

Reporter

Volume 32, July 2008, International



Supelco Ionic Liquids

The Dawning of a New Era in GC Phase Technology



The evolution of GC Phase Technology from Substituted Polysiloxane Polymers and Polyethylene Glycols in the 1950s, to Bonded Phases in the 1980s, and on to our new Ionic Liquid phases today.

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Roberto Ferrari
European Sales
Development Specialist,
Gas Chromatography
and Analytical Standards

Dear Colleague,

Supelco is pleased to announce the launch of the first of a series of diionic liquid (IL) gas chromatography (GC) columns – a breakthrough in GC stationary phase technology.

In gas chromatography just two types of stationary phases/columns are typically used with a few exceptions:

1. Columns containing a substituted polysiloxane polymer phase, the origin of which can be traced back to the 1950s and the very birth of the GC technique
2. Columns coated with a polyethylene glycol phase, a phase that has remained virtually unchanged nearly as long as GC has been practised

In recent years room temperature Ionic Liquids (RTILs) have been investigated and evaluated as GC stationary phases; however this work focused on monocationic ionic liquids, which did not exhibit the desired chromatographic characteristics and had narrow working ranges. In this issue of the Reporter, Supelco is delighted to announce the launch of di- and polycationic ionic liquid GC stationary phases/columns that expand the selectivity/polarity range and elevate the temperature at that polarity. For the first time, previously unachievable separations are now possible!

Launched in Europe at the 32nd International Symposium on Capillary Chromatography (ISCC) in Riva del Garda, Italy, this new GC stationary phase technology generated considerable interest.

Supelco/Sigma-Aldrich are welcoming GC chromatographers to test and evaluate these new IL GC columns to fully explore possibilities! We are keen to work with you on what's possible!

Learn more about this new and innovative GC phase on page 16 of this edition of Reporter.

Yours sincerely,

Roberto Ferrari
European Sales Development Specialist,
Gas Chromatography and Analytical Standards



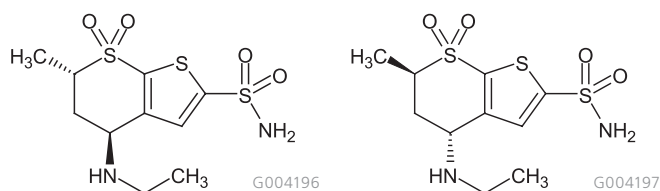
Improving the Chiral Separation of Dorzolamide

A Case Study of Supelco's Chiral Analytical and Screening Services

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In the mid 1980s and early 1990s, several pharmaceutical companies began to explore the development of carbonic anhydrase inhibitors for the topical treatment of glaucoma. Dorzolamide, a water soluble sulphonamide possessing two chiral centres, emerged as a product of these research efforts (1).

Figure 1 Structures of Dorzolamide and its Enantiomer



Dorzolamide

[(4R, 6R)-4-(Ethylamino-5,6-dihydro-6-methyl-4H-thieno[2,3-b]thiopyran-2-sulphonamide 7,7-dioxide, monohydrochloride)]

Although the development of enantiomerically pure chiral drugs such as dorzolamide has recently proven extremely beneficial to the treatment of various ailments, manufacturers face the challenge of chiral separation. Previous efforts focusing on the chiral separation of dorzolamide from its undesired chiral enantiomer [(4R, 6R)-4-(Ethylamino-5,6-dihydro-6-methyl-4H-thieno[2,3-b]thiopyran-2-sulphonamide 7,7-dioxide, monohydrochloride)] have been complicated. The chromatographic mode of separation described in the 2006 United States Pharmacopeia, for instance, involves a derivatisation of racemic dorzolamide with chiral reagent (S)-(-)- α -methylbenzyl isocyanate prior to separation on a non-chiral silica phase (2). Elimination of this derivatisation step would both conserve time and decrease expenses associated with the synthesis and analysis of dorzolamide.

In an effort to simplify the chiral separation of dorzolamide from its undesired enantiomer without prior derivatisation, a recent study employed Astec CHIROBIOTIC™ and CYCLOBOND™ columns in a chiral screen of the two dorzolamide enantiomers.

The Astec CHIROBIOTIC phases consist of macrocyclic glycopeptides linked covalently to a silica surface by five covalent bonds. These phases possess broad selectivity and can differentiate between small variability in chemical structure, making them valuable in the separation of a wide array of chiral molecules. Unlike most other chiral phases, they are remarkably stable and effective in reversed phase (RP), normal phase (NP), polar organic (POM), and polar ionic (PIM) chromatographic modes, without memory effects. The CHIROBIOTIC phases mainly rely on strong anionic or cationic binding, hydrogen bonding, and π - π complexation to achieve separation of various enantiomers (3). Conversely, the CYCLOBOND phases rely on

inclusion as the retention mechanism in reversed phase. Inclusion complexing arises due to apolar segments of chiral molecules interacting with the apolar cyclodextrin cavity. While apolar segments may occupy the inside of the cavity, more polar segments of the analyte may interact through dipole-dipole interactions, hydrogen bonding, and steric interactions at the mouth of the cavity, allowing the cyclodextrin phases to distinguish between isomers differing in stereochemistry. The CYCLOBOND phases thrive in reversed phase and polar organic modes, and some (CYCLOBOND I 2000 DMP and DNP) are also compatible with normal phase mode (4).

A sample consisting of a 1:1 mixture of dorzolamide hydrochloride and its enantiomer [(4R, 6R)-4-(Ethylamino-5,6-dihydro-6-methyl-4H-thieno[2,3-b]thiopyran-2-sulphonamide 7,7-dioxide, monohydrochloride)] (dorzolamide hydrochloride Related Compound A [2]) was tested in a chiral screening protocol employing six of the Astec CHIROBIOTIC and CYCLOBOND phases most likely to give positive results: CHIROBIOTIC V2 (Vancomycin), CHIROBIOTIC T (Teicoplanin), CHIROBIOTIC TAG (Teicoplanin Aglycone), CYCLOBOND I 2000 (β -cyclodextrin), CYCLOBOND I 2000 DNP (β -cyclodextrin, 3,5-Dinitrophenyl carbamate) CYCLOBOND I 2000 HP-RSP (β -cyclodextrin, High Performance R,S-hydroxypropyl ether) (25 cm x 4.6 mm I.D., 5 μ m particle size). Mobile phases encompassing reversed-phase (70:30, 20 mM ammonium acetate, pH 4.0:acetonitrile), and polar ionic (100:0.1:0.1, methanol:acetic acid:triethylamine) chromatographic modes of operation were applied to the CHIROBIOTIC phases. The CYCLOBOND phases were screened in the reversed-phase mobile phase as well as a mobile phase referred to as polar organic mode (95:5:0.3:0.2, acetonitrile:methanol:acetic acid:triethylamine). Screening was executed on the Waters 2690 Separations Module utilising a Waters 996 Photodiode Array Detector (UV at 220 and 254) and Waters Empower Acquisition Software (2002 Version).

Although the development of enantiomerically pure chiral drugs has proven extremely beneficial to the treatment of various ailments, manufacturers face the challenge of chiral separation.

Subsequent to initial screening, positive results were confirmed and optimised on the Agilent 1100 series HPLC utilising a VWD detector with a UV wavelength of 254 nm.

Table 1 summarises the results of the primary screen. The summary table shows evidence of enantiomeric selectivity observed on both the CHIROBIOTIC V2 (V2) and CHIROBIOTIC TAG (TAG) in polar ionic mode and on the V2 in reversed-phase. Near baseline resolution was revealed on the V2 in polar ionic mode, while only partial separation was observed on both the TAG in polar ionic mode and the V2 in reversed phase. Unlike the V2 and TAG, the CHIROBIOTIC T showed no separation in both reversed-phase and polar ionic modes of operation.





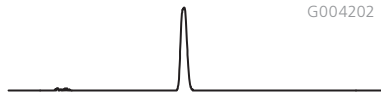


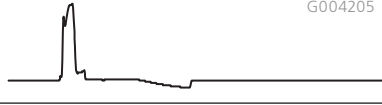
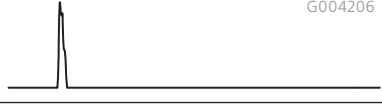

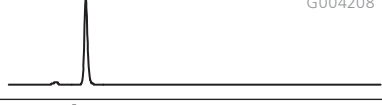
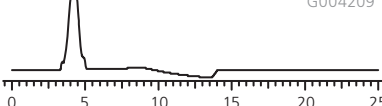
(continued on page 4)



Unlike the CHIROBIOTIC phases, the CYCLOBOND columns in the screen showed no positive results. The dorzolamide enantiomers were unretained on both CYCLOBOND I 2000 and CYCLOBOND I 2000 HP-RSP and unresolved on the CYCLOBOND I 2000 DNP in reversed-phase. Polar organic mode produced no discernible peaks on the CYCLOBOND phases.

Because the CHIROBIOTIC V2, under polar ionic conditions, produced the best resolution of the dorzolamide enantiomers in the primary screen, it was selected for optimisation purposes. Consecutive attempts at optimisation, including a decrease in flow rate from 1.0 mL/min down to 0.25 mL/min and a decrease in temperature from 25°C down to 10°C, succeeded in increasing resolution; however

Table 1 Primary Screen Summary of Dorzolamide and its Enantiomer on Astec CHIROBIOTIC and CYCLOBOND phases

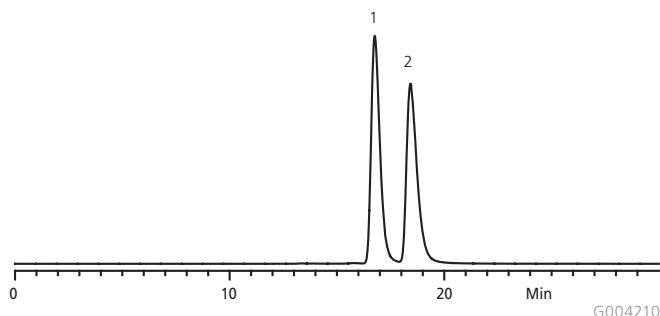
	Column	Mode	Mobile Phase	Result	
	G004198	CHIROBIOTIC TAG	RP	70:30, 20 mM NH ₄ OAc (pH 4.0):ACN	No Retention
	G004199	CHIROBIOTIC TAG	PIM	100:0.1:0.1, MeOH:HOAc:TEA	Partial Separation
	G004200	CHIROBIOTIC V2	RP	70:30, 20 mM NH ₄ OAc (pH 4.0):ACN	Partial Separation
	G004201	CHIROBIOTIC V2	PIM	100:0.1:0.1, MeOH:HOAc:TEA	Separation
	G004202	CHIROBIOTIC T	RP	70:30, 20 mM NH ₄ OAc (pH 4.0):ACN	No Separation
	G004203	CHIROBIOTIC T	PIM	100:0.1:0.1, MeOH:HOAc:TEA	No Separation
	G004204	CYCLOBOND I 2000	RP	70:30, 20 mM NH ₄ OAc (pH 4.0):ACN	No Retention
	G004205	CYCLOBOND I 2000	POM	95:5:0.3:0.2, ACN:MeOH:HOAc:TEA	Uncertain result
	G004206	CYCLOBOND I 2000 HP-RSP	RP	70:30, 20 mM NH ₄ OAc (pH 4.0):ACN	No Retention
	G004207	CYCLOBOND I 2000 HP-RSP	POM	95:5:0.3:0.2, ACN:MeOH:HOAc:TEA	Uncertain result
	G004208	CYCLOBOND I 2000 DNP	RP	70:30, 20 mM NH ₄ OAc (pH 4.0):ACN	No Separation
	G004209	CYCLOBOND I 2000 DNP	POM	95:5:0.3:0.2, ACN:MeOH:HOAc:TEA	Unknown

0 5 10 15 20 25

baseline resolution was not quite achieved. The volatile salt ammonium formate was employed to sharpen the peaks and enhance resolution. As seen in **Figure 2**, when coupled with the mobile phase 0.05 w% ammonium formate in methanol, these temperature and flow rate changes produced near baseline resolution. Ultimately, **Figure 3** shows that slight modification of the ammonium formate mobile phase to include the addition of water and pH adjustment (5:95, 20 mM ammonium formate, pH 4.0:methanol) gave baseline resolution of the dorzolamide enantiomers with the original temperature and flow rate (25°C, 1.0 mL/min).

Figure 2 Analysis of Dorzolamide and its Enantiomer on CHIROBIOTIC V2

column: CHIROBIOTIC V2, 25 cm x 4.6 mm I.D., 5 µm particles
mobile phase: 0.05 w% ammonium formate in methanol
flow rate: 0.25 mL/min
temp.: 10°C
det.: UV at 254 nm
injection: 2 µL
sample: 1.0 mg/mL in methanol
1. Dorzolamide
2. [(4R, 6R)-4-(Ethylamino-5,6-dihydro-6-methyl-4H-thieno[2,3-b]thiopyran-2-sulphonamide 7,7-dioxide, monohydrochloride)]

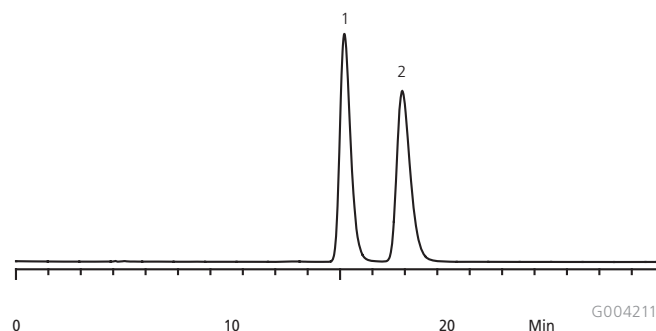


Of the six columns screened, the best resolution of the dorzolamide chiral enantiomers, was conclusively on the CHIROBIOTIC V2 stationary phase under polar ionic mode conditions. The vancomycin phase contains two ionic sites, making it especially good for the separation of both acidic and basic molecules. Decreasing the pH to 4.0 enhanced the ionic interactions between the secondary nitrogen attached to one chiral centre of the analyte and the carboxylic acid groups of the CHIROBIOTIC V2 stationary phase. Conformational differences cause one enantiomer to have a slightly stronger affinity to the stationary phase than the other, thus enhancing chiral separation of the dorzolamide enantiomers.

As in this separation of the dorzolamide enantiomers, chiral separations that may be achieved without extra derivatisation steps save considerable amounts of time. Since decreasing time ultimately saves money, modern industry constantly focuses efforts on seeking simple procedures aimed at increasing their efficiency. For this reason, the polar ionic mode on the Chirobiotic Chiral Selective Phases (CSPs) that provides fast and efficient separations is extremely popular.

Figure 3 Analysis of Dorzolamide and its Enantiomer

column: CHIROBIOTIC V2, 25 cm x 4.6 mm I.D., 5 µm particles
mobile phase: 5:95, 20 mM ammonium formate (pH 4.0):methanol
flow rate: 1.0 mL/min
temp.: 25°C
det.: UV at 254 nm
injection: 2 µL
sample: 1.0 mg/mL in methanol
1. Dorzolamide
2. [(4R, 6R)-4-(Ethylamino-5,6-dihydro-6-methyl-4H-thieno[2,3-b]thiopyran-2-sulphonamide 7,7-dioxide, monohydrochloride)]



References

- 1] J. Borrás et. al. *Bioorg. Med. Chem.* 1999, 7, 2397–2406.
- 2] United States Pharmacopeia, 29th rev.; United States Pharmacopeial Convention: Washington, DC. 2005; 756–757.
- 3] CHIROBIOTIC Handbook, 5th ed.; T406120, JEV, Supelco, 595 North Harrison Road, Bellefonte, PA 16823.
- 4] CYCLOBOND Handbook, 7th ed.; T406119, JEU, Supelco, 595 North Harrison Road, Bellefonte, PA 16823.

Featured Products

Description	Cat. No.
Astec CHIROBIOTIC V2 Chiral HPLC Column 25 cm x 4.6 mm I.D., 5 µm particles	15024AST

Did you know?

Supelco now offers **Chiral Screening Services** to assist customers in analytical method development and purification. The services consist of screening of Astec chiral columns, method optimisation and purification of enantiomers. Enantiomers are identified as (+) or (-) using the Chiralysers optical rotation detection system.

For more information on chiral screening services, method development or chiral purification, please visit our website at sigma-aldrich.com/astec, or contact us by e-mail EurTechServ@sial.com



Rapid, Sensitive Methods in Manufacturing Cleaning Validation Using Ascentis® Express HPLC Columns

Wayne Way wayne.way@sial.com

Contributed Article

The following was generated by an outside source using Sigma-Aldrich products. Technical content provided by: **S. Bannister, M. Talbott, F. Hanciles** –Xcelience LLC, Tampa, FL

Verification of the removal of drug residue from multi-product manufacturing equipment is required by GMP regulations. The suitability of applied analytical methods is judged with a combination of sensitivity, selectivity, and because the release of such equipment is dependent on time. The FDA does not set quantitative acceptance specifications, but the commonly used limit is based on not more than 0.1% of a dose carried over into a single dose of the next product. Translation of this into an analytical limit combines the total product contact area, the mass (or volume) of product contacting the surface, the mass (or volume) of each dose unit, the sampled area, the rinse volume and the fraction of the rinse sample used for analysis. The requisite limits are commonly measured in ng/mL of injected sample.

The ubiquity of HPLC in drug analysis makes it an attractive choice for cleaning validation. Methods qualified for cleaning validation are often adaptations of drug-substance methods. The original methods are capable of determining the drug and its related impurities, but the ability to simultaneously measure multiple closely related analytes comes at the expense of run time and is not needed in cleaning validation.

This work was undertaken to investigate the use of rapid gradients using recently introduced fused core particle columns on conventional instrumentation in the development of general-purpose methods for

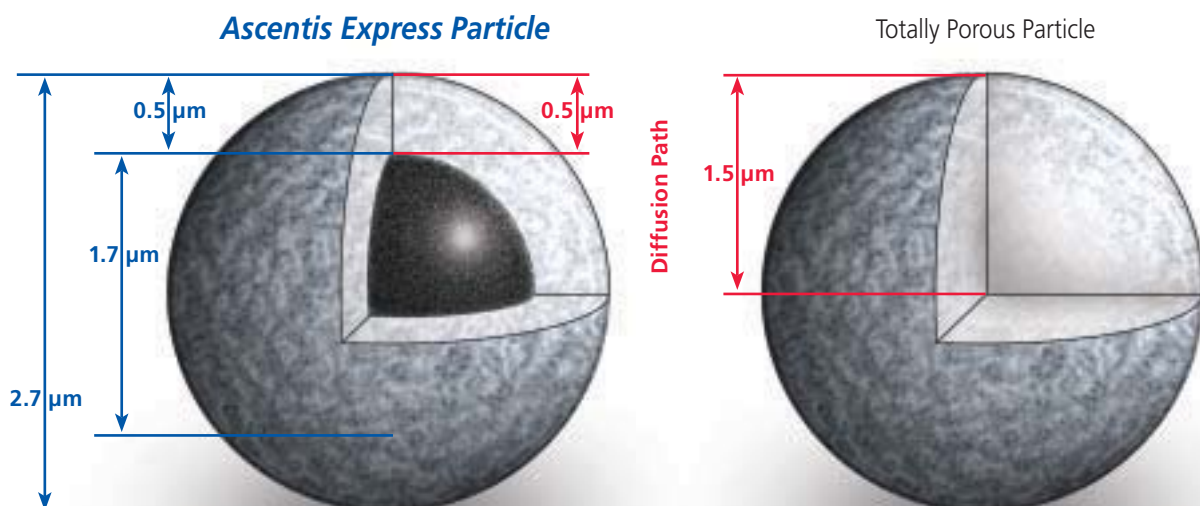
cleaning validation. The benefits include high sensitivity and reductions in the time needed to set up and run the method.

Resolution, limits of detection and quantitation, and run time in HPLC analyses are improved by reducing the width of eluted bands. Contributions to bandwidth include both column (particle size, packing structure and resistance to mass transfer in the stationary and mobile phases) and extracolumn volumes (injection, unswept volumes and tubing). Columns packed with 5 μm fully porous particles have been the standard for conventional HPLC for twenty-five years. Smaller-particle packings (3 μm) have been available almost as long and offer higher efficiency (lower band dispersion) on conventional instrumentation, but require higher pumping pressures due to lower bed permeability. Efficiency can be further increased by the use of particles smaller than 3 μm but only with the use of instrumentation optimised with respect to both pressure and extra-column effects.

Supelco recently introduced reverse-phase packings based on 2.7 μm silica particles in which a 0.5 μm layer of 90Å porous silica was deposited onto a 1.7 μm solid spherical core (**Figure 1**). Advantages of columns packed with these particles include high efficiency, lower backpressure and smaller efficiency losses with increasing velocity due to improved mass-transfer kinetics in the shallow porous layer. The narrow particle size distribution allows the use of larger pore column frits, which, combined with the greater stability of the packed bed, produces longer column lifetimes in routine use.

The high resolving power of gradient elution in the analysis of closely related substances is the result of the reduction of peak width as a band moves through the column. The back of the band

Figure 1 Fused-Core Structure of Ascentis Express Compared to Totally Porous Particles



is accelerated by the stronger solvent. A broad gradient will elute a wide range of substances and a steep gradient will elute them quickly.

Figure 2 Acidic and Neutral Drug Panel

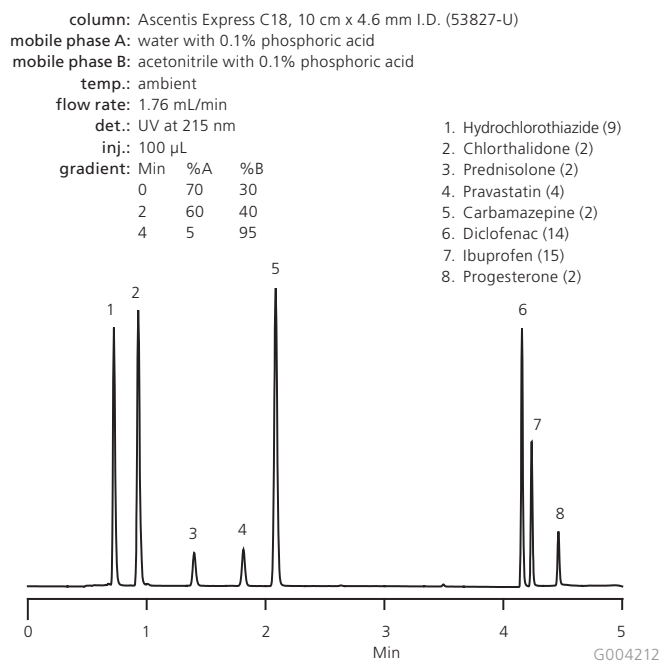
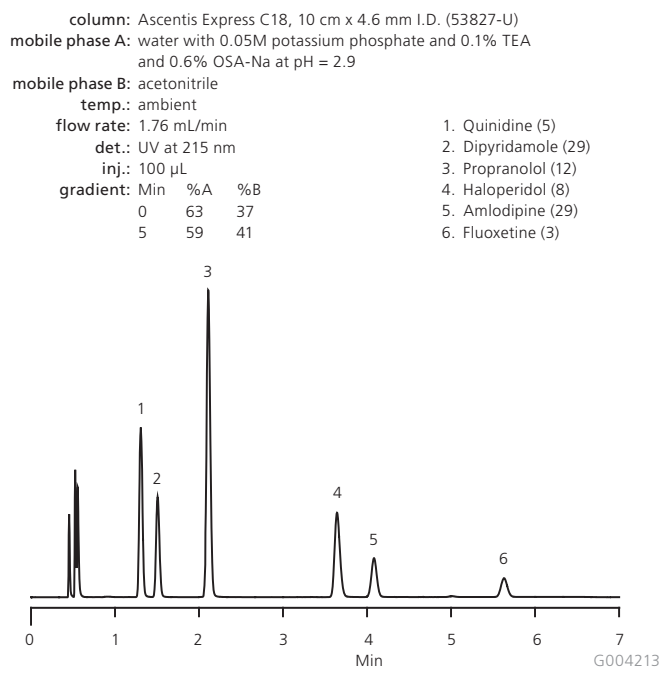


Figure 3 Basic Drug Panel



Versatile Separations

To judge the utility of Ascentis Express columns in cleaning validation, an Agilent 1100 component system with standard components (including a 10 mm/13 μ L flow cell) was used to develop a short gradient separation using Ascentis Express C18, 10 cm x 4.6 mm for each of two panels: eight acidic or neutral drugs and six basic drugs. For each separation, the flow rate was 1.76 mL/min, detection was at 215 nm, and 100 μ L injections were made of aqueous solutions representing the final equipment rinse. The separations are shown in **Figures 2 and 3**. Limits of detection (ng/mL) are listed next to each analyte in **Figures 2 and 3**.

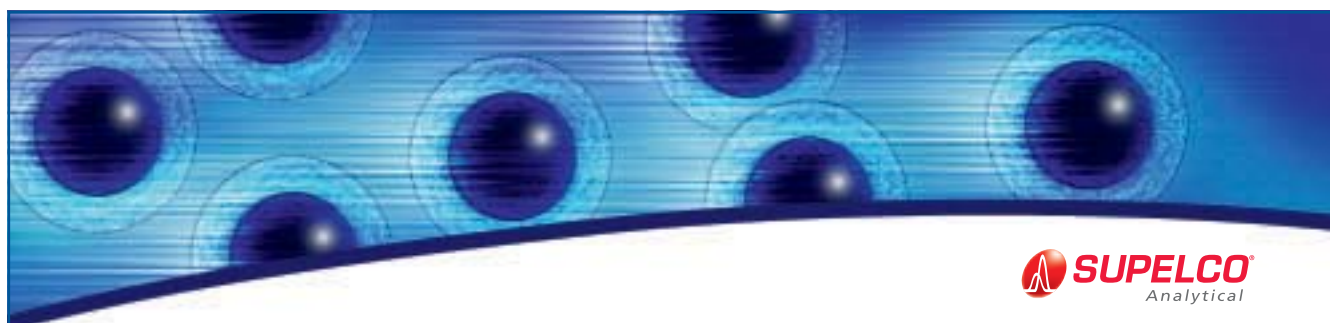
These separations demonstrate the capabilities of robust Ascentis Express columns on conventional instrumentation in the rapid analyses of multiple drugs at low ppb levels suitable for development as methods for cleaning validation in multi-product manufacturing facilities.

Featured Products

ID	Length	Ascentis	Ascentis
(mm)	(cm)	Express C18	Express C8
Ascentis Express Columns			
2.1	3	53802-U	53839-U
2.1	5	53822-U	53831-U
2.1	7.5	53804-U	53843-U
2.1	10	53823-U	53832-U
2.1	15	53825-U	53834-U
3.0	3	53805-U	53844-U
3.0	5	53811-U	53848-U
3.0	7.5	53812-U	53849-U
3.0	10	53814-U	53852-U
3.0	15	53816-U	53853-U
4.6	3	53818-U	53857-U
4.6	5	53826-U	53836-U
4.6	7.5	53819-U	53858-U
4.6	10	53827-U	53837-U
4.6	15	53829-U	53838-U

Related Information

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



Ascentis® Express HILIC HPLC Columns

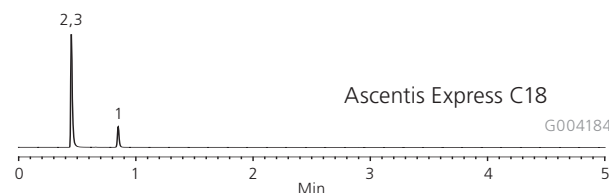
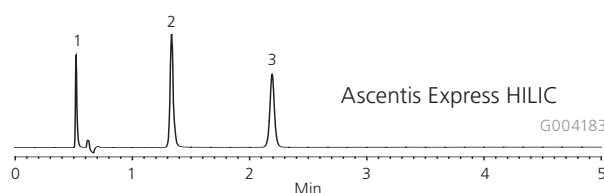
A breakthrough in HPLC column performance.

Key Benefits of HILIC

- Retention of highly polar analytes like metabolites
- Increased MS sensitivity
- Orthogonal selectivity to C18

Analysis of Polar Molecules on Ascentis Express HILIC and C18

columns: Ascentis Express HILIC, 10 cm x 2.1 mm I.D., 2.7 µm particles (53939-U)
 Ascentis Express C18, 10 cm x 2.1 mm I.D., 2.7 µm particles (53823-U)
 mobile phase: 10:90; 100 mM ammonium formate, pH 3.0 with concentrated formic acid:acetonitrile
 flow rate: 0.4 mL/min
 temp.: 35°C
 det.: UV at 254 nm
 injection volume: 1 µL
 1. Acenaphthene, 80 µg/mL in mobile phase
 2. Adenosine, 35 µg/mL in mobile phase
 3. Cytosine, 75 µg/mL in mobile phase



High Performance HPLC Interconnects

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Supelclean™ Sulfoxide SPE for the Extraction of PCBs and other Aromatic Compounds in Oil

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Contributed Article

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Introduction

Polychlorobiphenyls (PCBs) were once heavily used as an indestructible coolant and insulating fluid in transformer and capacitor oils, and also as a stabilising additive for a variety of products such as lubricating oils, hydraulic fluids, flame retardants, paints and adhesives. However, because of their high toxicity and resistance to environmental degradation (persistent organic pollutant, POP), production and distribution of PCBs have been banned since the 1970s.

Because of their stability and persistence in the environment, PCBs are still monitored routinely and heavily regulated. A common sample matrix encountered in PCB analyses is oil used in dielectric, hydraulic, and heat transfer systems. There are numerous sample prep techniques currently available for PCB analysis in transformer oil ranging from sulphuric acid extraction (1) to SPE cleanup using silica gel, Florisil® (2), and/or Alumina (3). Most of these techniques are able to achieve lower limits of detection in the range of 5–10 ppm. However, as more transformers are decontaminated and waste sites undergo treatment/remediation, lower limits of quantitation will be required to accurately determine PCB levels. This is a challenge because endogenous hydrocarbons found in transformer oil behave similarly to PCBs during sample preparation. As a result, they are often co-extracted with PCBs and can interfere with subsequent GC-MS analyses and possibly damage the GC instrument.

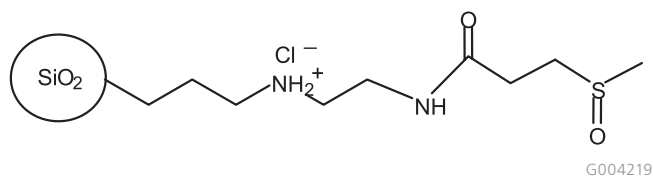
Researchers have found that dimethylsulfoxide (DMSO) liquid-liquid extraction (LLE) is an effective means of separating PCBs (aromatic hydrocarbons) from aliphatic hydrocarbons (transformer oil) prior to GC-MS analysis (4). Although effective, LLE is often tedious, time consuming and not greatly amenable to higher throughput applications. Based on the same extraction principles behind the DMSO LLE approach, we discuss the utility of a sulfoxide-bonded SPE stationary phase towards the extraction of PCBs from transformer oil. Using this new SPE phase, we are able to achieve quantitation levels below 0.5 ppm (mg/kg).

Supelclean Sulfoxide SPE – How it Works

Supelclean Sulfoxide SPE consists of a patent pending silica-bonded sulfoxide (-SO) phase (**Figure 1**). The technology was specifically developed for the extraction of polychlorinated biphenyls (PCBs) and related aromatic compounds from transformer, waste and mineral oil. Under normal-phase conditions, PCB retention is facilitated via interaction between the SPE phase's electrophilic sulfur atom and the pi-electron cloud formed from aromatic rings inherent with PCBs.

The phase is first conditioned with acetone to remove residual moisture from the phase. This is a critical step. Any residual moisture on the phase will negatively affect resolution and selectivity during extraction. The sulfoxide phase is then equilibrated with hexane and a diluted oil sample (1:1 v/v with hexane) is loaded onto the packed tube. Increasing volumes of hexane are then applied. As the hexane wash solvent passes through the cartridge, PCBs are preferentially retained/retarded on the SPE phase whereas endogenous sample interferences (e.g., long chain hydrocarbons) are eluted from the phase in the early fractions. Subsequent fractions are then eluted and collected in later fractions for subsequent GC-QMS or GC-HRMS analysis.

Figure 1 Supelclean Sulfoxide SPE



Extraction and Analysis of PCBs in Transformer Oil

6 g of Supelclean Sulfoxide SPE was packed into a glass 20 mL SPE cartridge (17 mm I.D. x 137 mm L) (**Figure 2**). Commercial insulation oil (Japan Industrial Standard JIS C2320-19 99, insulating oil, Class 1-2/4, paraffin oil) was spiked with a Kanechlor PCB mix at the total levels of 3.7 ppm (mg/kg) and diluted with hexane (1:1 v/v). The oil samples were extracted using the procedure described in **Table 1**, and analyzed via GC-QMS using a 5% phenyl/ 95% methylpolysiloxane column and Agilent 5973N MSD (5).

(continued on page 10)



Separation of PCBs and Aliphatic Hydrocarbon Interferences Prior to GC Analysis

PCBs were extracted from oil and analysed via GC-QMS using the procedure described in **Table 1**. **Figure 3** describes the elution profile of PCBs vs. transformer oil (aliphatic hydrocarbons). As described in **Figure 3**, aliphatic hydrocarbons (oil interferences) are poorly retained on the sulphoxide SPE phase and elute off the packed bed within the first 10–12 mL elution fraction. The retained chlorobiphenyl congeners (CBs) are more strongly retained and elute in the second 25 mL fraction.

Excellent Recovery and Lower Quantitation Levels Achieved

Insulation oil was spiked with PCBs at the total level of 3.7 mg/kg, extracted using Supelclean Sulphoxide SPE and analysed via GC-QMS using the procedure described in **Table 1**. Recovery was determined against ^{13}C -labelled PCB internal standards. An average Recovery \pm RSD of $98.5 \pm 4.2\%$ was achieved for mono- to octa-chlorobiphenyls (**Table 2**). Concentrations of nona- to deca-chlorobiphenyls in the sample were lower than detection limits of the GC-QMS system. Mono- and di-chlorobiphenyls in other samples having lower PCB concentrations were not determined due to elution overlap with the

Figure 2 Supelclean Sulphoxide SPE Tube

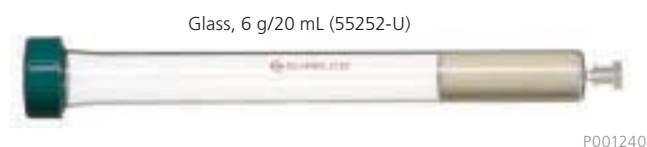


Table 1 Supelclean Sulphoxide SPE Extraction Method for PCBs in Transformer Oil

Supelclean Sulphoxide SPE Tube, Glass 6 g/20 mL (55252-U)

1. Condition the SPE phase with 20 mL acetone (removes residual moisture from the phase).
2. Equilibrate the SPE phase with 40 mL of hexane.
3. Load 0.4 mL diluted oil sample.
4. Elute aliphatic hydrocarbons (oil interferences) with 12 mL hexane.
5. Elute PCBs with 25 mL hexane.
6. Collect PCB fraction and concentrate under nitrogen for subsequent GC-QMS analysis (5).

Figure 3 Elution Profile of Oil Interferences and PCB Congeners from Sulphoxide SPE

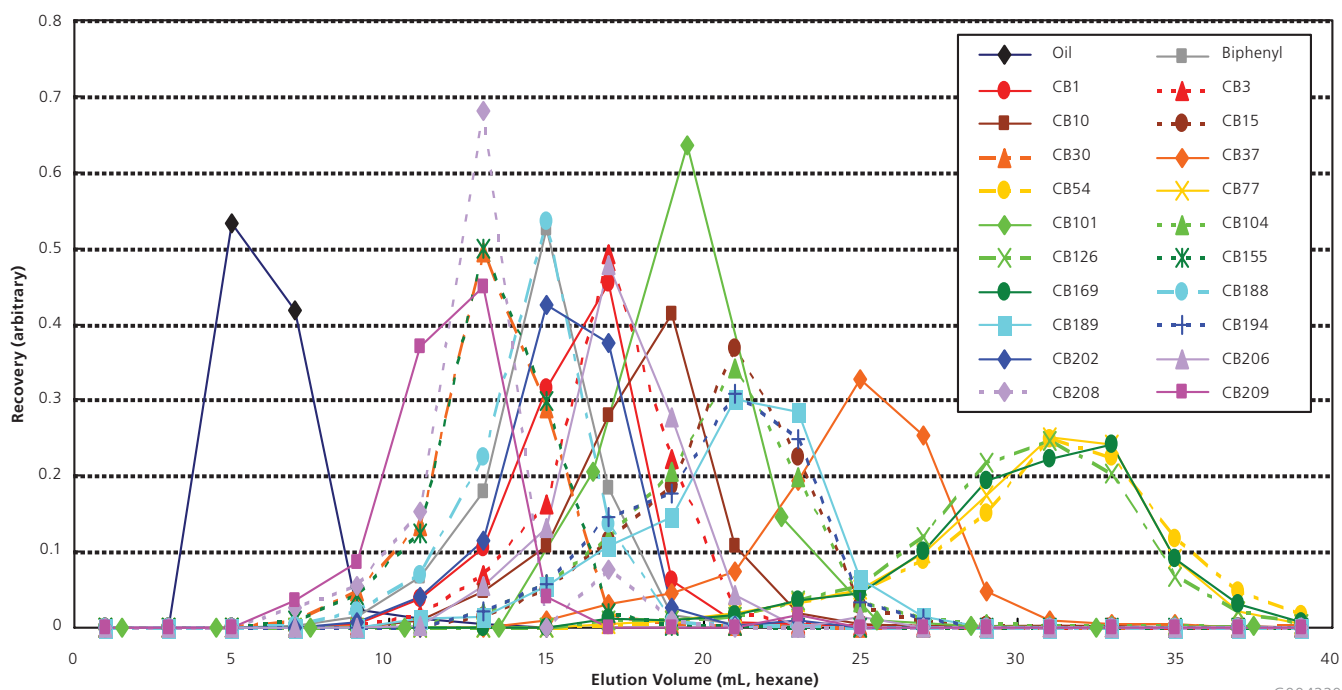


Table 2 Observed Concentrations of PCB Homologues of a PCB-fortified Insulation Oil Sample (n = 3).

		mono-CBs	di-CBs	tri-CBs	tetra-CBs	penta-CBs	hexa-CBs	hepta-CBs	octa-CBs	nona-CBs	deca-CBs	total CBs
Conc. mg/kg	Mean	0.045	0.29	0.80	0.87	0.90	0.35	0.55	0.13	nd	nd	3.9
	SD	0.003	0.05	0.08	0.06	0.08	0.002	0.01	0.004	–	–	0.13
Recovery %	Mean	102	91	92	108	106	95	97	97	95	9	–
	SD	5.2	2.6	5.1	5.4	6.8	2.8	2.7	3.3	1.2	3.4	–

tail-end of oil interferences during sulphoxide SPE processing. This is of minor concern because the primary PCB homologues of concern in transformer oil samples consist of the tri- to heptachlorobiphenyls. Note that using the assay described in this report, spike levels at the range of 0.045–0.9 mg/kg (ppm) for the individual PCBs were able to be determined.

Conclusion

In this report, we demonstrated the utility of a new silica-bonded sulphoxide SPE phase for the normal-phase extraction of PCBs (and possible related aromatic compounds) from difficult sample matrices such as transformer oils. Because aliphatic hydrocarbons are often co-extracted with PCBs using conventional SPE methods, lower limits of detection (< 5 ppm) are often difficult to achieve. Sulphoxide SPE allows for the user to separate aliphatic hydrocarbon interferences from PCBs prior to GC analysis using a generic/simple method. By removing this key matrix interference prior to analysis, detection limits of less than 0.5 ppm are readily achieved.

References

- 1] Copland et al. *Environ. Sci. Technol.* 1982, 16, 121–124.
- 2] Solid Phase Extraction of PCBs from Transformer Oil and Waste Oil and Analysis By Capillary GC, Supelco Application Note 67, 1998, T395067A.
- 3] Storr-Hansen et al. *Chemosphere* 1992, 24, 323–333.
- 4] Larsen et al. *Chemosphere* 1991, 23, 1077–1084.
- 5] Numata et al. *Anal. Chem.* 2003, 75, 1450–1457.

Featured Products

Description	Cat. No.
Supelclean Sulphoxide SPE	
Glass SPE Tube, 6 g/20 mL (17 mm I.D. x 137 mm L), pk 5	55252-U
Polypropylene SPE Tube, 3 g/6 mL, pk 30	55253-U
Bulk, 100 g	55254-U

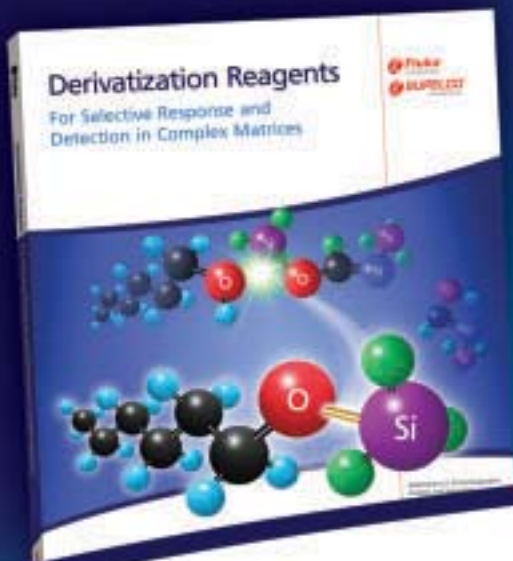
Related Products

Description	Cat. No.
Empty Glass SPE Tube (17 mm I.D. x 137 mm L) with PE frit, 20 mL, with PE frit, luer cap, and screw-top cap, pk 5	55255-U
Frit Insertion Tool for 20 mL Glass SPE tube	55257-U
Large Volume Reservoir (25 mL) for 6 mL SPE tubes, PP, pk 30	54258-U
Large Volume Reservoir (25 mL) for 6 mL SPE tubes, PTFE, pk 3	54259-U
SLB-5ms Capillary GC Columns	
15 m x 0.10 mm I.D., 0.10 µm	28466-U
20 m x 0.18 mm I.D., 0.36 µm	28576-U
30 m x 0.25 mm I.D., 0.25 µm	28471-U
30 m x 0.53 mm I.D., 0.50 µm	28541-U
30 m x 0.53 mm I.D., 1.0 µm	28559-U
SPB-608 Capillary GC Columns	
30 m x 0.25 mm I.D., 0.25 µm	24103-U
30 m x 0.53 mm I.D., 0.50 µm	25312
Equity-1701 Capillary GC Columns	
15 m x 0.10 mm I.D., 0.10 µm	28343-U
30 m x 0.25 mm I.D., 0.25 µm	28372-U
30 m x 0.53 mm I.D., 0.50 µm	28391-U
30 m x 0.53 mm I.D., 1.0 µm	28394-U

Related Information

For more information, please request the Supelclean Sulphoxide Data/ Instruction Sheet, T707009, and *Analysis of PCBs in Transformer Oil with a Sulphoxide Bonded SPE Phase and GC-MS*, T408040, on the attached post card. These publications are available in electronic form only. Be sure to include your email address on the request form.

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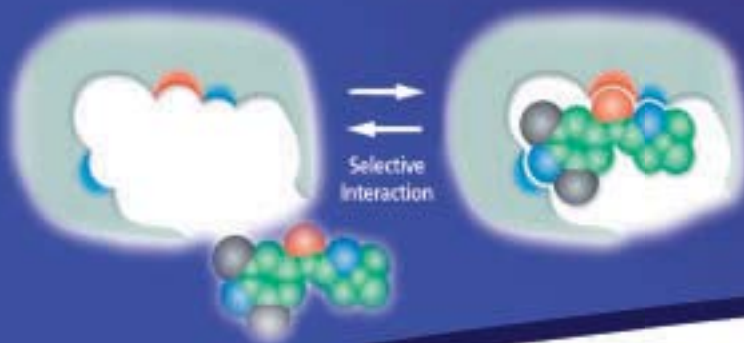
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Validation of the Radiello® Diffusive Sampler for the Measurement of Formic and Acetic Acids in Museum Environments

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Fondazione Salvatore Maugeri-IRCCS, Centro di Ricerche Ambientali, Padua/Italy



Introduction

The assessment of potential damages to indoor cultural heritage, due to some air-borne pollutants, is a major and growing concern for curators and conservators. For years, objects of art in museums have been placed in showcases.

Besides security reasons, this is mainly done to protect these objects against contamination by dust particles and deterioration by gaseous pollutants, a field of growing interest for conservators. In recent years, formic acid and acetic acid were also assumed to be harmful for several objects of art, containing e.g. copper or lead. Measurements inside museum showcases via SPME technique showed that these acids can accumulate and reach high concentrations. Most of the showcases are made of wood and/or composite materials as MDF (Medium Density Fiberboard), which can be considered as sources of low molecular weight volatile organic compounds.

This research is especially focused on formic acid and acetic acid. The aim of the work was the development of a monitoring technique based on diffusive sampling and its validation in environmental conditions typical of museum showcases.

The Measurement

Measurements inside microenvironments such as the museum showcases can easily be done by means of diffusive samplers, which require only little space and no electrical power. The Radiello samplers provide validated methods for the diffusive sampling and the analysis of various gaseous pollutants. To evaluate and validate the applicability of the diffusive sampling of gaseous acetic and formic acids, a radiello diffusive sampler, containing chemi-adsorbing cartridges (RAD166) impregnated with triethanolamine (TEA) in a blue diffusive body (RAD1201) was tested. The quantification was made by ion chromatography.

Analysis

The analytical procedure involves recovery of the acids from the chemi-adsorbing cartridges by means of water extraction (5 ml) followed by two 1-min mechanical stirring steps, between which the extracts are left to settle one hour. The analysis of aqueous extracts is done by ion chromatography, quantifying the corresponding ions (formate and acetate) by means of a conductivity detector with a suppressor module. A peak resolution acetate/formate of 1.95 is achieved.

Exposure Chamber Tests

Two exposure experiments were carried out in order to determine the sampling rates. The temperature and air humidity were kept constant at 25°C and 47% Relative Humidity, respectively. The two

The diffusive sampler

The theoretical principle of a general diffusive sampler is based on Fick's first law:

$$\frac{dm}{dt} = D \cdot S \cdot \frac{dC}{dl}$$

where **m** is the mass of the compound being adsorbed, **t** is the time of exposure of the sampler, **D** is the diffusion coefficient of the compound, **S** is the diffusive surface area, **C** is the ambient air concentration, **l** is the distance from the diffusive surface to the adsorbent layer.

In radiello samplers the diffusive surface is cylindrical, thus the diffusion equation is the following:

$$\frac{dm}{dt} = D \cdot 2\pi r h \cdot \frac{dC}{dr}$$

where **r** and **h** are the radius and the height of the cylinder, respectively. When using diffusive sampling, the atmospheric concentration **C** of target Compounds is determined by means of the following equation:

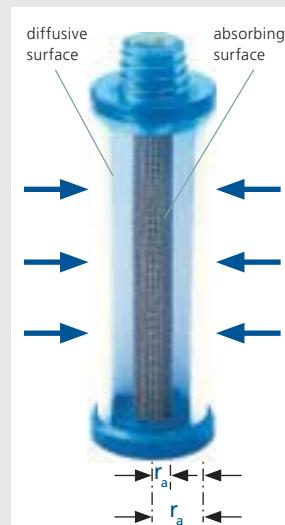
$$C = \frac{m}{t \cdot Q}$$

where **Q**, the sampling rate, needs to be experimentally determined under specified conditions (exposure time, concentration, temperature, humidity...). The adsorbed mass **m** may need to be corrected by subtracting the blank value, that is the mass recovered on an unexposed sampler. In order to adsorb formic and acetic acids, a microporous polyethylene cylinder (5.8 mm diameter, 60 mm height) was used as the inner chemiadsorbing cartridge: the reactant is the triethanolamine (TEA), which is impregnated onto the microporous polyethylene. The underlying hypothesis is that TEA react with acids diffusing towards the chemiadsorbing cartridge by forming a reaction adduct $(\text{HOCH}_2\text{CH}_2)_3\text{NH}^+\text{RCOO}^-$.

experiments were carried out by applying two different levels of concentration:

Low level: 128 µg/m³ of formic acid, 160 µg/m³ of acetic acid

High level: 1248 µg/m³ of formic acid, 1564 µg/m³ of acetic acid



(continued on page 14)



During each experiment, four groups of samplers (6 replicates each) were exposed to the test concentration for variable periods: 24hours, 2days, 4days and 7days.

The test atmospheres were generated by means of capillary injection of an aqueous mixture of the two organic acids in a nitrogen stream, followed by a further gaseous dilution.



Results

A series of six unexposed samplers were analysed in order to assess the blank levels (**Table 1**) and hence the detection limits of the measurement method, defined as three times the standard deviations. For each compound and concentration level a set of sampling rate values was calculated. Values in **Tables 1 and 2** represent the average and standard deviation of six individual values obtained for a specified exposure time.

Table 1 blank value

compound	blank values (µg)	st. deviation (µg)	7-days sampling detection limit (µg·m ⁻³)
Formic acid	1.69	0.07	0.2
Acetic acid	1.21	0.14	0.4

Table 2

concentration (µg·m ⁻³)	24-hour sampling rate (ml/min)	2-day sampling rate (ml/min)	4-day sampling rate (ml/min)	7-day sampling rate (ml/min)
Formic acid sampling rate (average values and standard deviations)				
128	80.4±1.3	93.0±5.0	101.4±3.7	91.2±3.9
1248	86.7±4.2	109.9±4.9	113.9±5.3	111.6±2.8
Acetic acid sampling rate (average values and standard deviations)				
160	92.8±4.1	101.0±4.9	102.0±3.7	98.0±4.0
1564	95.6±4.9	101.0±4.5	99.0±3.8	96.6±2.0

The analysis of variance (ANOVA) with two-level factors (concentration/time of exposure) shows that formic acid sampling rate is significantly affected by both concentration and exposure time. On the other hand, acetic acid sampling rate shows a weaker dependence on exposure time, but no statistically significant dependency on concentration.

Therefore, a couple of sampling rate values have to be used for formic acid 7-day sampling, while the average acetic acid sampling rate, for the same exposure time, comes out to be 97.3±3.1 ml/min.

The formic acid sampling rates show an unexpected behaviour, especially at low concentration. In fact, taking into account the higher value of the diffusion coefficient of that compound, higher sampling rate values, compared to acetic acid, would have been expected.

Conclusions and Perspectives

The measurement method allows the determination of concentrations above one thousand µg·m⁻³ of both formic acid and acetic acid inside museum showcases, with detection limits below 0.5 µg·m⁻³. The method will be a powerful tool in the assessment of concentrations of low molecular weight organic acids inside museum showcases, as well as for establishing "threshold values" for the protection of art objects.

Future developments will involve the evaluation of other parameters which could influence the sampling rates in typical museum conditions, such as air speed, which is likely to be slow.

In addition, the behaviour of formic acid needs to be further investigated, in order to try an explanation of the observed deviations from simple "Fick's law" behaviour of the sampler.

* Acknowledgement

The authors would like to thank A.F. Locateli Godoi, R. Godoi, S. Potgieter Vermaak, R. Van Grieken of the Micro and Trace Analysis Centre at the University of Antwerp for their support in this study.

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A Revolution in GC Phase Technology: Ionic Liquid Stationary Phases

Alternative and Extended Selectivities at Higher Temperatures

Leonard Sidisky, Mike Buchanan, mike.buchanan@sial.com

Introduction

Supelco is proud to announce that it has developed a novel GC stationary phase technology utilising the unique properties of di- and polycationic liquids based on the research of Professor Daniel Armstrong, University of Texas at Arlington. In this article the first of a series of ionic liquid GC columns is presented.

What are ionic liquids?

An ionic liquid is a liquid that essentially contains only ions. It is a salt whose melting point is below normal room temperature. It remains liquid at this and higher temperatures.

- Unique combinations of cations and anions
- Remain liquid over a wide temperature range
- Very low volatility
- Wide range of polarities up to highly polar
- Broadest range of solvation interactions of any known solvent
- Provides new selectivities when used as stationary phase in GC

Why are di- and polycationic ionic liquid stationary phases beneficial for GC?

With the successful use of ionic liquids as viable GC stationary phases, analysts will be able to perform previously unachievable separations.

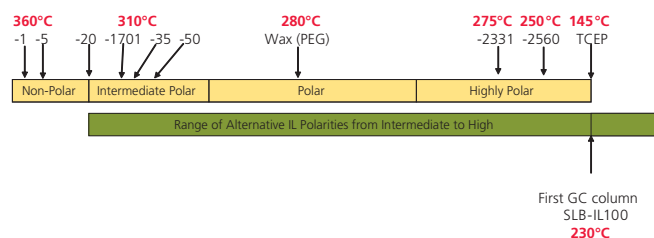
Figure 1 shows the range of polarities of today's classical and ionic liquid GC stationary phases. Due to the unique combinations of cations and anions, ionic liquids can be tailored to provide a variety of GC stationary phases with new and alternative polarities expanding today's range of selectivities. In addition the polarity of ionic liquid stationary phases can extend beyond what is possible with traditional GC stationary phases. The ability of ionic liquids to remain liquid over a wide temperature range expands the GC column operating temperature range compared to traditional stationary phases such as substituted polysiloxane polymers or polyethylene glycols.

Benefits of ionic liquids as GC stationary phases:

- Increased maximum temperatures
- Very low volatility, providing low column bleed
- Stable retention times
- Long column life
- Highly polar nature, expanding the polarity scale upwards
- Novel selectivities, allowing new and improved applications

Previous work with ionic liquids as GC phases focused on mono-cationic ionic liquids, which did not exhibit the desired chromatographic characteristics, or had narrow working ranges. Current research is extended to a variety of di- and poly-cationic ionic liquids.

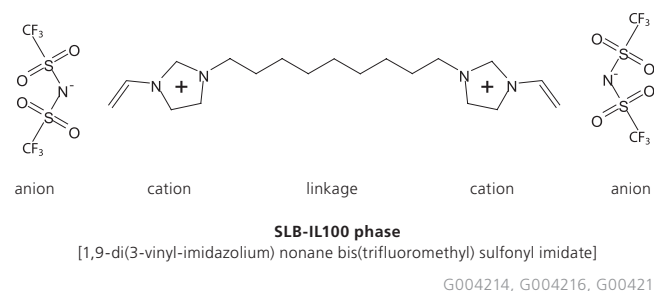
Figure 1 Polarity range of classic and ionic liquid GC stationary phases. Maximum operating temperatures are given in red.



What is the chemical basis of the new selectivities of ionic liquid GC columns?

Whereas the chemical structures of existing GC phases allow limited modification (changing the pendant group on polysiloxane polymers or adjusting the length of polyethylene glycols, for example), the chemical structure of ionic liquids permits numerous opportunities for modification. As shown in **Figure 2**, the components that can be modified include the cation, linkage and anion. Variation of these results in new and alternative selectivities.

Figure 2 Components of a Dicationic Ionic Liquid Stationary Phase



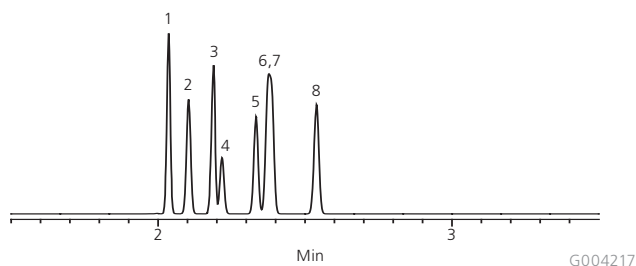
The SLB™-IL100: the first new generation ionic liquid GC column from Supelco

The SLB-IL100 is the first in this new range of unique columns that utilise ionic liquid phase chemistry (for the structure of this ionic liquid see **Figure 2**). The SLB-IL100 column demonstrates the desired characteristics that ionic liquids are predicted to possess; namely, a higher maximum temperature compared to non-ionic liquid columns with similar polarity and selectivity. It has a polarity roughly equivalent to that of the traditional TCEP (tris-cyanoethoxypropane) phase. Similar selectivity to TCEP is demonstrated by the BTEX (benzene, toluene, ethyl benzene and xylenes) separation but the higher maximum temperature of the SLB™-IL100 allows the column to be also used for FAME separations. Specifically, the SLB-IL100 has a 230°C maximum temperature, resulting from the robustness and low volatility of the phase, whereas traditional TCEP columns with

Figure 3 BTEX and n-Alkanes on the SLB-IL100

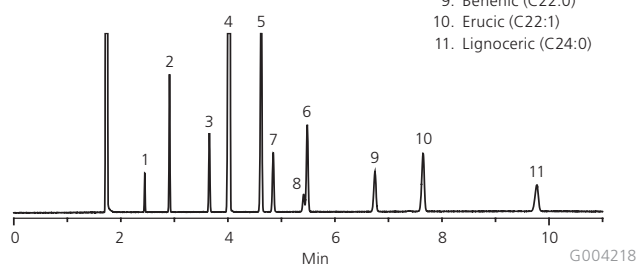
column: SP-IL100, 30 m x 0.25 mm I.D., 0.20 μ m (28884-U)
 oven: 110°C
 inj.: 250°C
 det.: FID, 250°C
 carrier gas: helium, 26 cm/sec @ 110°C
 injection: 0.1 μ L, 300:1 split
 liner: 4 mm I.D., split, cup
 sample: NEAT mixture containing varying percentages of each component

1. Undecane
2. Benzene
3. Tridecane
4. Toluene
5. Ethyl benzene
6. p-Xylene
7. m-Xylene
8. o-Xylene

**Figure 4** Rapeseed Oil FAMES on the SLB-IL100

column: SP-IL100, 30 m x 0.25 mm I.D., 0.20 μ m (28884-U)
 oven: 180°C
 inj.: 250°C
 det.: FID, 250°C
 carrier gas: helium, 30 cm/sec @ 180°C
 injection: 1 μ L, 100:1 split
 liner: 4 mm I.D., split, cup
 sample: Rapeseed oil FAME mix, 5 mg/mL total FAMES in methylene chloride

1. Myristic (C14:0)
2. Palmitic (C16:0)
3. Stearic (C18:0)
4. Oleic (C18:1n9c)
5. Linoleic (C18:2)
6. Linolenic (C18:3)
7. Arachidic (C20:0)
8. cis-11-Eicosenoic (C20:1)
9. Behenic (C22:0)
10. Erucic (C22:1)
11. Lignoceric (C24:0)



equivalent polarity and selectivity have a 140°C maximum temperature. The SLB-IL100 column is expected to broaden the range of applications that can be performed on highly polar columns.

Example Applications on the SLB™-IL100

BTEX and n-Alkanes

Figure 3 illustrates the separation of benzene, toluene, ethyl benzene, and the xylene isomers (BTEX) in the presence of C11 and C13 n-alkanes. The high polarity/selectivity of the ionic liquid phase results in the elution of toluene after C13 at 110°C. This is desirable because the aliphatic fraction of gasoline consists of n-alkanes up to C13. Therefore, the quantitation of aromatics in products based on gasoline (such as mineral spirits) requires a column with a polarity and selectivity able to separate the aromatic fraction from the aliphatic fraction. As shown, the SLB-IL100 has this necessary polarity and selectivity.

Rapeseed Oil FAMES

Figure 4 illustrates the separation of the fatty acids (analysed as FAMES) found in a rapeseed oil sample. Rapeseed oil contains a variety of saturated and mono-, di-, and tri-unsaturated fatty acids ranging in carbon length from C14 to C24. The elution of C18:3 after C20:0 and C20:1 is typically observed with highly polar phases.

Based on the rapeseed oil elution pattern and other characteristics, it has been determined that the SLB-IL100 phase is virtually equivalent in polarity and selectivity to the TCEP phase, currently one of the highest polarity/selectivity GC phases, but with a higher maximum operating temperature and superior phase stability.

Outlook for Ionic Liquid GC Phase Technology

The patented and successful use of ionic liquids as viable GC stationary phases heralds a new and exciting chapter in GC phase technology. Now analysts will be able to perform previously unachievable separations with the potential to go way beyond applications possible using traditional phases. For example, an ionic liquid phase with a polarity and selectivity similar to that of Carbowax® 20M, but with a maximum temperature over 300°C, is just one of many possibilities currently being investigated. Look for additional Supelco ionic liquid phases to be introduced in the coming months. This is truly an exciting time in GC phase development!

Sigma-Aldrich/Supelco is the first-to-market with this new, innovative, and patented (US 2008/0027231 A1; other patents pending) technology, developed in conjunction with Professor Daniel Armstrong (University of Texas at Arlington).

SLB-IL100 Specifications

Phase: non-bonded; 1,9-di(3-vinyl-imidazolium) nonane bis(trifluoromethyl) sulphonyl imidate
 Temp. Limits: Sub-ambient to 230°C

Related Information

Please inquire for SLB IL-100 GC column information, testing and availability. Please contact Roberto Ferrari at: roberto.ferrari@sial.com

Improve GC Reproducibility by Using FocusLiner™ Inlet Liners

Robert F. Wallace bob.wallace@sial.com

Introduction

Poor sample reproducibility observed by chromatographers from one consecutive injection to another may be an indication that small variations in the injection volume have occurred. Placing a small plug of either glass or quartz wool inside an inlet liner has historically been used to overcome this. However, this procedure does have distinct drawbacks. FocusLiner inlet liners are specifically designed to exhibit the benefits of using a wool plug without the drawbacks.

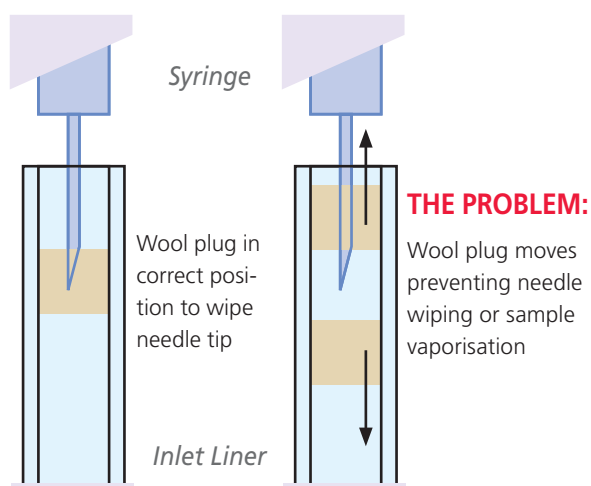
With a FocusLiner inlet liner, the chromatographer can be assured that the wool plug is always in the correct position.

The Problem with Wool Plugs in Traditional Liners

In addition to preventing non-volatile material from entering the column, a wool plug exhibits two benefits that assist in reducing injection volume variability. 1) The increased surface area facilitates the maximum vaporisation of the sample. 2) Any droplets formed on the outside of the needle are wiped off. Both of these benefits require that the needle tip penetrate the wool plug. Therefore, the position of the wool plug in the injection liner is critical. Unfortunately, there is no guarantee that once a liner is installed in the injector, the wool plug will stay in the correct position.

The wool plug can be easily dislodged without the chromatographer's knowledge. As shown in **Figure 1**, a common cause of wool plug displacement within the liner is that repeated injections progressively move the wool plug until no further contact with the needle is made. A sudden change in the inlet pressure, like changing the septum, can also result in the movement of the wool plug.

Figure 1 The Problem – Wool Plug in Traditional Inlet Liner



Relocation of the wool plug from the correct position can be characterised by excessive tailing of the solvent peak, as shown in **Figure 2**. As shown in **Figure 3**, sharp solvent peaks are only observed when the wool plug is in the correct position to wipe the needle tip.

Figure 2 Tailing solvent peak – wool plug in incorrect position

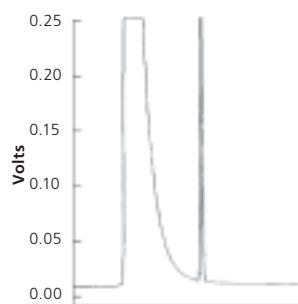
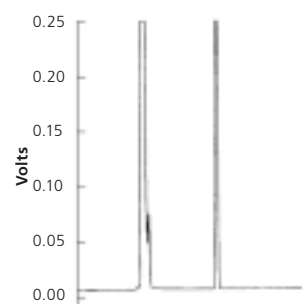


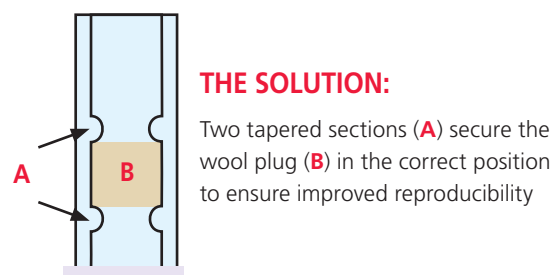
Figure 3 Sharp solvent peak – wool plug in correct position



Overcoming the Drawbacks

FocusLiner inlet liners utilise an innovative design that overcomes the drawbacks observed with the use of wool plugs in traditional inlet liners. With FocusLiner inlet liners, the wool plug is held in position by two tapered sections. As shown in **Figure 4**, these tapered sections secure the wool plug in the correct position, even after repeated injections and exposure to sudden pressure changes. With a FocusLiner inlet liner, the chromatographer can be assured that the wool plug is always in the correct position. This will ensure that the needle tip penetrates the wool plug, wiping any residual liquid sample from the needle tip while providing sufficient surface area for maximum volatilisation of the sample.

Figure 4 The Solution – FocusLiner Inlet Liner



Sample Precision

The effect on sample precision (measured as % RSD) caused by the position of the wool plug in the liner was measured. A 4 mm I.D. traditional inlet liner with the wool plug moved to the centre was evaluated against a 4 mm I.D. FocusLiner inlet liner. Another frequently used split liner was also evaluated. This liner design substitutes the wool plug with a sintered glass frit, which can be either fixed or removable. In this experiment a 4 mm I.D. fixed frit liner was used.

As shown in **Figure 5**, when the wool plug is moved to the centre of the traditional inlet liner, % RSD values are in the 8–10% range. Impressively, the Focus-Liner inlet liner was able to achieve % RSD values for the same probe compounds in the 0.2% range! This is up to 50 times lower than those measured with the traditional inlet liner.

The fixed sintered glass frit liner is also unable to match the precision provided by the FocusLiner inlet liner. This result is not surprising as a key element to achieving good sample reproducibility is the needle tip being wiped during injection. Therefore, liners with fixed or removable frits can only be used with limited success.

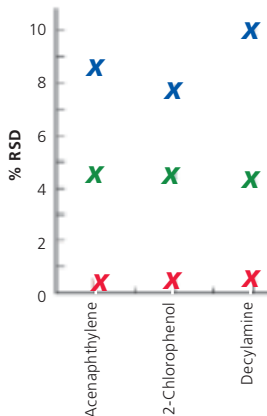
Sample Accuracy

Sample accuracy is also a critical factor in providing confidence in sample quantitation. Peak areas for probe compounds using the FocusLiner inlet liner were found to be, on average, 25% higher than a liner where the wool plug is positioned incorrectly.

Solvent peak tailing is also observed if the wool plug is incorrectly positioned, caused by slow vaporisation near the cool septum cap as the needle is wiped during withdrawal. As shown in **Figure 2**, a tailing solvent peak may interfere with the quantitation of peaks that elude shortly after the solvent peak. As shown in **Figure 3**, the correct position of the wool plug results in sharp solvent peaks and more accurate quantitation of peaks that elude shortly after the solvent peak.

Figure 5 % RSD of different wool plug positions

4 mm I.D. liner
w/wool in centre of liner
w/fixed sintered glass frit
FocusLiner



Conclusion

Poor reproducibility and severe tailing may be observed if the needle tip is not wiped during injections. As the sample is being delivered from the syringe, droplets will form that wet the syringe needle tip. The volume of sample that remains on the needle tip varies from injection to injection. The key to the improved reproducibility provided by the FocusLiner inlet liner is the proper positioning of the wool plug in the liner, allowing the needle tip to be wiped. The use of a FocusLiner inlet liner provides precise, accurate and reliable sample injections, resulting in improved reproducibility.

References

R.F. Wallace, Supelco, Reporter, August 2006; Volume 24.4: 11

Featured Products¹

Description	Cat. No.
Agilent® 5890/6890/7890	
FocusLiner™ for	
Split/Splitless, 78.5 x 6.3 x 4.0 mm	2879805-U
Split/Splitless, 78.5 x 6.3 x 4.0 mm, single taper	2879905-U
PerkinElmer® AutoSystem™ and Clarus	
Split/Splitless, 92 x 6.2 x 4.0 mm	2879205-U
Split/Splitless, 92 x 6.2 x 4.0 mm, single taper	2879105-U
Shimadzu®14/15A/16 with SPL-14 Injector	
Split/Splitless, 99 x 5.0 x 3.4 mm	2878105-U
Split/Splitless, 99 x 5.0 x 3.4 mm, single taper	2877805-U
Shimadzu 17A with SPL-17 Injector	
Split/Splitless, 95 x 5.0 x 3.4 mm	2878605-U
Split/Splitless, 95 x 5.0 x 3.4 mm, single taper	2878405-U
Varian® 1075/1077 Injector	
Split, 72 x 6.3 x 4.0 mm	2875405-U
Split, 72 x 6.3 x 4.0 mm, single taper	2874805-U
Varian 1078/1079 Injector	
Split/Splitless, 54 x 5.0 x 3.4 mm, single taper	2875705-U
Varian CP-1177 Injector	
Split/Splitless, 78.5 x 6.3 x 4.0 mm	2879805-U
Split/Splitless, 78.5 x 6.3 x 4.0 mm, single taper	2879905-U

1. All of these FocusLiner inlet liners are packs of 5 and packed with quartz wool. Additional pack sizes can be viewed on our website sigma-aldrich.com/focusliner

Related Information

Our full line of FocusLiner inlet liners can be viewed online at sigma-aldrich.com/focusliner. For more information on inlet liners, request the *Capillary GC Inlet Liner Selection Guide*, T196899 (BBB) and the poster *Selecting the Appropriate Inlet Liner*, T404081 (HCH).

LC-MS Mobile Phase Additives – Tips & Tricks

Shyam Verma shyam.verma@sial.com

LC-MS is becoming a routine analytical tool in research and industrial laboratories. The demand on sensitivity, specificity and speed of analysis requires use of high-purity chemicals for sample preparation, mobile phase and post-column additives. Additives are used to suppress unwanted signals to selectively enhance the signal of particular compounds in a mixture, for example glycosidic species in a mixture of peptides. Salts can suppress ionisation in ESI sources.

Acids – The Most Common Additives

Volatile, low molecular weight organic acids like formic and acetic acid are commonly used as additives in LC-MS mobile phase. Their primary advantage is that they improve ionisation and resolution of a wide range of molecules (1).

Overcoming the TFA Suppression Effects

The ionisation-suppressing effects of trifluoroacetic acid (TFA) can be partly overcome by addition of other LC-MS compatible organic acids, like formic or propionic acid (2).

Mobile phases for HPLC of proteins and peptides usually contain TFA to control the pH and improve peak shape and resolution. TFA enhances retention by ion pairing with the peptide and improves peak shape by reducing silanol interactions (3). However, TFA has adverse effects on MS detection. Its high surface tension prevents efficient spray formation and TFA ions in the gas phase ion-pair with the peptide basic group suppressing their ionisation and reducing the MS signal (4, 5).

The Neutral Salts

The neutral volatile salts, ammonium acetate and ammonium formate, offer a much broader influence on analyte separation and ionisation than do acids (6). Their use, of course, is dictated by the particular LC-MS separation objective.

It may be necessary under certain circumstances to use more neutral conditions, either because the analytes are sensitive to acids or do not exhibit optimal resolution at low pH. When acids are not suitable, volatile salts like ammonium formate or acetate may be the additives of choice. However, limited solubility of the salt in organic solvents, changing pH value during a gradient and the mildly acidic pH provided by the salt that permits both positive and negative ion mode detection are issues of concern (6).

Sodium Adduct Formation

Formation of alkali adduct is associated with decrease in sensitivity. When adduct formation tendency is strong, addition of small and defined amounts of sodium ions (mostly pre-column) can help to obtain uniform and stable molecular ions for detection in LC-MS (7). In addition to sensitivity, stability and perhaps specificity of the molecular ion are also important. The ability to form alkali adducts is useful for quantifying certain classes of molecules and for selec-

tively enhancing the LC-MS signals. However, their true benefit, particularly that of sodium ion, needs further studies.

The Complete Product Range

Sigma-Aldrich offers a wide range of high-purity additives for LC-MS applications in addition to pure CHROMASOLV® solvents and ready-to-use blends. This includes the most commonly used acids, bases, volatile salts and a sodium source (see Featured Products below). All products are of high purity, usually puriss p.a., and are tested for LC-MS applications.

References

- 1] Emmert J., *Analytix*, 2006, no. 2, 8.
- 2] Emmert J. and Rueck A., *Analytix*, 2006, no. 3, 16.
- 3] "Eliminate TFA and Improve Sensitivity of Peptide Analysis by LC-MS", Supelco Application Note 168 (T302168).
- 4] Apffel A., Fisher S., Goldberg G., Goodley P.C., Kuhlmann F.E., *J. Chromatography, A*, 1995, 712, 177–190.
- 5] Wang G., Cole R.B., *J. Am. Soc. Mass Spectrom.*, 1996, 7(10), 1050–1058.
- 6] Emmert J. and Leitner A., *Analytix*, 2006, no. 4, 9.
- 7] Emmert J. and Waelti T., *Analytix*, 2006, no. 5, 6.

Featured Products

Description	Pkg. Size	Cat. No.
Eluent Additives for LC-MS		
Trifluoroacetic acid, puriss* p.a.	50 mL	40967
Trifluoroacetic acid, puriss p.a.	10 x 1 mL	40967
Formic acid, puriss p.a.	50 mL	56302
Acetic acid, puriss p.a.	50 mL	49199
Propionic acid, puriss p.a.	50 mL	49916
Ammonium formate, puriss p.a.	50 g	55674
Ammonium acetate, puriss p.a.	50 g	49638
Sodium citrate tribasic dihydrate, puriss p.a.	50 g	61333
Ammonium bicarbonate, puriss p.a.	50 g	40867
Ammonium hydroxide solution 25%, puriss p.a.	100 mL	44273
Triethylamine, puriss p.a.	50 mL	65897
LC-MS CHROMASOLV® Blends		
Water with 0.1% ammonium acetate	2.5 L	34674
Methanol with 0.1% ammonium acetate	2.5 L	34670
Acetonitrile with 0.1% ammonium acetate	2.5 L	34669
Acetonitrile with 0.1% formic acid	2.5 L	34668
Selection of LC-MS Solvents and Blends		
2-Propanol CHROMASOLV LC-MS	1, 2.5 L	34965
Water with 0.1% formic acid and 0.01% TFA	2.5 L	34677
Acetonitrile with 0.1% formic acid and 0.01% TFA	2.5 L	34676

* "puriss" quality grade is defined as >98.5% assay, <0.1% ash, and specification n + 0.001, d + 0.001 with no extraneous colour and a homogeneous appearance. "p.a." or pro analysis denotes a product with guaranteed trace impurity levels and/or suitability for the indicated analytical application.

Related Information

For more information, request KCT on the attached postcard and visit our website: sigma-aldrich.com/chromasolv

LC-MS CHROMASOLV® - The Highest Quality Solvents and Blends

Solvent Blend	Pack Size	Cat. No.
Water with 0.1% TFA LC-MS CHROMASOLV	2.5 L	34978-2.5L-R
Acetonitrile with 0.1% TFA LC-MS CHROMASOLV	2.5 L	34976-2.5L-R
Methanol with 0.1% TFA LC-MS CHROMASOLV	2.5 L	34974-2.5L-R

LC-MS CHROMASOLV Solvents and Blends offer:

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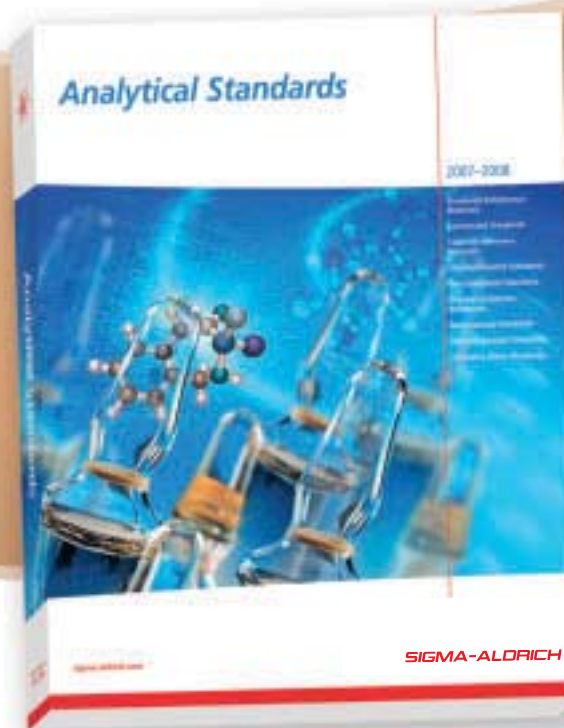
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New! US EPA Method 8270 LCS Mixes with Improved Stability

Steve Cecil, Jim Walbridge, Vicki Yearick vicki.yearick@sial.com

Low spike recoveries for EPA Method 8270 “Semivolatile Compounds by GC/MS” are often seen for aniline and benzidine compounds in the LCS* mixes. Sigma-Aldrich laboratory studies of these low recoveries have determined the cause to be interactions of aniline and benzidines with other mix components and/or the sample matrix. In addition to reactive stability, our studies show low recoveries are also seen for these compounds with increased exposure to oxygen, light and temperature.

Sigma-Aldrich chemists have designed two new Supelco brand 8270 LCS Spike Mix formulations to better meet the spike-recovery requirements when performing semi-volatile assays utilising SW-846 methodologies. These new 78-component LCS spiking standards, one with 100 µg/mL and a high concentration (HC) version with 200 µg/mL, are engineered for the improved stability need in today’s environmental laboratories.

- Both LCS spiking solutions are specially formulated to increase analyte stability including anilines and benzidines, while still providing for a water-soluble matrix.
- Our chemists have also addressed temperature, light and oxygen stability issues.
 - Temperature has been determined in the Supelco laboratory studies to be the single largest contributor to shelf-life degradation. To ensure the integrity of our spiking mixes, we ship them on dry ice. End users should store the solutions in the freezer at -15°C or colder, as noted on the spike mixes documentation.
 - Reactivity with oxygen is avoided by blanketing the mixes with an inert gas when preparing and ampulising the new mixes.
 - UV light degradation is minimised through the use of non-UV emitting lights during production and the use of amber glass for storage.
- Both spike mixes are offered in convenient 25 mL volumes and include detailed lot specific mix preparation and analytical testing results.

Description	Concentration	Pkg Size	Cat. No.
EPA 8270 LCS Spike Mix	100 µg/mL each component in methanol:dichloromethane:benzene (90:9.4:0.6)	25 mL	47064-U
EPA HC 8270 LCS Spike Mix	200 µg/mL each component in methanol:dichloromethane:benzene (80:18.75:1.25)	25 mL	40032-U
<i>Acenaphthene</i>	<i>Bis(2-ethylhexyl)phthalate</i>	<i>1,3-Dichlorobenzene</i>	<i>Hexachlorobenzene</i>
<i>Acenaphthylene</i>	<i>4-Bromodiphenyl ether</i>	<i>1,4-Dichlorobenzene</i>	<i>Hexachloro-1,3-butadiene</i>
<i>Aniline</i>	<i>Carbazole</i>	<i>3,3'-Dichlorobenzidine</i>	<i>Hexachlorocyclopentadiene</i>
<i>Anthracene</i>	<i>4-Chloroaniline</i>	<i>2,4-Dichlorophenol</i>	<i>Hexachloroethane</i>
<i>Azobenzene</i>	<i>4-Chlorodiphenyl ether</i>	<i>Diethyl phthalate</i>	<i>Indeno[1,2,3-cd]pyrene</i>
<i>Benz[a]anthracene</i>	<i>2-Chloroisopropyl ether</i>	<i>2,4-Dimethylphenol</i>	<i>Isophorone</i>
<i>Benzo[b]fluoranthene</i>	<i>4-Chloro-3-methylphenol</i>	<i>Dimethyl phthalate</i>	<i>2-Methyl-4,6-dinitrophenol</i>
<i>Benzo[k]fluoranthene</i>	<i>2-Chloronaphthalene</i>	<i>1,2-Dinitrobenzene</i>	<i>1-Methylnaphthalene</i>
<i>Benzo[ghi]perylene</i>	<i>2-Chlorophenol</i>	<i>1,3-Dinitrobenzene</i>	<i>2-Methylnaphthalene</i>
<i>Benzoic acid</i>	<i>Chrysene</i>	<i>1,4-Dinitrobenzene</i>	<i>o-Cresol</i>
<i>Benzo[a]pyrene</i>	<i>m-Cresol</i>	<i>2,4-Dinitrophenol</i>	<i>Naphthalene</i>
<i>Benzyl alcohol</i>	<i>p-Cresol</i>	<i>2,4-Dinitrotoluene</i>	<i>2-Nitroaniline</i>
<i>Benzyl butyl phthalate</i>	<i>Dibenz[a,h]anthracene</i>	<i>2,6-Dinitrotoluene</i>	<i>3-Nitroaniline</i>
<i>Bis(2-chloroethoxy)methane</i>	<i>Dibenzofuran</i>	<i>Di-n-octyl phthalate</i>	<i>4-Nitroaniline</i>
<i>Bis(2-chloroethyl)ether</i>	<i>Dibutyl phthalate</i>	<i>Fluoranthene</i>	<i>Nitrobenzene</i>
<i>Bis(2-ethylhexyl)adipate</i>	<i>1,2-Dichlorobenzene</i>	<i>Fluorene</i>	<i>2-Nitrophenol</i>
			<i>4-Nitrophenol</i>
			<i>N-Nitrosodimethylamine</i>
			<i>N-Nitrosodiphenylamine</i>
			<i>N-Nitrosodi-n-propylamine</i>
			<i>Pentachlorophenol</i>
			<i>Phenanthrene</i>
			<i>Phenol</i>
			<i>Pyrene</i>
			<i>Pyridine</i>
			<i>2,3,4,6-Tetrachlorophenol</i>
			<i>2,3,5,6-Tetrachlorophenol</i>
			<i>1,2,4-Trichlorobenzene</i>
			<i>2,4,5-Trichlorophenol</i>
			<i>2,4,6-Trichlorophenol</i>

* LCS (Laboratory Control Sample/Blank Spike) Spikes are typically required when sample matrix spike recoveries are determined to be outside the control limits. LCS standards are prepared by spiking known concentrations of target analytes into clean sample matrixes. The spiked LCS mix is then subjected to the same sample preparation and analysis protocols as the sample. LCS spike recoveries are calculated and used to determine the analytical accuracy of the method.

** Surcharges for dry ice shipment might apply

Did you know?

A safe and inexpensive method for removing the top from a 2 mL glass ampul is to use a Sigma-Aldrich ampul breaker (Z122904). Simply insert the top of the ampul into the breaker and snap off the top. The ampul top is retained in the breaker for safe disposal.



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Custom Standards



Are Your Vials & Inserts Compatible?

Ron Shawley ron.shawley@sial.com

The I.D. of 2 mL vials can vary by as much as 0.3 mm. As a result of this variation, your vial insert may not properly fit, costing you time, money and causing frustration.

Due to a lack of worldwide standards, this problem is magnified when comparing products manufactured in the US and Europe. These differences are shown in **Table 1**.

To ensure these parts fit together properly, we recommend that your vials and inserts be purchased from the same manufacturer.

Sigma-Aldrich offers a variety of compatible vial and insert products. For help with product selection, email Sigma-Aldrich Technical Service at EurTechServ@sial.com or visit our website at sigma-aldrich.com/supelco

Table 1 Differing Specifications for US & European Vials & Inserts

	US Manufacturer		EU Manufacturer	
	Vial I.D.	Insert O.D.	Vial I.D.	Insert O.D.
Standard I.D. (mm)	5.04–5.06	4.58–4.68	5.18–5.22	4.95–5.00
Large I.D. (mm)	5.94–6.08	5.67–5.77	6.18–6.21	5.98–6.00

Compatible Vials & Inserts from US and European Manufacturers

Description	Pk. Size	Cat. No.
US Crimp Neck Vial, Large Opening, 2 mL, 12 x 32 mL		
Clear glass	100	27058
	1000	27059
US Glass inserts		
0.2 mL, 6 mm x 29 mm with bottom spring	100	24721
0.25 mL, 6 x 31 mm	100	24717
European Crimp Neck Vial, Large Opening, 1.5 mL, 11.6 x 32 mm		
Clear glass	100	SU860055
	1000	854964
European Glass inserts		
0.1 mL, 5.7 x 29 mm with bottom spring	100	SU860066
0.1 mL, 6 x 31 mm	100	SU860067

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