines

Problems can occur over time within the split lines of the injection port. Injection of sample volumes in excess of the liner capacity can result in flashback, which can contaminate the split line. This can then result in "ghost peaks" in subsequent chromatographic analyses. Septa particles and sample residues can build up in split lines and cause a plug, which will result in problems maintaining proper flow to the inlet. Finally, on some GC systems, to access the inlet liner, a large nut to which the split line is attached must be removed. Frequent manipulation of this nut can result in kinking and/or breakage of the split line.

Purifiers

While not injection port items, purifiers are mentioned in this article because they have a direct affect on the quality of the carrier gas entering the injection port. The three contaminants that need to be removed from a carrier gas stream are hydrocarbons, moisture, and oxygen. Using a multi-bed purifier that removes multiple contaminants, or several single-bed purifiers that each remove a separate contaminant, are both viable options. Replacement of purifiers when they become saturated will ensure a continuous supply of clean carrier gas into the injection port and GC system.



Conclusion

GC users often overlook or disregard the importance of preventative maintenance, and only act when a problem is obvious. There are several important injection port components that require attention to reduce the risk of problems. These items should be checked and replaced regularly, particularly when installing a new column. Be proactive with your maintenance today, or be sorry with your results tomorrow.



Related Information

For more information on maintenance, request T112853 (AIP), *Capillary GC Troubleshooting Guide: How to Locate Problems and Solve Them*.

Did you know...?

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Capillary Column Ferrule Selection and Installation

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Introduction

To provide a leak-tight seal, the ideal gas chromatography (GC) ferrule will accommodate column outside diameter (O.D.) variations, seal with minimum torque, and not stick to the column or fittings. The proper selection of any GC ferrule should be based on compatibility with the column material, temperature, connection requirements, and variation in column diameter. Supelco offers Supeltex™ and CapSeal Bullet™ ferrules to provide a leak-free connection for any application.

Choosing the Correct Ferrule Material

A proper ferrule for capillary GC is designed to seal tightly on all columns having the same nominal O.D., and should be made of material compatible with the column. As shown in Table 1, ferrule composition will dictate reusability and allowable injector and detector temperature. In multiple-use applications, the ferrule must resist deformation and the affects of high temperature. If you are using temperature-programmed analyses, the ferrule also must be resilient enough to maintain a good seal when column temperature and corresponding back pressure increase. Supeltex M-2A ferrules are multiple-use ferrules.

The outside diameters of equal O.D. columns often differ because tubing dimensions cannot be absolutely controlled. Soft ferrules, such as those made of graphite, usually are flexible enough to adjust to these variations and will not stick to fittings or columns due to their natural lubricating characteristics. Graphite is one of the most thermally stable and chemically resistant materials. In a natural or vacuum environment, it does not melt when heated. In the presence of oxygen and temperatures above 450° C, graphite can oxidize. Ideal for capillary GC applications with its versatility, Supeltex M-4 (flexible graphite) ferrules provide great sealability, ensuring a leak-tight seal for extended periods at high temperature.

The CapSeal Bullet ferrule, with its innovative design, provides a refined graphite ferrule with a clean sharp

profile, and minimal flashing to ensure maximum sealing surface and minimal possibility of column contamination during installation. The graphite body in an aluminum base makes it easy to remove the ferrule from any fitting. The CapSeal Bullet ferrule allows low-torque sealing (effective at 1/8-turn past fingertight), and has a temperature limit of 450° C for both isothermal and programmed temperature use, under normal conditions.

Choosing the Correct Ferrule I.D.

Following the selection of the ferrule material best designed for your capillary column, next will be the selection of a size that matches the column. Before tightening, ferrules should fit snugly around the column because loose ferrules may crack or damage the column wall during tightening. This can cause leaks or column breakage after several heating and cooling cycles. Use 0.4 mm I.D. ferrules with 0.25 mm I.D. and smaller I.D. fused silica columns, 0.5 mm I.D. ferrules with 0.32 mm I.D. columns, and 0.8 mm I.D. ferrules with 0.53 mm I.D. columns.

Choosing the Correct Ferrule Shape

After selecting a ferrule material and I.D. that best fits the application, it is critical to use the proper ferrule shape, as shown in Figure 1, that is compatible with the column nut. An improper nut and ferrule combination will create dead volume between the ferrule and the portion of the injection port that is not swept by the carrier gas. This can result in poor chromatography as evidenced by fronting peaks and band broadening. To ensure the chromatography is the best it can be just follow these few suggestions. If you are using the original nut supplied for the injector and non-MS detector end of an Agilent® GC instrument, choose a Supeltex short design ferrule. For the MSD source nut of an Agilent instrument, the recommended ferrule is the Supeltex long design. On PerkinElmer® and Varian® GC instruments, the nut supplied with the instrument will use the Supeltex long design ferrule.

Column Installation

Correct installation of the ferrule is critical to column performance. Once the ferrule is properly sized, squarely cut

Table 1. Ferrule Properties

Ferrule	Composition	Temp. Limit	Characteristics
Supeltex M-2A	VESPEL™ SP-21 (85% polyimide/15% graphite)	400 °C	Conforms easily to capillary columns, ensuring an effective seal with less chance of breakage. High reusability.
Supeltex M-4	Flexible graphite	450 °C	Seals at 1/4 turn past fingertight. Maximum surface contact. Minimal chance of column contamination during installation. Will not stick to column. Limited reusability.
CapSeal Bullet	Graphite in aluminum base	450 °C	Seals at 1/8 turn past fingertight. Minimum flashing. Will not stick to column or fitting. High reusability.

Figure 1. Column Ferrules



the sealed capillary column end. To make the cut, Supelco recommends using a specialized tool, such as a Capillary Cleaving™ Tool or Shortix® Tubing Cutter, and inspecting the cut with a magnifier, such as a Coddington Magnifier or Lenscope Illuminated Magnifier, to verify the cut is square. Column fragments or ferrule particles can contaminate the column, causing it to adsorb active analytes. Therefore, always keep the column end pointed toward the floor when cutting the end, or while installing the nut and ferrule. This will allow column fragments and ferrule particles to fall away from the column. Install the nut and ferrule on the column according to your instrument instructions. After ferrule installation, remove stubborn particles that may remain in the column end by cutting about one inch from the end of the column. Do this each time a ferrule is installed.

When installing a fused silica column in the injector or detector port, follow the instrument manufacturer's recommended insertion distances. Otherwise, column performance may be adversely affected. Attach the column by inserting inlet and detector ends and handtightening the fittings. Use a wrench for final tightening. To avoid column damage, do not over-tighten.

Using the appropriate ferrule does not automatically ensure a leak-free seal. Whether installing a column in the GC or connecting it to a GC-MS system, failure to check all connections may cause unreliable analytical results. Installation is complete when an electronic leak detector, such as a GOW-MAC® leak detector, proves that the system is leak-free.

Conclusion

Selecting the best ferrule for your analyses need not be a complicated issue. If you want to reduce the possibility of leaking connections, and minimize your experience at digging stuck ferrules out of fittings, we recommend the simple approach presented in this article. Proper ferrule selection and installation can save hours of wasted time and effort each and every time a new capillary GC column is installed.

Featured Products

Description	Ferrule I.D.	Pack Size	Cat. No.
Capillary GC Ferrules, Short Design[▲]			
Supeltex M-2A	0.4 mm	10	24803-U
Supeltex M-2A	0.5 mm	10	24806-U
Supeltex M-2A	0.8 mm	10	24801-U
Supeltex M-4	0.4 mm	10	24811-U
Supeltex M-4	0.5 mm	10	24809-U
Supeltex M-4	0.8 mm	10	24808-U
CapSeal Bullet	0.4 mm	10	23864
CapSeal Bullet	0.5 mm	10	23865
CapSeal Bullet	0.8 mm	10	23866
Capillary GC Ferrules, Long Design[▼]			
Supeltex M-2A	0.4 mm	10	24826-U
Supeltex M-2A	0.5 mm	10	24824-U
Supeltex M-2A	0.8 mm	10	24823-U
Supeltex M-4	0.4 mm	10	28025-U
Supeltex M-4	0.5 mm	10	28026-U
Supeltex M-4	0.8 mm	10	28027-U
CapSeal Bullet	0.4 mm	12	23488
CapSeal Bullet	0.5 mm	12	23489
CapSeal Bullet	0.8 mm	12	23490

NOTE: Other pack sizes available at sigma-aldrich.com/gc

[▲] Designed to fit original column nuts (except MSD source nuts) that ship with Agilent GCs.

[▼] Designed to fit original nuts that ship with PerkinElmer and Varian GCs. Also fit Agilent MSD source nuts.

Related Products

Description	Cat. No.
Retractable Capillary Cleaving Tool	23814
Capillary Cleaving Tool	23740-U
Shortix Tubing Cutter	21386-U
Coddington 20X Magnifier	23139
Lenscope 10X Illuminated Magnifier	23135
Agilent Column Nut (use w/short design ferrules), Pk. 2	24833-U
Agilent MSD Source Nut (use w/long design ferrules), Pk. 5	28034-U
GOW-MAC Mini Leak Detector, 115 V/60 Hz	22807
GOW-MAC Mini Leak Detector, 230 V/50 Hz (CE approved)	22808

Related Information

For more information on capillary GC ferrules and fittings, request *The Supelco Guide to Leak-Free Connections*, T100741 (AXR) and *Improved Design Graphite Ferrules Help Ensure Leak-Free Connections for Capillary GC*, T394016 (AOR). These publications are available in electronic form only. Please provide email address on the request form to ensure delivery.

Did you know...?

Using liquids to detect gas leaks is not practical, especially in a capillary GC system. Even a small amount of liquid leak detector that seeps into a fitting, or through the septum, can damage your column. To help find leaks quickly without risk of contaminating your system, use an electronic gas leak detector. These units can easily and quickly pinpoint gas leaks too small to detect with a soap solution.

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GC-SH is a relatively straightforward technique, and the methodology, as it applies to residual solvents in pharmaceuticals, is described and validated in specific monographs (1-3). These guidelines recommend both the types of solvents and the acceptable levels of residual solvents in pharmaceuticals and formulations to help ensure consumer safety.

New Headspace Grade Solvents

When developing a GC-SH method, such parameters as sample solvent, extraction temperature, extraction time, sample volume and headspace volume are optimized (4, 5). The composition and purity of the sample solvent significantly affects the recovery and quality of the chromatogram (see Figure 1). Sigma-Aldrich offers solvents specifically developed for GC-HS applications. The purity and handling specifications of these solvents

meet the requirements of United States Pharmacopoeia (USP), European Pharmacopoeia (Ph.Eur.), as well as the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH).

The new GC-SH product line includes water and four most commonly used organic solvents: dimethyl sulphoxide (DMSO), N,N-dimethylformamide, N,N-dimethylacetamide, 1,3-Dimethyl-2-imidazolidone. Dimethyl sulphoxide is specified in USP and Ph.Eur. for water-insoluble substances. Water is the preferred solvent for water-soluble solutions, as described in USP and Ph.Eur. monographs. All solvents are microfiltered at 0.2 µm and packed under inert gas for longer shelf life.

USP and Ph.Eur. Residual Solvent Standards

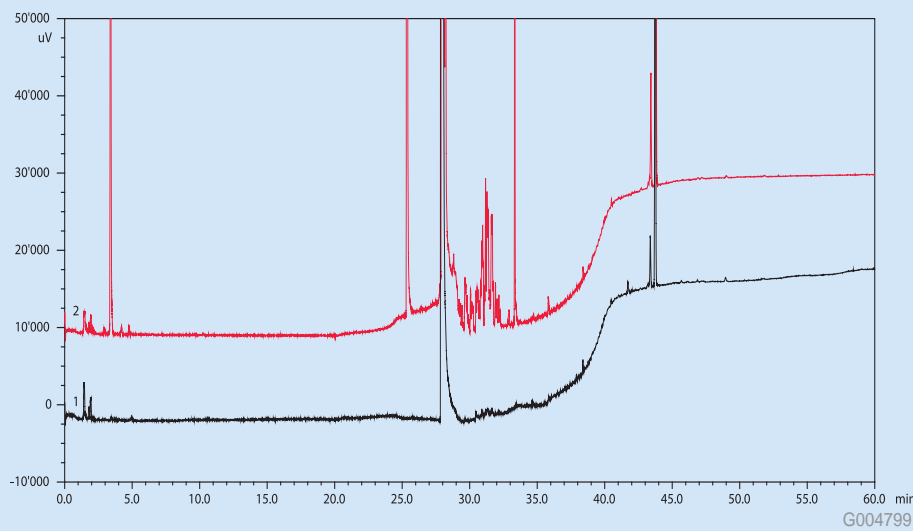
The residual solvents described in the ICH guidelines are analyzed by direct injection of three separate mixtures, divided by their individual classes (I, II or III). We offer calibration standards specifically for this application. These standards can be tailored in combinations and concentrations to meet specific customer needs through our Custom Chemical Standards Program.

Capillary GC Columns for Residual Solvent Analysis (6)

No single column is capable of separating all 61 solvents listed in the ICH guidelines. The Supelco GC column

OVI-G43 is specially prepared and tested to meet the requirements of USP Method 467 and the Ph.Eur. general method for determining residual solvents in pharmaceutical preparations. This column will separate residual solvents for research purposes or qualitative analysis. The USP and Ph. Eur. methods also specify using a deactivated 5 m guard column. In order to ensure analysis under optimum conditions, we strongly suggest the use of a deactivated guard

Figure 1. Headspace Gas Chromatogram of Two DMSO Grades: GC-HS Grade (Black Trace) and Conventional Grade (Red Trace) [2]



column (Cat. No. 25339) with the OVI-G43 column. For further information, please consult Supelco Technical Bulletin 933 (FLX).

References

1. United States Pharmacopeia, 31st Edition (2008), <467> Residual Solvents.
2. Ph.Eur. 6.0 (2008) Method 2.4.24, Identification and control of residual solvents.
3. ICH Guideline Q3C, Impurities: Guideline for Residual Solvents, The Fourth International Conference on Harmonization, July 17, 1997.
4. Camarasu, C. C. Residual Solvents Determination in Drug Products by Static Headspace-Gas Chromatography. *Chromatographia* 2002, 56, S137–S143.
5. Lee, C. R.; Nguyen van Dau, C.; Krstulovi, A. M. Artefact formation in the determination of residual solvents according to a method of the European Pharmacopeia. *Int. J. Pharm.* 2000, 195, 159–169.
6. Supelco Technical Bulletin 933. Capillary GC column choices for residual solvent analyses using direct injection or solid phase microextraction (SPME).

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GC-HS Solvents

Description	Boiling Point	Pack Size	Cat. No.
N,N-Dimethylacetamide, puriss. p.a. for GC-HS	166 °C	1 L	44901
Dimethyl sulphoxide, puriss. p.a. for GC-HS	189 °C	1 L	51779
N,N-Dimethylformamide, puriss. p.a. for GC-HS	153 °C	1 L	51781
1,3-Dimethyl-2-imidazolidinone puriss. p.a., for GC-HS	225 °C	1 L	67484
Water, puriss. p.a. for GC-HS	100 °C	1 L	53463

Residual Solvent Standards

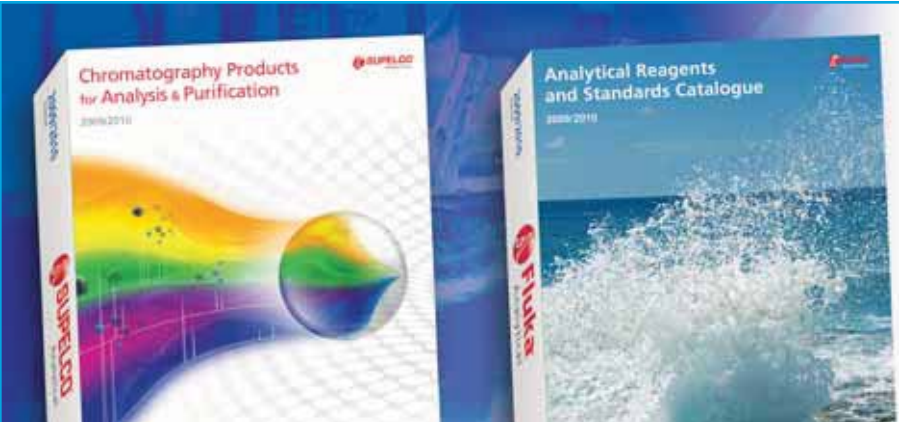
Description	Pack Size	Cat. No.
Class 1 Residual Solvent Standard in DMSO	25 x 2.5 mL	861255
Class 2 Residual Solvent Mix in DMSO	25 x 2.5 mL	861256
International USP 467 Mix	1 mL	47632-U
USP 467 OVI Mix 1, 24th ed.	1 mL	47545-U
USP 467 OVI Mix 2 w/o Benzene each component in DMSO	5 x 1 mL	4M4626-U
USP 467 OVI Mix 2, 24th ed. in DMSO	1 mL	47546-U

For customized standards, please contact us by e-mail at customstandards@sial.com

GC Columns for Residual Solvent Analysis by Headspace GC

Description	Pack Size	Cat. No.
OVI-G43, 30 m x 0.53 mm I.D., d, 3.0 µm	1 each	25396
OVI-G43 Guard Column, 5 m x 0.53 mm I.D.	1 each	25339

A complete product listing can be found online at sigma-aldrich.com/gc-hs



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Gas Chromatography

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Micro Volume Vial Inserts

techservice@sial.com

Micro volume vial inserts, when used in conjunction with 12 x 32 mm autosampler vials, allow for maximum sample recovery and easier sample removal. This is because their conical shape decreases the surface area inside the vial.

Micro volume vial inserts can be used with screw top, crimp top, or snap top vials. They are available in glass and polypropylene. Glass is the traditional choice. However, polypropylene inserts are recommended for pH-sensitive samples, greater solvent compatibility, biological samples, and ionic samples because of the material's inertness.

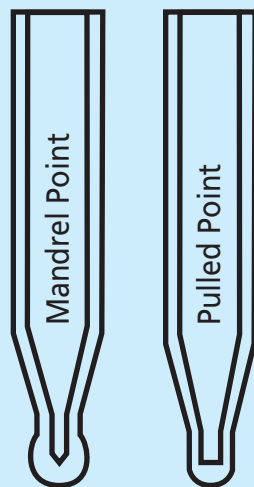
There are three styles of inserts available: conical, conical with spring, and shell style (Figure 1). Conical inserts are available with or without springs. The plastic spring acts as a shock absorber, minimizing damage to the autosampler needle. Further, the conical insert with spring raises the sample up inside the vial during needle penetration, which maximizes sample recovery. Shell style inserts are the most economical inserts available, and they provide a larger sample capacity (up to 350 μL) than conical inserts. However, their flat bottom design yields less efficient sample recovery than a conical insert.

All conical styles of inserts are offered with the traditional pulled point as well as the improved mandrel point. Pulled point inserts are more economical, but mandrel

point inserts provide a more pointed and uniform tip that enables better sample recovery (Figure 2).

Sigma-Aldrich offers a wide selection of vial inserts as well as technical support to help you choose the best style and material for your applications. For help with product selection, please contact our Technical Service group by email at techservice@sial.com, or visit us on the Web at sigma-aldrich.com/vials.

Figure 2. Mandrel Point vs. Pulled Point



G004789

Figure 1. Vial Insert Styles



Featured Products

Dimensions	Volume	Description	Style	Pkg. Size	Cat. No.
Inserts for Standard Opening, 12 x 32 mm vials					
4 x 25 mm	50 μL	Clear glass, conical with bottom spring	Mandrel point	100	27400-U
5 x 29 mm	150 μL	Clear glass, conical with polyflange	Mandrel point	100	24705
5 x 29 mm	150 μL	Clear glass, conical with bottom spring	Mandrel point	100	24707
5 x 30 mm	150 μL	Clear glass, conical with bottom spring	Pulled point	100	Z291730-1PAK
5 x 30 mm	150 μL	Polypropylene, conical with bottom spring	Mandrel point	100	24708
5 x 31 mm	100 μL	Clear glass, requires metal spring	Mandrel point	200	24703
5 x 31 mm	250 μL	Clear glass, flat bottom	Flat bottom	200	24701
Inserts for Large Opening, 12 x 32 mm vials					
6 x 29 mm	150 μL	Clear glass, conical with top spring	Mandrel point	100	24719
6 x 29 mm	200 μL	Clear glass, plastic bottom spring	Mandrel point	100	24721
6 x 29 mm	200 μL	Polypropylene, conical with bottom spring	Mandrel point	100	24722
6 x 29 mm	250 μL	Clear glass, conical point	Mandrel point	100	24717
6 x 31 mm	250 μL	Clear glass with flange	Mandrel point	100	27407
6 x 31 mm	250 μL	Polypropylene with plastic flange	Mandrel point	100	27409
6 x 31 mm	350 μL	Clear glass, flat bottom	Flat bottom	100	24715

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Figure 1. Clearly Labeled Separate Source Standards



E001058

Aldrich, can ease the audit process. Sigma-Aldrich's Supelco brand Separate Source standards are clearly labeled (Figure 1) and certified by our QC department as meeting or exceeding U.S. Environmental Protection Agency (EPA) requirements for use alongside our Primary

Standards (Figure 2). This gives the laboratory and auditors the necessary assurance your Separate Source protocols meet critical inspection.

The Separate Source standards are written into many contracts, specified in US EPA SW-846, and as part of the accreditation by The NELAC Institute (TNI). Separate Source standards are required verification or primary calibration standards. These standards are mandated to be sourced from a different vendor or from a different lot than that used for the daily verification standard. This covers a wide range of US Environmental Protection Agency (EPA) methods. Sigma-Aldrich Separate Source standards offer laboratories the convenience of dealing with a single vendor

for obtaining separate standards, as well as eliminating the need to buy and inventory raw materials from which to prepare their own separate source standards.

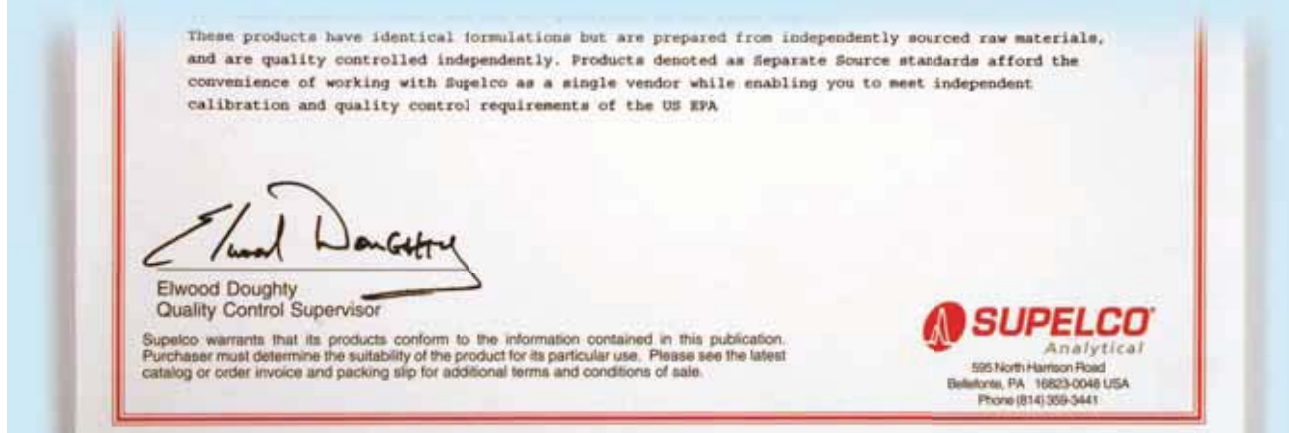
Sigma-Aldrich Separate Source standards are pairs of product having identical composition, but are prepared from independently sourced raw materials, and are independently quality controlled. Our internal processes prevent the use of the same raw material lot for both the primary and secondary standards. This can be observed by comparing the raw material lot numbers on the Certificate of Analysis that accompanies each individual standard in the pair.

Our Separate Source standards include neat components, and single- and multi-component solutions. They are available for the US EPA drinking water, wastewaters, and solid waste methods. The standards are identified by the words "Separate Source" and SS in the product description and by the catalog number (4- and 4S-, 5 and 5S-, 8- and 8S-). For example, 5S02111 is the Separate Source standard for 502111.

As a convenience for our customers, Sigma-Aldrich's Custom Standards chemists can easily tailor a Separate Source standard and Primary standard pair to meet your exact specifications. We are also able to formulate a custom Separate Source standard to match your Primary Standard from a different vendor. For additional information, please contact our Technical Service department at techservice@sial.com.

To view a current list of ready-to-ship Supelco brand Separate Source reference standards, please visit our web site sigma-aldrich.com/separate-source.

Figure 2. Certified Separate Source Standards Meet or Exceed EPA Requirements



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TraceCERT: Traceable Certified Reference Materials for Ion Chromatography

Juerg Wuethrich and Vicki Yearick
vicki.yearick@sial.com



Ion chromatography (IC) is an analytical technique that is used to separate and quantify from percent to ppm-levels of common anions and cations in aqueous samples. Separation occurs via differential interaction with an ion-exchange resin. Conductivity is the most common detection method and in some cases UV detection is also used. The sensitivity of IC makes it also ideal for low-ppm level quantification or trace analysis. Subsequently, IC requires high-purity eluents and well-defined certified reference materials (CRM).

Because measurement results are directly affected by the quality of the CRM, the choice of the appropriate CRM producer is a matter of trust. One indicator of the technical and administrative competence of a CRM producer is inspection and accreditation by an independent authority. The Sigma-Aldrich production facility in Switzerland is accredited as a reference material producer by the Swiss Accreditation Service according to ISO Guide 34 and also under ISO/IEC 17025 (1, 2). This double accreditation is called the "Gold Standard" for CRM producers and represents the highest achievable level of quality assurance.

With the first products developed under these double accredited conditions Sigma-Aldrich launches its **TraceCERT**® calibration standards program for IC and also for spectrometry. **TraceCERT** thereby stands for Traceability and Certified representing that these CRM are suitable for even the most challenging applications, and also fulfill the needs of laboratories working in a regulated environment. These CRMs are not only traceable to an accepted reference, but are also of well-defined purity and have a properly calculated measurement for uncertainty. All details are described in a comprehensive certificate that is designed according to ISO Guide 31 (3). The certificate is available electronically from our web page by entering the product number and corresponding lot number. As a unique feature for these IC standards, we list the most common trace impurities that are relevant for the ion chromatographic separation (13 elements for cation standards and 7 inorganic anions for anion standards).

More issues related to reference materials and CRM production are described in Issues 4-8 of our 2008 Analytix Newsletters, covering the following topics: traceability, uncertainty assignment, high-purity starting materials handling, production, packaging and stability studies. (Articles are available at our web site: sigma-aldrich.com/ic)



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TraceCERT: IC Standards 1000 mg/L, 100 mL HDPE pkg.

Description	Matrix	Cat. No.
Anion standards		
Bromide	water	43147
Chloride	water	39883
Chromate	water	40121
Cyanide	water	90157
Fluoride	water	77365
Iodide	water	41271
Nitrate	water	74246
Nitrite	water (pH ~11)	67276
Phosphate	water	38364
Sulfate	water	90071

Description	Matrix	Cat. No.
Cation standards		
Ammonium	water	59755
Barium	0.1% nitric acid	87142
Cadmium	0.1% nitric acid	69679
Calcium	0.1% nitric acid	39865
Cobalt	0.1% nitric acid	49594
Copper	0.1% nitric acid	40786
Lead Standard	0.1% nitric acid	51777
Lithium Standard	0.1% nitric acid	59878
Magnesium	0.1% nitric acid	89441
Manganese	0.1% nitric acid	51439
Nickel	0.1% nitric acid	42637
Potassium	water	53337
Sodium	water	43492
Strontium	0.1% nitric acid	42151
Zinc	0.1% nitric acid	67902

As a leading supplier of high-quality products for all areas of analytical chemistry, Sigma-Aldrich offers several products to complement the **TraceCERT** standards product line. These additional products include certified eluent concentrates, high-purity water developed specifically for IC analysis, certified multi-element standards (PRIMUS) and an IQ/OQ/PQ kit for verification of linearity, repeatability, and carryover within the ion chromatographic system. To learn more about our complete line of IC products, please visit our website sigma-aldrich.com/ic.

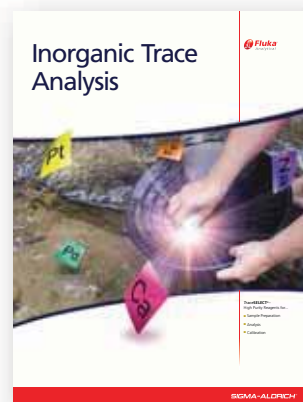
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2. ISO Guide 34, General requirements for the competence of reference material producers, 2000
3. ISO Guide 31, Reference materials - Contents of certificates and labels, 2000
4. **TraceCERT** Traceable Certified Reference Materials. Part 1: Swiss Precision Meets Analytical Competence. *Analytix* 2006 issue 5, 4-5.
5. **TraceCERT** Traceable Certified Reference Materials. Part 2: What Traceability Really Means. *Analytix* 2007 Issue 1, 7-9.
6. **TraceCERT** Traceable Certified Reference Materials. Part 3: Challenges in the Characterization of High-purity Starting Materials. *Analytix*, 2007 Issue 2, 4-5.
7. **TraceCERT** Traceable Certified Reference Materials. Part 4: Production, handling and Storage of High-precision Calibration Solutions. *Analytix* 2007 Issue 3, 6-8.
8. **TraceCERT** Traceable Certified Reference Materials. Part 5: Reliability is a Matter of Proper Uncertainty Calculation. *Analytix* 2007 Issue 4, 8-10.

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