

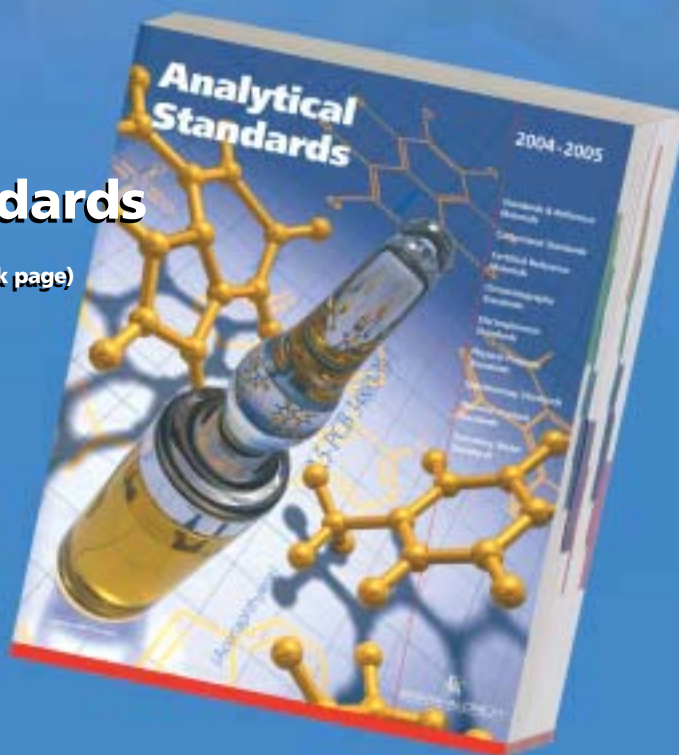
the Reporter

FOR EUROPE MAGAZINE

Volume 11 May 2004 International issue

SUPELCO

The NEW Standards Catalogue (See back page)



Discovery Bio HPLC Columns

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Introducing Discovery DSC-MCAX

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The Alumina Chloride PLOT for the Analysis of Freons (See Page 19)



New Standards for Residue Analysis: Fluoroquinolones

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HPLC/LC

New Series: Discovery Column Selection by Separation Problem

SPME/SPE

The Importance of Ion-Exchange Capacity in Mixed-Mode SPE

GC

Achieve Consistent Results with Applications Containing Complex Hydrocarbon and Alcohol Mixtures

Standards & Reagents

New Standards for Residue Analysis: Fluoroquinolones


SIGMA-ALDRICH

Editorial

Your One-Stop Supplier in Chromatography

Dear Chromatographer,

The challenges posed by HPLC are becoming more and more demanding and analysts need the right application solutions for classical HPLC as well as for LC-MS.

A typical C18 phase, traditionally the chromatographer's best friend, is sometimes not the ideal choice for an application or is only suitable/applicable with tremendous efforts e.g. with regard to the composition and gradient of the mobile phase: complex solvent mixes and addition of ion pair reagents.

Generally chromatographers will try everything to get a good analysis on a C18 column; but the method development takes longer and the methods are often less robust and reproducible. The selectivity is simply not the right one for the separation problem. To attain a better performance a significant alteration in the selectivity of the phase is appropriate. The switch from one brand's C18 column to another brand's C18 rarely provides the selectivity difference needed to get a significant change in the separation/elution.


The full range of Discovery™ Columns

More suitable, alternative selectivities can provide an easier and quicker method development that leads to more reliable, simpler and robust methods that can also be efficiently transferred to other labs if needed. New phases like the

Discovery™ line of columns offer a broad range of selectivities for small molecules like drug substances and for large molecules such as proteins and peptides. Choosing the right selectivity from a range of columns based on silica, zirconia and polymer can be the key to simpler method development and a more robust application. Therefore it is worth considering an alternative selectivity into consideration early in the method development cycle to save time and effort.

Solvents and Buffers for LC-MS

With the growing popularity and use of LC-MS there is an increasing demand for reliable low bleed columns and suitable chemicals. By choosing a virtually no bleed column in conjunction with high purity mobile phase solvents and ingredients the background noise in chromatograms and mass spectra can be minimised. Using Discovery C18 or Bio Wide Pore C18 columns in conjunction with Fluka/RdH LC-MS solvents and buffers will give a more hassle free analysis with less background. These form the right LC-MS application solution for today's demanding needs.



Klaus Buckendahl
Sales Development Manager Liquid Chromatography Europe



Meet Supelco

27th International Symposium on Capillary Chromatography

Palazzo dei Congressi – Riva del Garda - Italy. May 31 – June 4, 2004

The Symposium will cover a variety of techniques:

- Capillary Gas Chromatography
- Micro Liquid Chromatography
- Electromigration Methods
- Microfabricated Analytical Systems – Lab-on-a-chip
- Column Technology
- Coupled and Multidimensional Techniques
- Comprehensive Techniques
- Sampling Systems
- Sample Preparation
- Trace Analysis
- Automation

The Symposium will cover applications such as:

- Environmental Applications
- Energy, Petrochemistry and Industrial Applications
- Biomedical and Pharmaceutical Applications
- Analysis of Natural Products, Food, Flavours and Fragrances

For further Information and Updates, please consult the website www.richrom.com



New Series: Discovery Column Selection by Separation Problem

Starting with this new series in the Reporter, Supelco presents guidelines for narrowing down the candidate columns based on your separation problem or challenge. Not only does Discovery help you develop the best HPLC methods, it will also solve common HPLC problems. The majority of HPLC separation problems fall into two categories:

Peak Shape and / or Efficiency Related Problems

Discovery high-quality particle and bonded phase technology improve efficiency by eliminating unwanted secondary interactions. Removing these secondary interactions also removes sources of variation, making separations developed on Discovery columns reproducible column to column and lot to lot.

Retention and / or Selectivity Related Problems

The Discovery functionalised reversed-phases have different, unique bonded phase chemistries. Analyte molecules have different affinities to the different bonded phases and interact with them to differing degrees. An increase in affinity toward the bonded phase relative to the mobile phase increases retention, while a decrease in affinity decreases retention. Discovery functionalised reversed phases can be more sensitive to differences between analyte molecules than a C18, and can therefore distinguish between them and give greater resolution.

Separation Problems Addressed by Discovery Columns

The following pages show examples of how Discovery columns can solve the most common HPLC separation problems. These are representative and are intended for guidance, the solution may be a different Discovery phase than we've presented.

See How Discovery Can Solve These Common HPLC Problems

Use these Problem Solution Guidelines along with the Column Screening Data to choose the right Discovery phase to meet your separation criteria.

1. Poor retention or not enough retention of polar compounds
need to eliminate ion-pair additives
2. Too much and too little retention on the same run
3. Too much resolution or wasted space in the chromatogram
4. Poor resolution of closely-eluting compounds
5. Switching of critical peak pair
6. Broad or tailing peaks, small peaks elute in tail of larger peak
7. Lengthy analysis time

Choosing a Discovery Phase Discovery Solves HPLC Problems

Problem 1: Poor Retention of Polar Compounds How does Discovery solve this problem?

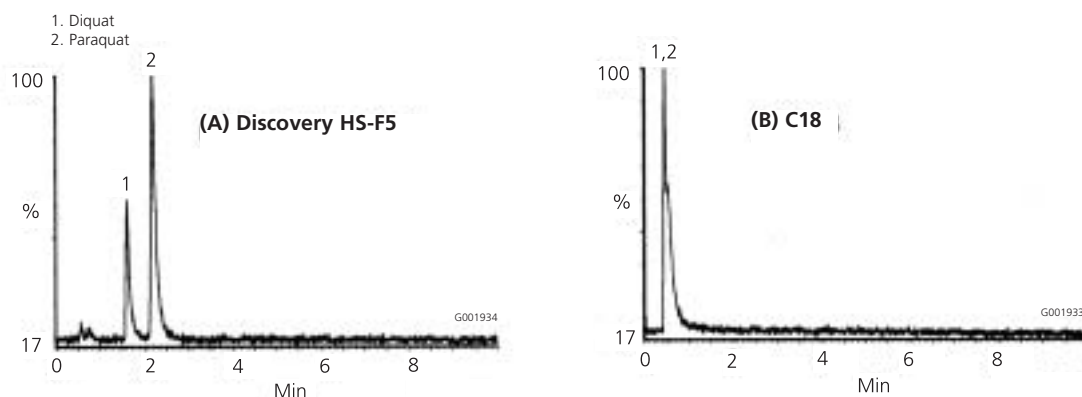
The different phase chemistries of the Discovery family give enhanced retention of polar compounds compared to a C18. By using one of the functionalised reversed-phases, you can obtain a different separation based on unique combinations of polar and hydrophobic retention. Note that there are many paths to take, and different Discovery phases to choose that will solve a problem. We have just highlighted a few examples.

Demonstration 1: Enhanced retention of polar quaternary amines on Discovery HS F5

As shown in Figure 1, quaternary amines are not well retained on C18 without ion pairing. By changing the stationary phase to the Discovery HS F5 column, adequate retention and peak shape were obtained. Note that this separation is done with volatile, mass spec friendly mobile phases and no ion-pair reagents are used. The separation was done on a 5cm x 2.1mm ID column packed with 3µm Discovery HS F5 particles; ideal for LC/MS work.

Figure 1. Longer Retention of Polar Quaternary Amines on Discovery HS F5

Column: (A) Discovery HS F5 or (B) C18, 5cm x 2.1mm ID, 3µm particles
Mobile Phase: 95:5, 5mM Ammonium Formate (pH 3.5 with Formic Acid); MeOH
Flow Rate: 0.3ml/min
Det.: MS, (+) ESI mode
Temp.: 60°C
Inj.: 3µl, 50µg/ml each compound in mobile phase



Your Problem Solving Partner in Chromatography

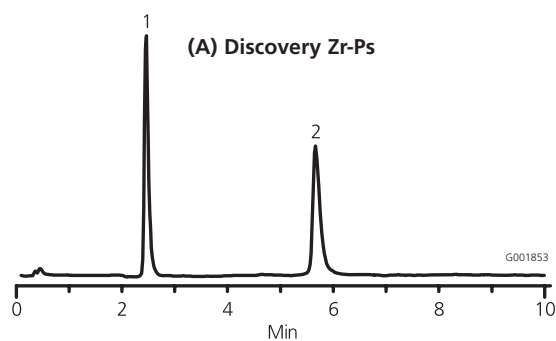
Demonstration 2: Enhanced retention of polar quaternary amines on Discovery Zr-PS

As shown in Figure 2, there are often multiple Discovery solutions to an HPLC problem. Discovery Zr-PS gives another example of enhanced quaternary amine retention compared to a C18. Here, natural ionic interactions from the Zr-PS particles enhance retention.

Figure 2. Paraquat and Diquat on Discovery Zr-PS vs C18

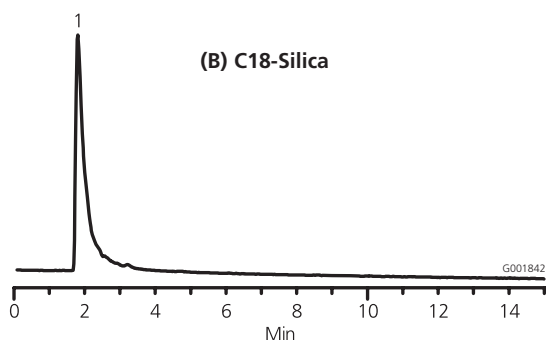
Column: Discovery Zr-PS, 7.5cm x 4.6mm ID, 3µm particles (65741-U)
 Mobile Phase: Mobile Phase: 25mM H₃PO₄, 25mM NH₄F (pH 8.0 with NH₄OH): CH₃CN, (50:50)
 Flow Rate: 3ml/min (2610psi)
 Temp.: 65°C
 Det.: 290nm
 Concentration: 50µg/ml each
 Inj.: 10µl

1. Paraquat
 2. Diquat



Column: C18-silica, 15cm x 4.6mm ID, 3µm particles
 Mobile Phase: 25mM H₃PO₄ (pH 7.0 with NH₄OH): CH₃CN, (95:5)
 Flow Rate: 1ml/min (2590psi)
 Temp.: 35°C
 Det.: 290nm
 Concentration: 50µg/ml each
 Inj.: 10µl

1. Paraquat and Diquat

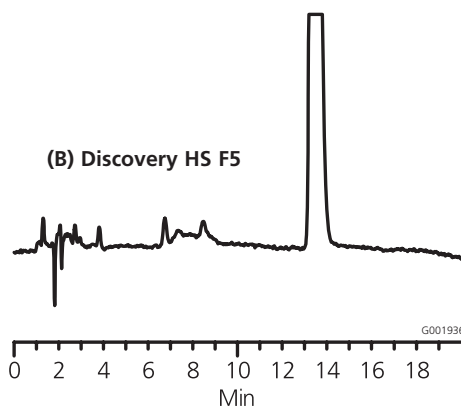
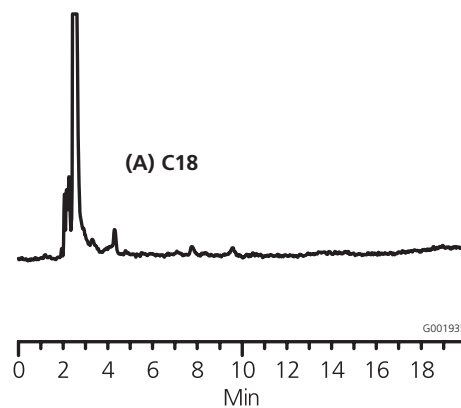


Demonstration 3: Poor retention of polar degradation products.

This example of changing the stationary phase to enhance retention shows the anti-hypertensive compound pindolol that has been degraded with UV light for 62 hours. Figure 3 shows that a C18 column gave poor retention of the parent compound. It was not able to re-solve early-eluting degradants from the parent compound. In contrast, Discovery HS F5 gave adequate retention of pindolol and resolved many more degradants that eluted prior to the parent peak.

Figure 3. Discovery HS F5 Gives Enhanced Retention of Pindolol and Degradation Products

Column: (A) C18 or (B) Discovery HS F5, 15cm x 4.6mm ID, 5µm particles
 Mobile Phase: 50:25:25, 10mM Potassium Phosphate (pH 6.8):CH₃CN: MeOH
 Flow Rate: 1ml/min
 Temp.: 35°C
 Det.: UV at 235nm
 Inj.: 5µl, UV-degraded Pindolol, 200µg/ml in 90:10, Water: MeOH

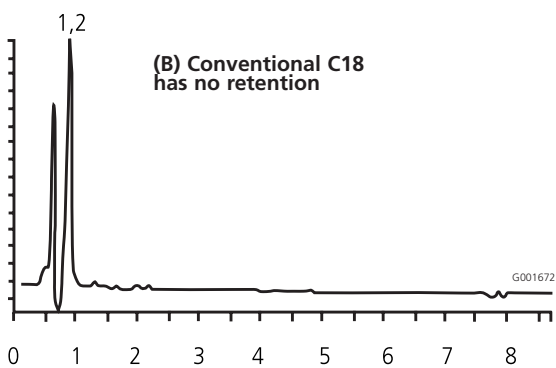
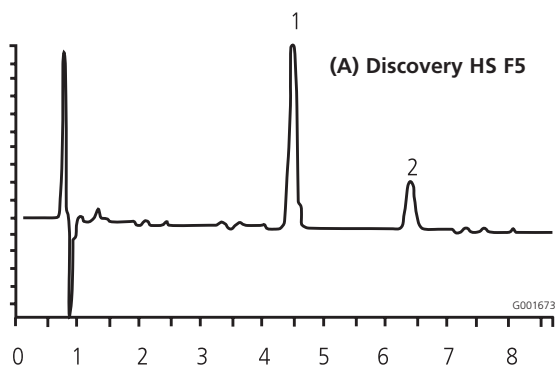


Demonstration 4: Poor retention of polar amines.

This example shows how changing the stationary phase from a standard C18 to a Discovery HS F5 column can enhance retention (Figure 4). Methcathinone, a psychoactive designer drug, is synthesised in clandestine labs by oxidation of ephedrine. Analysis and absolute identification are critical in criminal proceedings. A C18 column did not give adequate retention, even after much mobile phase manipulation. However, Discovery HS F5 gave adequate enhanced retention. Note also the high organic in the mobile phase for better desolvation in the MS.

Figure 4. Discovery HS F5 Provides Excellent Separation-Solutes Are Not Retained on C18

Column: (A) Discovery HS F5 or (B) conventional C18, 15cm x 4.6mm ID, 5µm particles
 Mobile Phase: 30:70, 10mM Ammonium Acetate (pH 6.98): CH₃ CN
 Flow Rate: 2.0ml/min
 Temp.: 35°C
 Det.: Photodiode Array
 Inj.: 5µl
 1. Methcathinone (100µg/ml)
 2. (+/-) Ephedrine (200µg/ml)



Demonstration 5: Poor retention of polar antibiotic compounds.

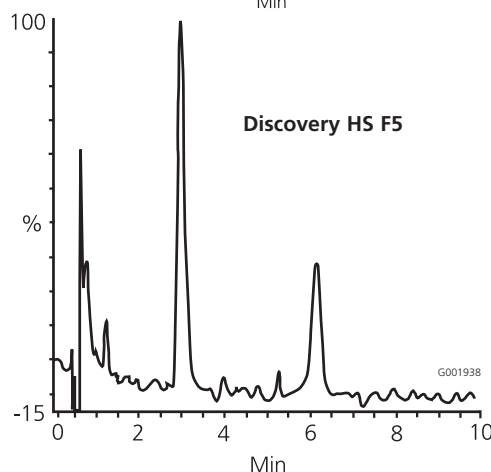
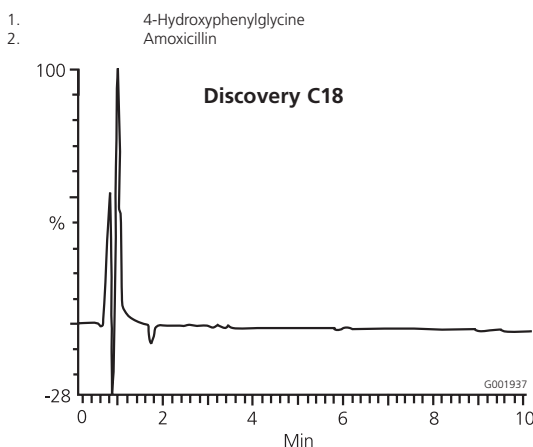
In this example, changing the stationary phase once again enhanced retention over a C18, this time for amoxicillin and an impurity. 4-Hydroxyphenylglycine is a common impurity of amoxicillin. Neither compound is retained by a C18 column. Both elute at the void volume. Conversely, on the Discovery HS F5, both compounds are retained and resolved, allowing reliable quantitation and purity profiling.

Conclusion

These examples show that if there is a problem with poor retention of polar compounds on a C18, a change in the stationary phase will likely give you enhanced retention and different selectivity.

Figure 5. Poor retention of polar Discovery HS F5 Gives Enhanced Retention of Antibiotic Compounds

Column: (A) C18 or (B) Discovery HS F5, 5cm x 4.6mm ID, 5µm particles
 Mobile Phase: 20:80, 0.1% Formic Acid in Water: MeOH
 Flow Rate: 1ml/min
 Temp.: 35°C
 Det.: UV photodiode array and MS
 Inj.: 10µl, each compound 50µg/ml in 0.1% formic acid



OFFER

FREE Trial of Discovery HS F5 see offer on page 7

Quote Promotion Code W90 to qualify for this offer.
 Offer valid until 31 May 2004

i Information Request.....1101

HPLC Article

Isocratic Elution of Analytes Differing Significantly in Polarity on Discovery® HS F5

Dave Bell dbell@sial.com

Role of Stationary Phase Chemistry Toward Retention

Retention and selectivity observed in chromatographic processes are the manifestation of the multiple types of interactions between the analytes and the stationary phase. Traditional alkyl phases such as C18 are designed to operate primarily by hydrophobic interactions and exhibit minimal polar interactions. These properties often provide for predictable method development and smooth operation, however, in many cases analytes that vary significantly in polarity require gradients to perform the analysis efficiently. Although gradient elution is acceptable practice for some applications, isocratic solutions are still favoured.

Polar reversed phase columns, by design, impart polar interactions of retention as well as the hydrophobic interactions available on traditional C18 phases. The additional polar interactions can be utilised to alter the selectivity for a given set of analytes. In this report, such manipulation of the polar and hydrophobic interactions provided by a pentafluorophenylpropyl stationary phase (Discovery HS F5) is demonstrated using the antiparkinsonian drug, selegiline, and two of its polar metabolites.

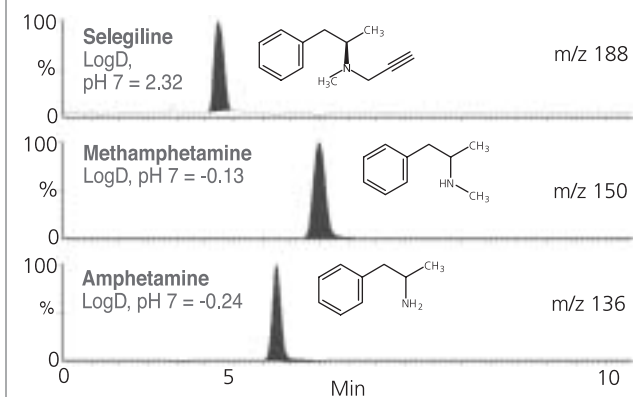
Retention and Separation on Discovery HS F5

Selegiline and two of its metabolites (amphetamine and methamphetamine) vary significantly in polarity (Figure A). Using alkyl stationary phase chemistries, low percentages of organic solvents are required to retain the polar amphetamines. Under the same conditions the relatively non polar selegiline solute is highly retained resulting in an overall run time of about 30 minutes.

Due to the polar interactions available on the Discovery HS F5 phase, the amphetamines can be retained using greater percentages of organic modifier. Under these conditions, selegiline and the amphetamine analytes elute in less than 5 minutes as shown in Figure A.

Figure A. Retention of Selegiline and Polar Metabolites on Discovery HS F5

Column: Discovery HS F5, 5cm x 4.6mm, 5µm particles
Cat. No.: 567513-U
Mobile Phase: 30:70, 10mM ammonium acetate (pH 4.0 w/ acetic acid): CH₃ CN
Flow Rate: 1ml/min
Temp.: 35°C
Det.: MS, ESI (+)
Inj.: 5µl
Sample: 10µg/ml each component in CH₃ OH



Conclusion

The availability of polar interactions provided by modern stationary phases such as the Discovery HS F5 provides new opportunities for optimal HPLC method development. In those cases where C18 phases provide too much resolution or inadequate retention of polar analytes, polar reversed-phases are likely to provide superior performance.

Related Products	Supplier	Cat. No.
Selegiline, R-(—)-Deprenyl hydrochloride	(Sigma)	M-003
Amphetamine	(Sigma)	A-2262
Methamphetamine	(Sigma)	M-5260
Ammonium Acetate, ReagentPlus™	(Aldrich)	43,131
Acetic Acid, glacial	(Aldrich)	33,882-6
Acetonitrile, LC-MS CHROMASOLV	(Riedel-de Haën)	34967
Methanol, HPLC Grade	(Aldrich)	27,047-4

For a complete listing of all Sigma-Aldrich products, log on to our website: sigma-aldrich.com

Did you know?

Due to the additional interactions available using polar reversed phase columns such as Discovery HS F5, the choice and concentration of buffer salts is critical to adequately control retention and separation compared to alkyl phases. For instance, the use of ammonium formate versus formic acid often results in improved peak shape and selectivity.

Ordering information

Prod. No.	ID (mm)	Length (mm)
Discovery HS F5		
3µm Particles		
567500-U	2.1	5.0
567502-U	2.1	10.0
567503-U	2.1	15.0
567504-U	4.6	5.0
567506-U	4.6	10.0
567507-U	4.6	15.0
5µm Particles		
567508-U	2.1	5.0
567510-U	2.1	10.0
567511-U	2.1	15.0
567512-U	2.1	25.0
567513-U	4.6	5.0
567515-U	4.6	10.0
567516-U	4.6	15.0
567517-U	4.6	25.0
NEW LC-MS Dimension! Discovery F5-3µm 3.3cm x 2.1mm		
567501-U	2.1	33

Information Request..... 1101

Discovery HS F5 Separates What C18 Cannot.



Discovery HS F5 Delivers

- Increased Retention vs. C18
- Unique Selectivity vs. C18
- Improved Resolution vs. C18

i Information Request.....1101

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25% OFF any Analytical
Discovery HS F5 HPLC column

Quote Promotion Code W91 to qualify for this offer.

Offer valid until 31st May 2004

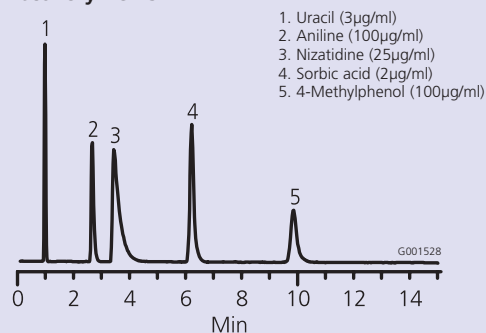
A Novel Fluorinated Reversed-Phase

Discovery HS F5 shows unique retention and selectivity compared to conventional C18 phases.

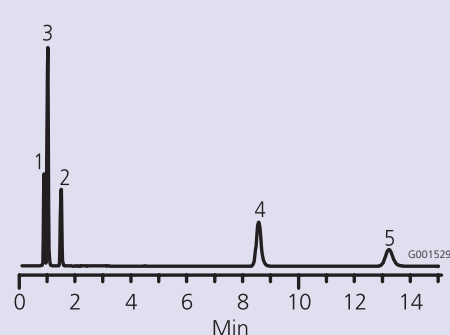
Note the elution reversal and the greater retention of aniline and nizatidine (peaks 2 and 3) on the F5 phase.

Columns: Discovery HS F5 and Conventional C18, 15cm x 4.6mm ID, 5µm;
Mobile Phase: 85% 10mM ammonium formate, 0.1% formic acid, pH 3.3:15% MeCN;
Flow Rate: 2ml/min; Temp.: 35°C; Detection: 254nm; Injection: 10µl.

Discovery HS F5



Conventional C18 Phase



Particle Properties and Efficiency in Ion-Exchange

Ion-exchange HPLC resolves compounds based on charge or charge distribution. It is widely used for separating proteins and peptides. Ideally, the column should be efficient and selective enough to resolve proteins with slight charge differences, such as protein isoforms and posttranslationally modified proteins. While selectivity can be adjusted by using different surface chemistries and/or mobile phase conditions, efficiency is related more to the particle structure, particle size and size distribution, and the physical arrangement of the particles in the packed column. Separations achieved using polymethacrylate-based ion-exchange columns give good recovery, high loading capacity, and outstanding chemical stability in the pH range 1-12. However, polymer-based materials often exhibit lower efficiency than their silica-based counterparts. Supelco has recently introduced a new polymethacrylate based anion-exchange column: Discovery BIO PolyMA-WAX. It features a diethylaminoethyl (DEAE) functional group, and complements the Discovery BIO PolyMA-SCX cation-exchange column. Both columns are developed based on latest particle technology aimed at improving column efficiency while maintaining selectivity, capacity, and recovery.

Competitive Efficiency of Discovery BIO PolyMA-WAX

The efficiency of Discovery BIO PolyMA-WAX was evaluated using a mixture of three proteins, aprotinin, apotransferrin, and hexokinase, at pH 8 under salt gradient conditions. Nine competitive, polymer-based anion-exchange columns (both Q-type and DEAE functional groups) from five leading manufacturers were compared under the same conditions. The ratio of peak height to peak width at half height was compared. Flow rate was adjusted to ensure constant linear velocity since column IDs varied. The gradient volume relative

to the column volume was also kept constant for fair comparison. Figure A presents the results of Discovery BIO PolyMA-WAX and the nine other columns. As the figure demonstrates, Discovery BIO PolyMA-WAX gave the highest efficiency for the three representative proteins tested. One of the main contributors to the higher efficiency is the smaller diameter of the Discovery BIO PolyMA particles. High efficiency means better sensitivity and resolution, and ultimately more peaks per unit time.

Efficiency of Discovery BIO PolyMA-WAX is demonstrated in the separation of hemoglobin variants and shown in Figure B. Hemoglobin (Hb) is the oxygen transporter in erythrocytes. It consists of four polypeptide subunits. Hemoglobin A0 is the principal hemoglobin, and A2 is minor hemoglobin (approximately 2% of the total hemoglobin) in adults. Hemoglobin S is hemoglobin found in patients with genetically transmitted hemolytic disease. Hemoglobin A0 and S differ in only one amino acid: Hb S contains valine instead of glutamate at position 6 of the b (beta) subunit. While all three variants share the same a (alpha) subunit, Hb A2 has a d (gamma) subunit instead of the b (beta) subunit of the Hb A0 and S. Figure B shows the separation of hemoglobins A2, S, and A0 on Discovery BIO PolyMA-WAX. Discovery BIO PolyMA-WAX is not only able to well resolve the three isoforms of hemoglobin, but also small impurities carried over by the sample. In comparison, the resolution on a popular brand name column is not as good due to its lower column efficiency.

Conclusion

Small particle size, narrow particle size distribution and uniform arrangement of particles provide the high efficiency observed on the Discovery BIO PolyMA-WAX column. The efficiency permits the column to separate proteins with slight differences in structure, and it has the ability to resolve complex protein mixtures.

Figure A. Column Efficiency of Various Proteins on Discovery BIO PolyMA-WAX vs. Competitive Polymer-Based Anion-Exchange Columns

Columns:	Discovery BIO PolyMA-WAX (polymethacrylate polymer, DEAE functional group), 5cm x 4.6mm ID, 5µm particles (59602-U); competitive columns of similar dimensions (Q = quaternary amine; DEAE = diethylaminoethyl functional groups). Competitive columns contain polymethacrylate particles unless noted otherwise.		
Mobile Phase:	(A) 50mM Tris-HCl (pH 8.0); (B) 50mM Tris-HCl, 0.5M NaCl (pH 8.0)		
Flow Rate:	2.41cm/min (linear velocity kept constant to compare columns of different ID)		
Temp.:	35°C		
Det.:	UV 280nm		
Gradient:	Column Volumes	%A	%B
	0	100	100
	2	100	0
	3	80	20
	15	40	60
	16	0	100

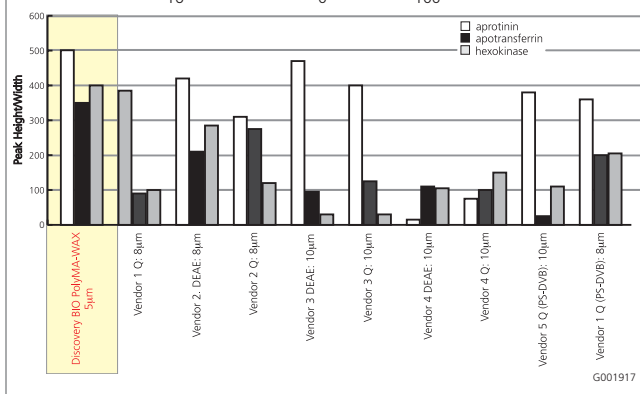
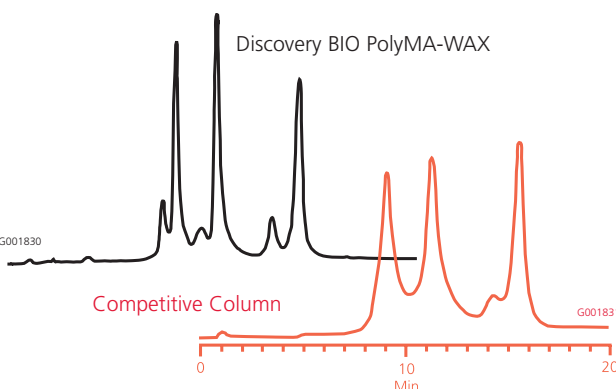


Figure B. Separation of Hemoglobin Variants on Discovery BIO PolyMA-WAX vs. Competitive Column

Columns:	Discovery BIO PolyMA-WAX (DEAE functional group), 5cm x 4.6mm ID, 5µm particles (59602-U); competitive DEAE column, 5cm x 5mm ID, 5µm particles		
Mobile Phase:	(A) 10mM Tris-HOAc (pH 8.0); (B) 10mM Tris-HOAc, 0.25M KCl (pH 8.0)		
Flow Rate:	0.5ml/min (Discovery column), 0.59ml/min (competitive column)		
Temp.:	35°C		
Det.:	UV 280nm		
Inj.:	5µg each compound		
Sample:	1. Hemoglobin A ₂	2. Hemoglobin S	3. Hemoglobin A ₀



NEW Discovery BIO HPLC Columns

Maximize the sensitivity
of your HPLC method
with longer column lifetime
and trouble-free operation.

Meeting the Challenges of Protein and Peptide Separations

Discovery BIO Wide Pore C18, C8, and C5 - Reversed-phase columns

- High efficiency for high sensitivity protein and peptide separations
- Exceptional column lifetime and reproducibility for reliable operation
- Ideal for LC-MS: bleed-free, capillary and microbore dimensions, excellent peak shape without TFA
- Scalable from capillary to preparative dimensions

Discovery BIO PolyMA-SCX and PolyMA-WAX - Ion-exchange columns

- High efficiency for high sensitivity protein and peptide separations
- Excellent protein recovery, higher than competitive phases
- Reliable high and low pH operation

Ordering information

Prod. No.	ID (mm)	Length (cm)
Discovery C18 5µm Particles		
50494721	2.1	5.0
569220-U	2.1	10.0
569229-U	2.1	12.5
50495521	2.1	15.0
504947-30	3.0	5.0
569221-U	3.0	10.0
569230-U	3.0	12.5
504955-30	3.0	15.0
504971-30	3.0	25.0
504947-40	4.0	5.0
569222-U	4.0	10.0
569231-U	4.0	12.5
504955-40	4.0	15.0
504971-40	4.0	25.0
504947	4.6	5.0
569223-U	4.6	10.0
569232-U	4.6	12.5
504955	4.6	15.0
504971	4.6	25.0
569224-U	10.0	25.0
569226-U	21.2	25.0

OFFER

35% OFF Discovery Bio
HPLC Columns

Quote Promotion Code W87 to qualify for this offer.
Offer valid until 31 May 2004

HPLC Article

Automated Column Switching Facilitates Method Development

SupelPRO™ Automated Fluidics Instruments Complement Method Development on Discovery HPLC Columns

Supelco's SupelPRO series are precision, electronically controlled, motorised valve instruments for repetitive fluid switching operations. Each SupelPRO instrument is self contained and incorporates a 2-position or multi-position port valve. Standard multi-position models include a 4-line BCD (binary coded decimal) port, and the 2-position models include the Level Logic (type of electrical signal). Power requirements: 100-240VAC, 50-60Hz (auto switching). All units shipped with standard US power cord. Other power cords are available on a custom basis.

All SupelPRO units are CE approved. SupelPRO 3-Column or 6-Column Selector

Select from among up to 3 columns or up to 6 columns. Useful for column selectivity comparisons, other column selection applications. Includes mounting clips and cover.

SupelPRO 2-Channel Selector with Bypass Valve

This 6-port, 3-position motorised valve is useful for selecting 1 of 2 connected columns, or flushing.

SupelPRO 11-Port, 10-Position Valve

Use this 11-port, 10-position valve to select from up to 10 inputs to 1 output, or select 10 outputs from 1 input.

SupelPRO 2-Position Valves

Available with 6 or 10 ports. Useful for a wide variety of applications, including sample clean up and back-flushing.

SupelPRO Solvent Selector Valve

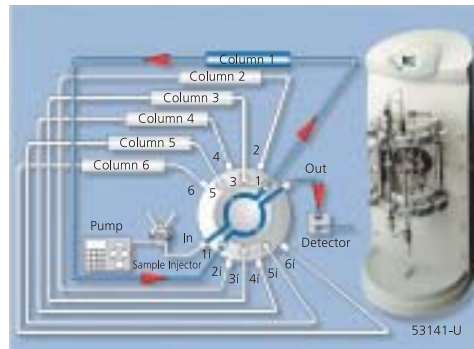
Allows automation from 6 inlets. Comes with factory installed 1/16" or 1/8" OD tubing and 1/4-28 fittings. Rated to 300psi (20 bar).

Ordering information

Description	Prod. No.
SupelPRO™ 3-Column or 6-Column Selector	
3-Column	
Stainless Steel	53140-U
PEEK	53142-U
6-Column	
Stainless Steel	53141-U
PEEK	53143-U
SupelPRO 2-Channel Selector with Bypass Valve	
Stainless Steel	53146-U
PEEK	53147-U
SupelPRO 11-Port, 10-Position Valve	
Stainless Steel	53152-U
PEEK	53153-U
SupelPRO 2-Position Valves	
6-Port	
Stainless Steel	53148-U
PEEK	53149-U
10-Port	
Stainless Steel	53150-U
PEEK	53151-U
SupelPRO Solvent Selector Valve	
1/16"	53144-U
1/8"	53145-U

TRADEMARKS: Discovery, Supelguard, SupelPRO-Sigma-Aldrich Co.

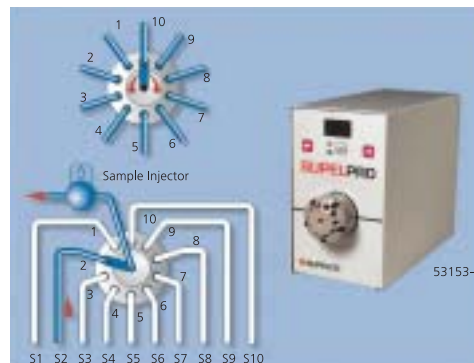
SupelPRO 3-Column or 6-Column Selector



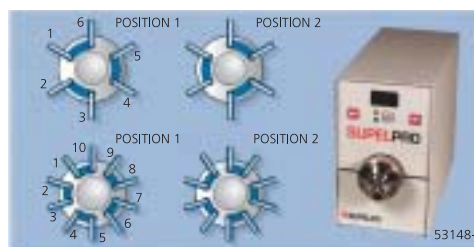
SupelPRO 2-Channel Selector with Bypass Valve



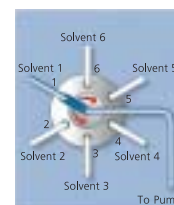
SupelPRO 11-Port, 10-Position Valve



SupelPRO 2-Position Valves



SupelPRO Solvent Selector Valve



SPE Article

The Importance of Ion-Exchange Capacity in Mixed-Mode SPE

Introduction

Most researchers who use SPE to process biological samples rely on traditional single-mode retention mechanisms to recover, clean up, and concentrate their compounds of interest prior to LC or GC analysis. Although single-mode SPE chemistries are often adequate, problems can easily arise. For example, reversed-phase chemistries (e.g., C18) can result in insufficient clean up. Although selectivity can be improved when using ion-exchange phases, the technology is often very sensitive to sample matrix effects. In this report, we will discuss the utility of mixed-mode SPE technology, and how ion-exchange capacity can play a critical role in many bio-analytical applications.

How Mixed-Mode SPE Works

Discovery DSC-MCAX (Mixed-Mode Cation Exchange) SPE utilises the dual retention mechanisms of both hydrophobic and electrostatic interaction to retain basic, acidic, neutral, and zwitterionic compounds from aqueous sample matrices such as biological fluids. Its broad affinity for such a wide range of compounds is due to the dual bondings (octyl, C8 & benzene sulphonic acid, SCX) contained within the packed-bed.

Although the technology was designed to drastically improve the selectivity/sample clean up of basic and zwitterionic compounds, DSC-MCAX is useful in other applications as well. Through the careful manipulation of pH and organic strength, the user can also fractionate basic and zwitterionic from acidic and neutral compounds, or adjust the protocol to permanently retain any unwanted basic and zwitterionic interfering components while eluting acidic and neutral compounds of interest. Table 1 summarises the generic protocol recommended for DSC-MCAX use.

Table 1. Recommended Generic Protocol for Discovery DSC-MCAX

1. Condition and equilibrate with methanol and 10mM potassium phosphate, pH 3-6
2. Load Sample
3. Wash off hydrophilic compounds/interferences with 10mM potassium phosphate, pH 3-6; and/or 1M acetic acid
4. Wash off hydrophobic compounds/interferences with methanol
5. Elute basic/zwitterionic compounds with 5% ammonium hydroxide in methanol

The Importance of Ion-Exchange Capacity

Most bioanalytical applications provide analytical support for drug metabolism/ pharmacokinetic studies. In some cases, parent drug compounds can metabolise into very polar compounds that are difficult to retain via reversed-phase SPE. As a result, two different retention mechanisms are required to retain both parent drug and metabolite(s).

In this application, piroxicam and piroxicam's highly polar metabolite, 2-aminopyridine, were spiked in human urine. Spiked samples were then extracted via Discovery DSC-MCAX using the recommended generic protocol, and analysed via HPLC-UV. The results were then compared against two competitor mixed cation phases run in parallel. Using the DSC-MCAX provided excellent clean up of biological samples such as urine (Figure A). Although greater than 80% recovery was observed for piroxicam on all three mixed-cation phases, recovery suffered for 2-aminopyridine on the two competitor phases. Unlike piroxicam, 2-aminopyridine's polar

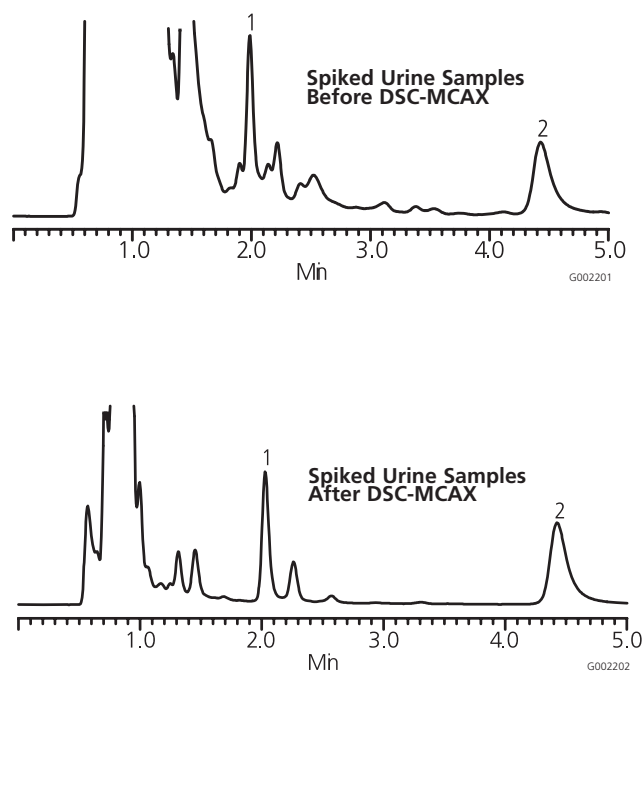
nature required cation exchange to be the dominant mode of retention. Discovery DSC-MCAX's high ion-exchange capacity allowed for excellent retention of both piroxicam and its polar metabolite 2-aminopyridine.

Conclusion

The dual retention properties of Discovery DSC-MCAX provide a broad affinity for a wide range of compounds. By controlling pH and organic strength, the technology offers superior clean up and selectivity relative to most single-mode SPE phases. DSC-MCAX's high ion-exchange capacity also allows for the selective recovery of polar basic compounds.

Figure A. Competitor Comparison of 2-Aminopyridine and Piroxicam from Human Urine

SPE Tube:	Discovery DSC-MCAX, 100mg/3ml
Cat. No.:	52783-U
HPLC Column:	Discovery HS F5, 15cm x 4.6mm, 5µm particle size
Cat. No.:	567516-U
Mobile Phase:	10mM potassium phosphate, pH 6: MeCN (85:15)
Flow Rate:	2ml/min
Temp.:	25°C
Det.:	UV, 220nm
Inj.:	Vol.: 10µl
1.	2-Aminopyridine (4µg/ml spike)
2.	Piroxicam (10µg/ml spike)



Efficiency of Absolute Recovery (n=4)	% Recovery \pm RSD	
	2-Aminopyridine	Piroxicam
Discovery DSC-MCAX	102 \pm 3.5%	101 \pm 1.2%
Leading Competitor A	30 \pm 52.5%	98 \pm 3.2%
Leading Competitor B	36 \pm 24.2%	83 \pm 4.3%

Related Products	Cat. No.
2-Aminopyridine (Aldrich)	A2880
Piroxicam	P5654
Potassium Phosphate, Monobasic	P0662
Acetonitrile, HPLC grade	27,071-7

Did you know...?

Strict pH control is critical when conducting ion-exchange SPE (e.g., mixed-mode, strong cation, weak anion, etc.). For example, when dealing with basic compounds, reducing the sample pH during loading will ionize the compounds' basic functional groups. When both sorbent and compound functional groups are in their ionized state, electrostatic interaction (compound retention) can occur. Conversely, increasing the pH during elution will neutralise the basic compounds' functional groups disrupting the compound and sorbent's electrostatic interaction.

Trademarks

Freon, Teflon - E.I. du Pont de Nemours & Co., Inc. Carboxen, CHROMASOLV, Discovery, Fluorcol, OMI, Thermogreen - Sigma-Aldrich Co. SCOTTY - Scott Specialty Gases, Inc.

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96-Well Starter Kit and PlatePrep Vacuum Manifold

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Starter Kit Includes:

- PlatePrep Vacuum Manifold
- 1 96 Sq. Well Collection Plate, 2ml, PP
- 2 Disposable Reservoir/Waste Trays, PVC
- 1 96 Sq. Well Piercable Cap Mat
- 5 Reagent Reservoirs
- 1 Cluster Tube Rack

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P000845

Ordering information	
Description	Prod. No.
96-Well Starter Kit with PlatePrep Manifold	575650-U

Discovery DSC-MCAX

Superior Sample Clean-Up



Introducing Discovery DSC-MCAX

(Mixed-Mode Cation Exchange) SPE Products

Discovery DSC-MCAX SPE utilizes the dual retention mechanisms of both hydrophobic and electrostatic interaction to retain basic, acidic, neutral and zwitterionic compounds from aqueous sample matrices. The packed bed comprises of both octyl (C8) and benzene sulphonic acid (SCX) bondings to offer one of the most selective SPE systems available.

- Achieve superior selectivity/sample clean-up when isolating basic/zwitterionic compounds.
- Experience greater and more reproducible recoveries in bioanalysis applications.
- Fractionate basic/zwitterionic compounds from acidic and neutral compounds.

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FREE DSC-MCAX MultiPak
3x 100mg/1ml + 3x 300mg/3ml
2616-U

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Description	Qty/pk.	Prod No.
DSC-MCAX,	50mg/1ml	108 57281-U
DSC-MCAX,	100mg/1ml	108 57282-U
DSC-MCAX,	100mg/3ml	54 57283-U
DSC-MCAX,	300mg/3ml	54 57284-U
DSC-MCAX,	300mg/6ml	30 57286-U
DSC-MCAX,	1g/6ml	30 57288-U
DSC-MCAX, 96-Well	25mg/well	1ea. 575639-U
DSC-MCAX, 96-Well	50mg/well	1ea. 575640-U
DSC-MCAX, 96-Well	100mg/well	1ea. 575641-U

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SUPELCO

Sample Preparation Article

The "Dioxin Prep System": A Multi-Layer Silica Gel Column Connected to a Dual-Layer Reversible Carbon Column for Rapid Determinations of PCDD/Fs*

Introduction

Reference methods (Methods US EPA 23 or 1613 B; European method EN-1948) for the quantitative analysis of the seventeen toxic 2,3,7,8-PCDD/Fs involve successive clean-up steps on various chromatographic adsorbents (multi-layer silica, Florisil, alumina, activated carbon) which considerably increase the time needed for analysis. Recently, Supelco launched a new preparation kit for rapid clean-up of dioxin samples, called the "Dioxin Prep System" and composed of a multi-layer silica gel column and a dual-layer carbon cartridge connected in series. This system aims at shortening considerably sample preparation time while maintaining high accuracy for performing PCDD/Fs analysis. To date, it has mainly been applied in Japan for a range of environmental samples such as stack emissions, fly ashes and waste waters 1, 2, 3. Before the Dioxin Prep System became commercially available in the UK, Corus UK and Hall Analytical Laboratories were invited to evaluate it alongside their existing dioxin analytical procedure that is derived from US EPA method 23 and is UKAS accredited (ISO 17025). Results from this evaluation are summarised in this paper.

The dioxin Prep System

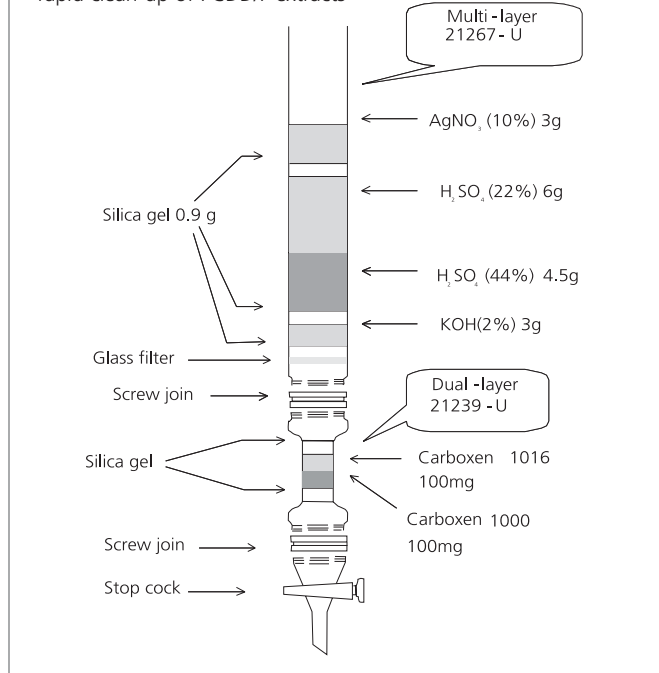
As shown in Fig. 1, the Supelco multi-layer silica gel column contained 7 layers of treated silica, which met the requirements of Japanese Industrial Standard Methods K-0311 and K-0312. The dual-layer carbon column was composed of two 100mg carbon layers, Carboxen 1016 (surface area 75 m²/g) and Carboxen 1000 (surface area 1200m²/g). Prior to clean-up, multi-layer silica gel and dual-layer carbon columns were pre-conditioned separately using 200ml of n-hexane, and 50ml toluene followed by 100 ml n-hexane, respectively. Extracts were applied to the Supelco combination columns and eluted

Figure 2. Picture of the Dioxin Prep System



with 200ml n-hexane. Multi-layer silica columns were disconnected and replaced by empty silica columns. Dual-layer carbon cartridges were then back-eluted using 80ml toluene to obtain PCDD/F fractions. Elution steps were all performed under vacuum (100-400mmHg; 3ml/min) using a vacuum manifold (Fig. 2: picture of Dioxin Prep System).

Figure 1. Schematic of the Supelco's Dioxin Prep System for rapid clean-up of PCDD/F extracts



Blanks and quality control (QC) materials were analysed both by Corus UK and Hall Analytical Laboratories to evaluate the efficiency of the clean-up using Supelco's Dioxin Prep System. Quantitation of 2, 3, 7, 8 substituted PCDD/Fs congeners was by isotope dilution using US EPA method 23 standard solutions. Samples were extracted by accelerated solvent extraction (Dionex ASE 200) using toluene. Analysis of cleaned-up extracts for PCDD/Fs was conducted by HRGC/HRMS using a 60m DB5-MS column and a Micromass Autospec Ultima high resolution mass spectrometer.

Results

The first step in the evaluation of the Dioxin Prep System consisted of analysing blank samples spiked with ¹³C¹²-labeled PCDD/Fs. After extraction and clean-up using the Dioxin Prep System, mean recoveries of ¹³C¹²-labeled internal standards ranged from 66 to 90% (Fig. 3), well within the acceptance criteria of the method US EPA 23. Relative standard deviation (RSD %; N=3) ranged from 10 to 20 %.

A QC material (waste dust from an iron making process) was analysed. Figure 4 shows the results obtained by Corus UK and Hall Analytical Laboratories using the Dioxin Prep System and

Figure 3. Recoveries of ¹³C₁₂-PCDD/Fs internal standards after analysis of blank samples cleaned-up using Supelco Dioxin Prep System

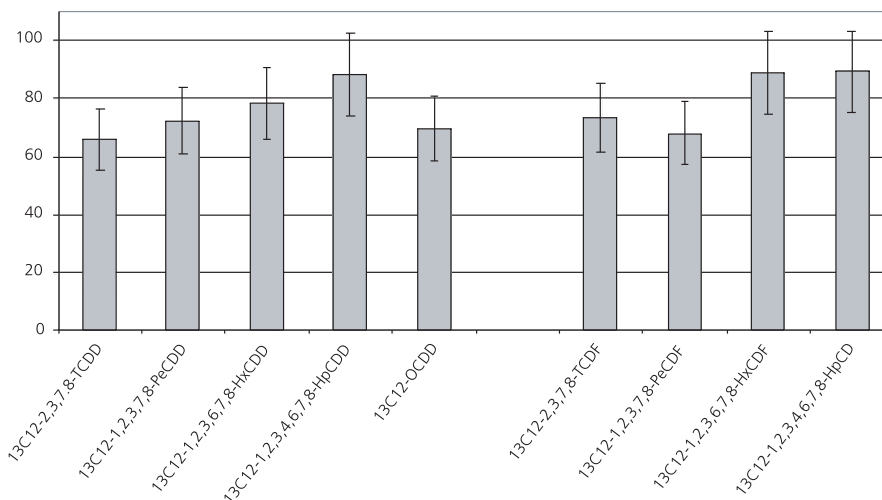
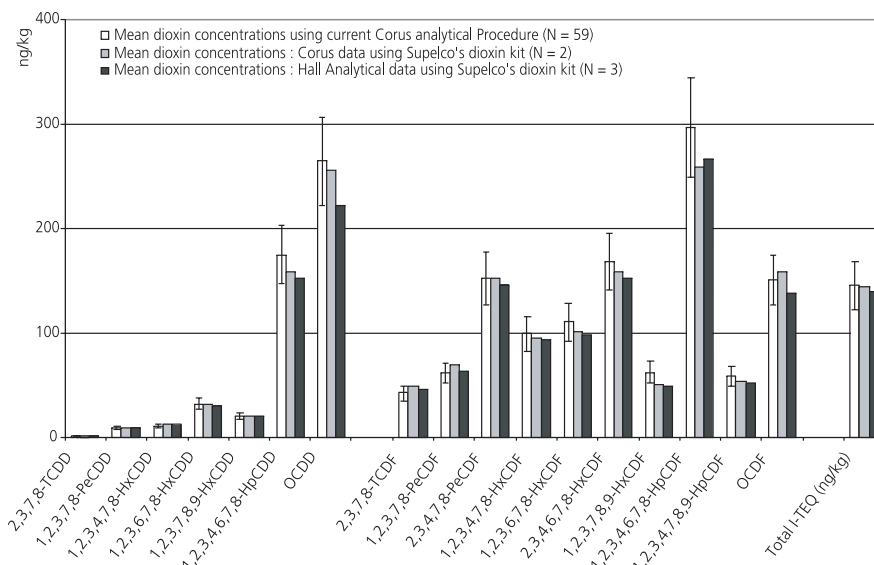


Figure 4. Analysis of a QC material by Corus UK and Hall Analytical Laboratories using the Supelco Dioxin Prep System. Dioxin results are compared with QC data from the replicate analysis of N = 59 samples using the existing Corus analytical procedure



compares it to the mean values of the QC data obtained using their current UKAS accredited procedure (N = 59 replicate analysis; RSD % ranged from 8 to 25%). Both laboratories obtained results well in agreement with the QC data showing the efficiency of the clean-up which enables accurate analysis of PCDD/Fs.

Conclusion

The Supelco Dioxin Prep System enabled determination of toxic PCDD/Fs for a complex environmental sample (waste dust from an iron-making process) with acceptable internal standards recoveries. The Dioxin Prep System provides the opportunity to perform a rapid clean-up step of environmental samples when compared with reference standard procedures.

*Eric Aries¹, David R. Anderson¹, Nicholas Ordsmith², Lisa Fitzpatrick² and Fiona Barclay²
 1 - Chorus UK, Rotherham 2 - Hall Analytical, Manchester 3 - Supelco UK

References

- Maeoka et al., 2001. Study on saving time for dioxin analysis based on JIS Method, 10th Symposium on Environmental Chemistry, p. 314-315.
- Matsumoto et al., 2000. Study on sample preparation for dioxins, 9th Symposium on Environmental Chemistry, p. 238-239.
- Matsumura et al., 2000. Simplifying sample preparation for dioxins, 8th Symposium on Environmental Chemistry, p.202-203.

Ordering information	
Description	Prod. No.
Dioxin Sample Preparation Kit	21296-U
PK5 Multi-layer Dioxin Tube	21267-U
PK5 Empty Dioxin Column	21222-U
44% H ₂ SO ₄ Coated Silica Gel, 100g	21334-U
10% Silver Nitrate Coated Silica Gel, 100g	21319-U
22% H ₂ SO ₄ Coated Silica Gel, 100g	21341-U
Stopcock for Multi-layer Dioxin tube	21268-U

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GC Article

Achieve Consistent Results with Applications Containing Complex Hydrocarbon and Alcohol Mixtures

Chemists faced with the challenge of developing a reliable method for the GC analysis of complex mixtures routinely contact Supelco as their problem-solving partner. One such analysis involves complex mixtures containing both non-polar hydrocarbons, as well as, polar alcohols. We discuss in this article the important factors you need to consider when developing and optimizing conditions for this type of application.

For any GC method to be successful, it must produce data of a known, desired quality that you can consistently reproduce. When developing such a method, the chromatographer must optimize conditions to produce the required resolution, retention, and analyte response. With complex hydrocarbon and alcohol mixtures, this can be time consuming. In today's fast paced laboratory, time is money, so you need proven performance and great technical advice early on in your method development.

Because of the repeated interest from our customers in this type of complex hydrocarbon and alcohol application, we developed a method using an Equity-1 column (30m, 0.53mm ID, 3.0µm film thickness, Cat. No. 28076-U). We recommend the Equity-1 as the column of choice when developing this type

of application due to the reproducible, consistent performance you will achieve with the improved Equity family of capillary GC columns. First, we optimized the conditions to produce the desired resolution, retention, and analyte response for a complex mixture of 32 hydrocarbons and alcohols. Figure A illustrates the optimized results on the first Equity column. The peak shapes of all the alcohols were excellent, indicating good column inertness. We achieved very good analyte response as well for both the hydrocarbons and alcohols. We started the GC oven temperature at 40°C in order to decrease the cycle time of the overall analysis. Despite the slightly higher starting temperature, the Equity-1 column was still able to separate 31 of the 32 compounds in this complex mixture. We resolved 3-methyl-1-butanol from 2-methyl-1-butanol (peak numbers 14 and 15), as well as, 3-methyl-3-pentanol from 4-methyl-2-pentanol and pentanol (peak numbers 16, 17, and 18.).

To demonstrate the consistent performance of the Equity columns, we then ran the same mixture under the identical optimized conditions on a second Equity-1 column. The second Equity-1 column is from another manufacturing batch, using a different polymer lot than the first column. Figure B illustrates the consistent performance we attained on the second Equity-1 column.

Figure A. Hydrocarbons and Alcohols on Equity-1, Column #1

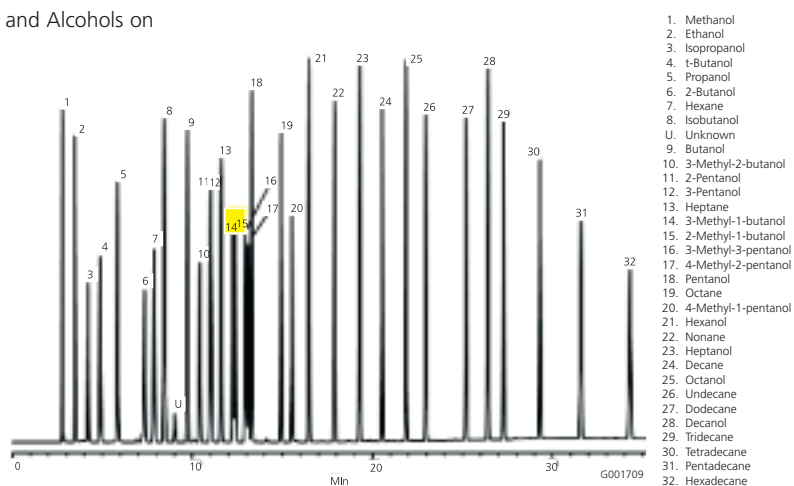
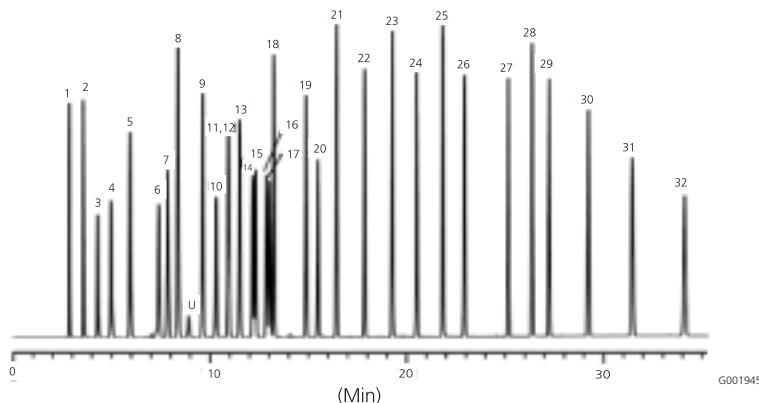
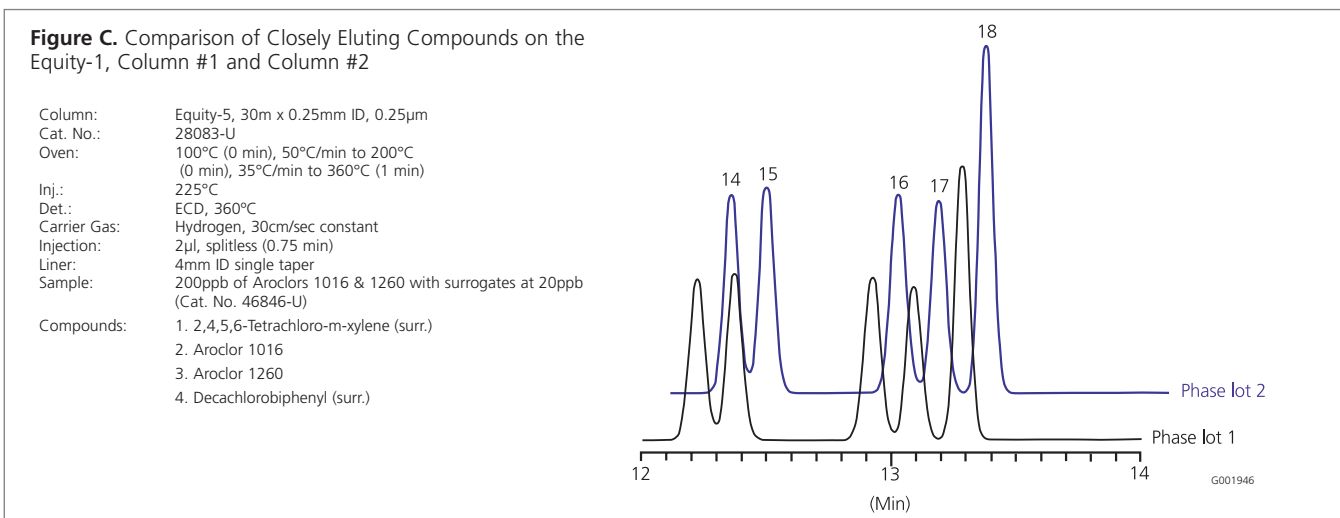


Figure B. Hydrocarbons and Alcohols on Equity-1, Column #2



We achieved very reproducible resolution on the second Equity-1 column. In fact, the separation pattern on the second column was virtually identical to the first, even for the most closely eluting peaks. See Figure C for a comparison of these critical separations

This data clearly illustrates the column-to-column reproducibility, across manufacturing batches and polymer lots, you can expect when using Equity columns. The new and improved columns will guarantee that the effort you spend to optimize your for key parameters such as resolution, retention,



As a check of retention, we compared the absolute retention times of each peak between the two columns (Table 1). The reproducibility of retention was excellent, with times matching within 3%. This made it easy to positively identify each peak on the second column. Finally, we compared the analyte response between the two columns by looking at the absolute area counts of each peak (Table 1). Area counts agreed within 5%, illustrating good reproducibility of analyte response between the two columns.

and analyte response, will be maintained when the time comes to replace the column or transfer the method.

Supelco delivers the consistent performance you demand with our new family of Equity capillary GC columns. We have developed an improved polymer that is very stable, uniform, and well characterised. The new manufacturing procedures for the Equity family are more stringent, resulting in superior product reproducibility. We verify the reproducibility and performance for each individual Equity column through improved testing procedures, which verify that the critical parameters of selectivity, retention, analyte response, and bleed meet our tight specifications.

Table 1. Comparison of Retention Times and Area Counts.

1	Methanol	2.763	2.845	423.65	409.28
2	Ethanol	3.466	3.555	488.78	511.93
3	Isopropanol	4.185	4.280	327.84	338.57
4	t-Butanol	4.860	4.959	506.14	494.89
5	Propanol	5.821	4.924	650.44	680.34
6	2-Butanol	7.324	7.382	455.19	457.45
7	Hexane	7.856	7.852	578.88	584.84
8	Isobutanol	8.422	8.353	972	1000
9	Butanol	9.707	9.615	746.21	780.57
10	3-Methyl-2-butanol	10.394	10.306	471.73	489.32
11-12	2-Pentanol & 3-Pentanol	11.008	10.924	942.18	978.18
13	Heptane	11.579	11.498	684.85	700.05
14	3-Methyl-1-butanol	12.222	12.158	507.21	522.49
15	2-Methyl-1-butanol	12.371	12.307	525.41	545.88
16	3-Methyl-3-pentanol	12.922	12.861	539.95	554.06
17	4-Methyl-2-pentanol	13.088	13.030	489.2	507.55
18	Pentanol	13.283	13.230	807.71	846.55
19	Octane	14.917	14.868	715.71	730.22
20	4-Methyl-1-pentanol	15.514	15.475	529.73	548.32
21	Hexanol	16.454	16.421	874.63	920.84
22	Nonane	17.875	17.840	767.86	790.65
23	Heptanol	19.281	19.255	859.94	905.96
24	Decane	20.529	20.497	755.91	784.34
25	Octanol	21.845	21.818	855.28	930.12
26	Undecane	22.949	22.913	750.31	782.72
27	Dodecane	25.186	25.143	744.88	776.67
28	Decanol	26.400	26.356	878.28	909.34
29	Tridecane	27.270	27.217	750.82	781.68
30	Tetradecane	29.293	29.220	736.31	766.26
31	Pentadecane	31.564	31.445	699.76	729.83
32	Hexadecane	34.288	34.100	666.03	698.22

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Ordering information

Equity-1 Capillary GC Columns

Phase: bonded; poly(dimethylsiloxane)

Temp. Limits: 0.25 and 0.32mm ID: -60°C to 325/350°C
0.53mm ID: -60°C to 300/320°C (<=1.5µm Df)
-60°C to 260/280°C (>1.5µm Df)

Prod. No.	Length (m)	D _f (µm)
0.20mm ID		
28041-U	12	0.33
28042-U	25	0.33
28043-U	10	1.2
0.25mm ID		
28044-U	30	0.10
28045-U	15	0.25
28046-U	30	0.25
28047-U	60	0.25
28048-U	15	1.0
28049-U	30	1.0
28050-U	60	1.0
28052-U	100	1.0
0.32mm ID		
28053-U	30	0.10
28054-U	15	0.25
28055-U	30	0.25
28056-U	60	0.25
28057-U	30	1.0
28058-U	60	1.0
28060-U	100	1.0
28061-U	30	2.0
28062-U	30	5.0
28063-U	60	5.0
0.53mm ID		
28064-U	15	0.10
28065-U	30	0.10
28067-U	15	0.5
28068-U	30	0.5
28069-U	15	1.0
28071-U	30	1.0
28072-U	15	1.5
28073-U	30	1.5
28074-U	60	1.5

Ordering information

28075-U	15	3.0
28076-U	30	3.0
28077-U	60	3.0
28079-U	15	5.0
28081-U	30	5.0
28082-U	60	5.0

Equity-5 Capillary GC Columns

Phase: bonded; poly(5% diphenyl/95% dimethylsiloxane)

Temp. Limits: 0.25 and 0.32mm ID: -60°C to 325/350°C
0.53mm ID: -60°C to 300/320°C (<=1.5µm Df)
-60°C to 260/280°C (>1.5µm Df)

Prod. No.	Length (m)	D _f (µm)
0.10mm ID		
28083-U	15	0.10
0.20mm ID		
28084-U	15	0.20
28085-U	30	0.20
28086-U	60	0.20
28087-U	12	0.33
0.25mm ID		
28088-U	15	0.25
28089-U	30	0.25
28090-U	60	0.25
28092-U	30	0.5
28093-U	15	1.0
28094-U	30	1.0
28095-U	60	1.0
0.32mm ID		
28096-U	15	0.25
28097-U	30	0.25
28098-U	60	0.25
28099-U	30	0.32
28195-U	30	0.5
28199-U	30	1.0
28251-U	60	1.0
0.53mm ID		
28252-U	15	0.5

Ordering information

28259-U	30	0.5
28263-U	60	0.5
28264-U	30	1.0
28265-U	15	1.5
28267-U	30	1.5
28268-U	30	3.0
28269-U	60	3.0
28278-U	15	5.0
28279-U	30	5.0
28293-U	60	5.0

Equity-1701 Capillary GC Columns

Phase: bonded; poly(14% cyanopropylphenyl/86% dimethylsiloxane)

Temp. Limits: 0.25 and 0.32mm ID: subambient to 280°C
0.53mm ID: subambient to 260°C

Prod. No.	Length (m)	D _f (µm)
0.25mm ID		
28371-U	15	0.25
28372-U	30	0.25
28373-U	60	0.25
28374-U	15	1.0
28378-U	30	1.0
28379-U	60	1.0
0.32mm ID		
28381-U	15	0.25
28382-U	30	0.25
28384-U	60	0.25
28386-U	15	1.0
28387-U	30	1.0
28388-U	60	1.0
0.53mm ID		
28389-U	15	0.5
28391-U	30	0.5
28393-U	15	1.0
28394-U	30	1.0
28395-U	15	1.5
28396-U	30	1.5

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GC Article

The Alumina Chloride PLOT for the Analysis of Freons

Traditionally, the analysis of Freon® has been done by packed column GC. However, the limited availability of packings able to do this application has created a problem. Alumina PLOT columns provide a better alternative to traditional packings, including the popular Fluorcol materials, for the analysis of Freon gases. Alumina PLOT columns are prepared using sub-micron, granular activated alumina (aluminum oxide). The polarity of the aluminum oxide provides a unique selectivity, for example, eluting small, unsaturated hydrocarbons after larger (or similar molecular sized) saturated hydrocarbons.

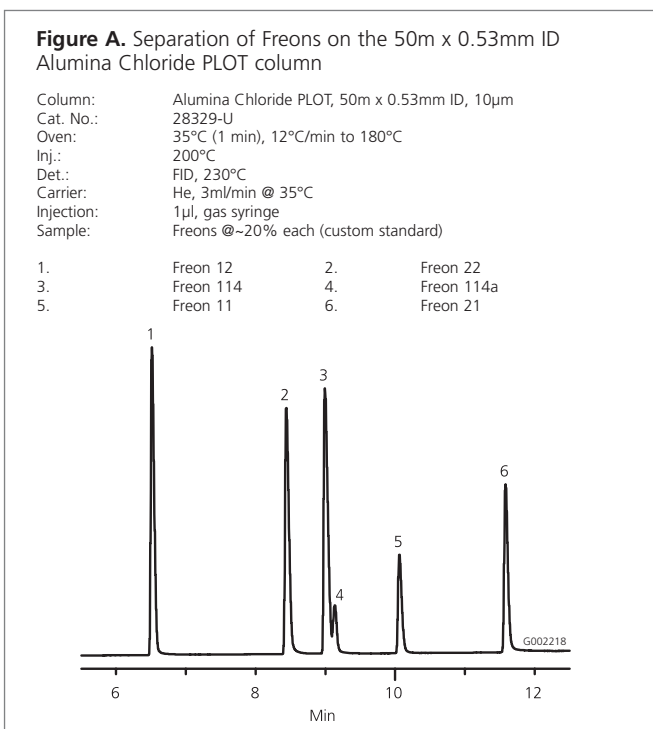
Ordering information		
Alumina Chloride	Max. Temp.	Prod. No.
Dimension		
30m x 0.32mm ID	180°C	28326-U
50m x 0.32mm ID	180°C	28327-U
30m x 0.53mm ID	180°C	28328-U
50m x 0.53mm ID	180°C	28329-U

Related Products	
Description	Prod. No.
Alumina Chloride GC Capillary PLOT Column	
50m x 0.53mm ID, 10µm	28329-U
Molecular Sieve 5A Water Vapor Trap	
200cc, 1/8" Fittings	20619
OMI Indicating Purifiers	
OMI-2 Purifier Tube ¹	23906
OMI-2 Tube Holder, 1/8" fittings ¹	23921
Seal Kit for OMI-2 Tube Holder (includes 2 Teflon seals and tool)	23917

First time users must order both purifier and corresponding holder. Holder is reusable.

For a complete listing of all Sigma-Aldrich products, log on to our website: sigma-aldrich.com

Figure A illustrates the separation of a typical Freon mixture on the Alumina chloride PLOT column. The column exhibits peak shape and resolution that is superior to the same application on Fluorcol packing. In addition, the polarity/selectivity of this column allows for the separation of the Freon 114 and 114a impurity.



i Information Request.....1106

Performance Tip

Minimize Instrument Downtime by Reducing Moisture in Carrier Gas Streams

To insure proper operation, Alumina PLOT columns must be regenerated after exposure to moisture. Limiting moisture exposure will help to minimize instrument downtime resulting from the regeneration procedure. Typically, moisture is introduced onto the column via the sample injection or through impure carrier gas. Unfortunately, the user has limited control over the amount of moisture present in a particular sample. Moisture in the carrier gas, however, can be controlled by following these steps:

- 1) Use carrier gas that is UHP (Ultra-High Purity) Grade or better. Typically, this means a minimum purity level of 99.9995%. Routinely check the cylinder volume and change out cylinders when the pressure gauge reaches 125psi.
- 2) Use ultra-high purity gas regulators. These regulators feature 316 grade stainless steel diaphragms, metal-to-metal diaphragm seals, and nickel-plated brass bodies. These features eliminate diffusion of contaminants (such

as moisture) from the room into the carrier gas. Additionally, these regulators are sealed to a helium leak rate of less than 2x10⁻⁸ cc/second.

- 3) Use gas regulators equipped with purge valves. When gas cylinders are changed, the gas regulator must be removed from the gas cylinder. A small amount of room air will fill the cylinder connector fitting. Once the valve on the newly attached full gas cylinder is opened, this slug of room air (containing moisture) is forced into the carrier gas line. The solution is to use a gas regulator that is equipped with a purge valve. A purge valve allows this small volume of gas to be expelled into the room instead of into the carrier gas line.
- 4) Use purifiers designed to remove moisture. A purifier that contains Molecular Sieve 5A adsorbent material will remove the trace amounts of moisture that exist in the gas cylinder. To determine when the purifier needs to be replaced, position an indicating purifier downstream.

Your Problem Solving Partner in Chromatography

Estrogen Metabolites on the Equity-5

This application demonstrates the use of a short, narrow-bore Equity-5 column for the separation of estrogen metabolites. This column provided excellent peak shape, response and resolution of the analytes. All compounds were analysed as methylated derivatives.

The estrogen metabolite standard was provided courtesy of the David and Alice Jurist Institute for Research at Hackensack University Medical Centre, Hackensack, NJ 07601. Affiliated with the Dept. of Medicine UMDNJ, Newark, NJ.

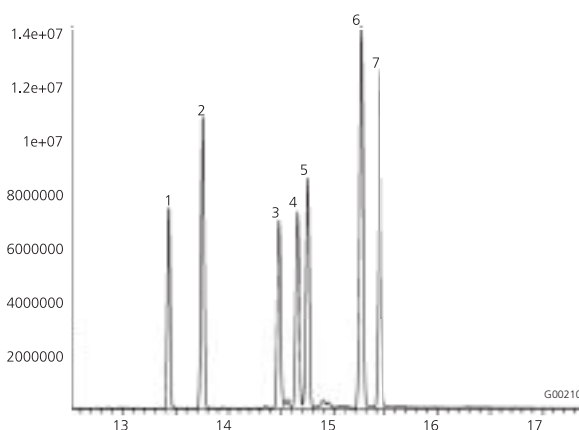
Key Words

Estrogen metabolites, estrogen, narrow-bore, Equity, 28087-U

Conditions

Column: Equity-5, 12m x 0.20mm ID, 0.33 μ m
 Cat. No.: 28087-U
 Oven: 80°C (1 min), 15°C/min to 260°C (1 min)
 Inj.: 300°C
 MSD: Interface: 270°C
 Scan Range: 50-550m/z
 Flow: Helium, 1.0ml/min constant
 Injection: 0.2 μ l pulsed splitless, (pulsed to 2.5ml/min until 0.5 min) split open at 1.5 min
 Liner: 2mm ID, straight
 Sample: 100ppm methylated estrogen metabolites in pyridine

Peak IDs
 1. Estrone
 2. Estradiol
 3. 2-Hydroxyestrone
 4. 16-Hydroxyestrone
 5. 4-Hydroxyestrone
 6. Estriol
 7. Epiestriol

**Pesticides on the Equity-5**

This application shows the separation of the 18 pesticides on the US EPA's Target Compound List (TCL) plus some additional compounds. These additional pesticides are of interest due to their frequent use or classification by the EPA as persistent,

bioaccumulative and toxic (PBT). Separation of all 25 pesticides was achieved on the Equity-5 in less than 30 minutes.

Key Words

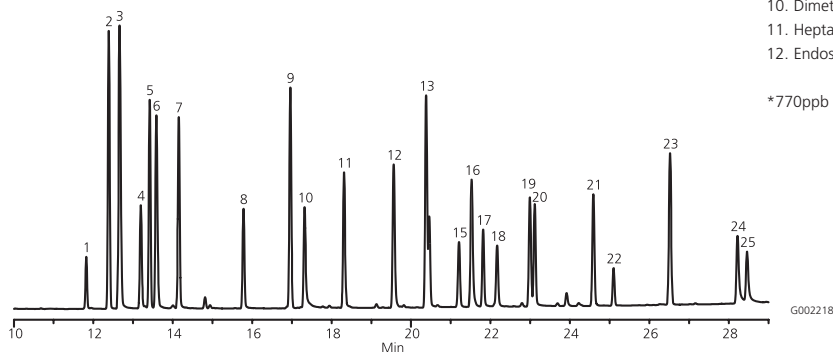
Pesticides, Equity, 28089-U, 48913

Conditions

Column: Equity-5, 30m x 0.25mm ID, 0.25 μ m
 Cat. No.: 28089-U
 Oven: 100°C (2 min) to 160°C @ 15°C/min, to 280°C @ 5°C/min (1 min)
 Inj.: 250°C
 Det.: ECD, 300°C
 Flow: Helium, 22cm/sec, constant
 Injection: 2 μ l, pulsed splitless (pulsed to 30psi for 0.2 min) Liner: 2mm ID, straight
 Compounds: pesticides
 Liner: 4mm ID single taper
 Sample: pesticides, 80-800ppb in hexane

Peak IDs
 1. Trifluralin, 80ppb
 2. α -BHC, 100ppb
 3. Hexachlorobenzene, 90ppb
 4. β -BHC, 100ppb
 5. γ -BHC, 100ppb
 6. Quintozene, 90ppb
 7. δ -BHC, 100ppb
 8. Heptachlor, 100ppb
 9. Aldrin, 100ppb
 10. Dimethylchlorthal, 85ppb
 11. Heptachlor epoxide, 100ppb
 12. Endosulfan I, 100ppb
 13. Dieldrin, 100ppb
 14. 4,4'-DDE, 100ppb
 15. Endrin, 100ppb
 16. Endosulfan II, 100ppb
 17. 4,4'-DDD, 100ppb
 18. Endrin aldehyde, 100ppb
 19. Endosulfan sulfate, 100ppb
 20. 4,4'-DDT, 100ppb
 21. Endrin ketone, 100ppb
 22. Methoxychlor, 100ppb
 23. Mirex, 120ppb
 24. cis-Permethrin*
 25. trans-Permethrin*

*770ppb mixed cis/trans isomers

**OFFER**

See offer on page 18

GC Article

Maximize Column Lifetime Using Supelco Carrier Gas Purifiers

Abstract

Oxygen, moisture, and hydrocarbon contaminants in carrier gas will lower GC performance and shorten column life. Even brief exposure to these carrier gas contaminants can damage small sections of the column. Once damage occurs, column degradation is irreversible. To insure maximum column life, you must protect it from carrier gas impurities at all times. Sources of carrier gas contamination include the gas cylinder, the cylinder changing process, fittings and regulators. Use of Supelco's recommended purifier products will eliminate carrier gas contaminants and maximize column life.

Sources of Carrier Gas Contamination

There are many sources of oxygen, moisture, and hydrocarbon contaminants. The primary source is the carrier gas cylinder. All grades of gas contain contaminants. The differences are in the level and types of contaminants measured. There is always a chance that even the highest purity gas contains contaminants that can damage your column. Although use of high purity gases reduces the risk of rapid damage, impurities in all grades of cylinder gas will shorten the life of your column.

A second source of contaminants is the cylinder changing process. Contaminants enter regulators and gas lines while they are disconnected. No matter what the cylinder changing process, a small amount of these contaminants unavoidably reaches the column when the system goes back on line. This results in the damage of a small section of the column. Once damaged, the column degradation process is irreversible.

A third source of contamination is from regulators, valves, and fittings in the system. Contaminants permeate through all regulator diaphragms unless they are made of metal. Valves and fittings present potential sources of leaks from connection and re-connection. Dirt and occasional over compression prevents proper reseal of the fitting. If fittings and tubing are made of different materials, leaks develop over time because of differing rates of expansion. Leaking fittings are an ongoing source of contaminants that shortens column life.

The Solution is Gas Purifiers

As long as contaminants are present in the GC system, columns will not last as long or perform as well as they should. To guard against this, the system must contain components designed to eliminate damaging contaminants. Gas purifiers are the only sure way to guarantee that the GC system is contaminant free.

Supelco recommends the use of four types of gas purifiers. Table 1 describes these purifiers and the contaminants they remove.

Proper Sequence of Gas Purifier Installation

The proper sequence of purifier installation is important for peak performance. Remove hydrocarbons first, followed by moisture, and then oxygen. Hydrocarbons bind with moisture and oxygen removal sites in moisture and oxygen traps reducing their effectiveness. Likewise, water binds to oxygen removal sites in oxygen traps reducing their effectiveness. Figure A illustrates the proper sequence of installation for carrier gas purifiers.

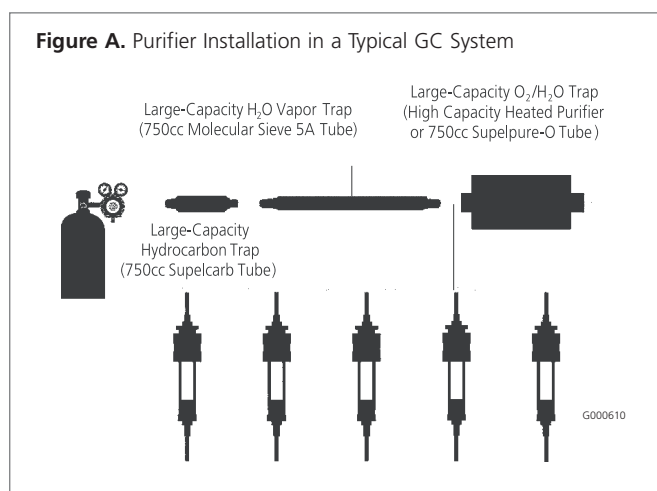
Purifiers improve the quality of even the highest purity carrier gas. As a final safeguard, Supelco recommends installing an OMI (Oxygen-Moisture Indicator) purifier tube just before the carrier gas line entering the GC. This protects against contaminants that may have entered through fittings in the gas lines. The OMI tube also gives a visible indication of contaminants. If it changes colour, you know the GC system has leaks or that the purifiers have expired and need replaced.

Name	Contaminants Removed	Size	Fitting	Cat. No.
Supelcarb™ HC	C3 and higher hydrocarbons	120cc	1/8"	24448
		120cc	1/4"	24449
		750cc	1/4"	24564
		750cc	1/2"	24565
Molecular Sieve 5A Traps	H ₂ O	200cc	1/8"	20619
		200cc	1/4"	20618
		750cc	1/4"	23991
		750cc	1/2"	23992
High Capacity Heated Purifier**	O ₂ , H ₂ O, CO ₂ & CO	32cc	1/8"	22396
			1/4"	22398
OMI™ -2	O ₂ , H ₂ O, CO ₂ , CO, NH ₃ , alkynes, halocarbons, halogens, & Hydrogen halides	15cc	1/8"	23906

** Can be used with He, N₂, Ar/CH₄ not compatible with air, O₂ or H₂.

Conclusion

You need carrier gas purifiers regardless of the gas purity used. Contamination is always present in the GC system. Sources include the gas cylinder, the cylinder changing process, fittings and regulators. Carrier gas purifiers are the only sure way to guarantee the lowest level of carrier gas contamination. Install purifiers in the right sequence for proper purification. Installation must include a hydrocarbon trap, followed by a high capacity moisture and oxygen purifier, and finally an OMI indicating purifying tube. Maximum GC column life is possible only if you use the right carrier gas purification products.



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Specification/Product Range

LC-MS solvent Prod. No.	Water 39253	Acetonitrile 34967	Methanol 34966	2-Propanol 34965	Ethylacetate 34972
Pack sizes	1 L	1 L / 2.5 L	1 L / 2.5 L	1 L / 2.5 L	1 L / 2.5 L
Assay (GC)		min. 99.9 %	min. 99.9 %	min. 99.9 %	min. 99.7 %
Fluorescence at 254nm	max. 1 ppb	max. 0.5 ppb	max. 1 ppb	max. 1 ppb	
Fluorescence at 365nm	max. 1 ppb	max. 0.5 ppb	max. 1 ppb	max. 1 ppb	
Chloride (Cl)	max. 0.000001 %				
Fluoride (F)	max. 0.000001 %				
Nitrate (NO ₃)	max. 0.00001 %				
Sulfate (SO ₄)	max. 0.00001 %				
Free acid		max. 0.001 %	max. 0.001 %	max. 0.001 %	
Free alkali (as NH ₃)		max. 0.0002 %	max. 0.0005 %	max. 0.0005 %	max. 0.0005 %
Non-volatile matter	max. 0.001 %	max. 0.0002 %	max. 0.0005 %	max. 0.0005 %	max. 0.0005 %
Water (Karl Fischer)		max. 0.01 %	max. 0.02 %	max. 0.05 %	max. 0.03 %
Transmittance at 200nm	min. 95%	min. 95%			
Transmittance at 230nm	min. 99%	min. 99%	min. 75%	min. 75%	
Transmittance at 260nm			min. 98%	min. 98%	min. 50%
HPLC gradient (254nm)	max. 1 mAU	max. 0.2 mAU	max. 2 mAU	max. 2 mAU	
Silver (Ag)	max. 0.1 ppm	max. 0.1 ppm	max. 0.1 ppm	max. 0.1 ppm	
Aluminum (Al)	max. 0.5 ppm	max. 0.5 ppm	max. 0.5 ppm	max. 0.5 ppm	
Barium (Ba)	max. 0.1 ppm	max. 0.1 ppm	max. 0.1 ppm	max. 0.1 ppm	
Calcium (Ca)	max. 0.1 ppm	max. 0.1 ppm	max. 0.1 ppm	max. 0.1 ppm	
Cadmium (Cd)	max. 0.05 ppm	max. 0.05 ppm	max. 0.05 ppm	max. 0.05 ppm	
Cobalt (Co)	max. 0.02 ppm	max. 0.02 ppm	max. 0.02 ppm	max. 0.02 ppm	
Chromium (Cr)	max. 0.02 ppm	max. 0.02 ppm	max. 0.02 ppm	max. 0.02 ppm	
Copper (Cu)	max. 0.02 ppm	max. 0.02 ppm	max. 0.01 ppm	max. 0.02 ppm	
Iron (Fe)	max. 0.1 ppm	max. 0.1 ppm	max. 0.1 ppm	max. 0.1 ppm	
Potassium (K)	max. 0.1 ppm	max. 0.1 ppm	max. 0.1 ppm	max. 0.1 ppm	max. 0.1 ppm
Magnesium (Mg)	max. 0.1 ppm	max. 0.1 ppm	max. 0.1 ppm	max. 0.1 ppm	max. 0.1 ppm
Manganese (Mn)	max. 0.02 ppm	max. 0.02 ppm	max. 0.01 ppm	max. 0.02 ppm	
Sodium (Na)	max. 0.1 ppm	max. 0.1 ppm	max. 0.1 ppm	max. 0.1 ppm	max. 0.1 ppm
Nickel (Ni)	max. 0.02 ppm	max. 0.02 ppm	max. 0.02 ppm	max. 0.02 ppm	
Lead (Pb)	max. 0.1 ppm	max. 0.1 ppm	max. 0.02 ppm	max. 0.1 ppm	
Tin (Sn)	max. 0.1 ppm	max. 0.1 ppm	max. 0.1 ppm	max. 0.1 ppm	
Zinc (Zn)	max. 0.1 ppm	max. 0.1 ppm	max. 0.1 ppm	max. 0.1 ppm	
Particle test	+	+	+		
LC-MS suitability test	+	+	+	+	+

Standards and Reagents Article

New Standards for Residue Analysis: Fluoroquinolones

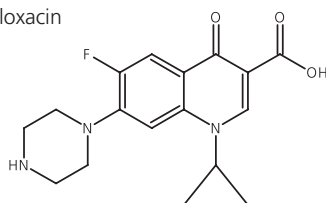
Fluoroquinolones are quinolones that have an added fluorine atom to improve their effectiveness. These compounds have a broad spectrum antimicrobial activity against Gram-negative as well as Gram-positive bacteria, and an excellent tissue distribution. They act by deactivating the bacterial enzymes necessary for DNA transcription. The fluoroquinolones have a broad range of therapeutic indications. For instance, they are given to neutrogenic patients as prophylaxis. In veterinary medicine, fluoroquinolones are used as treatment and metaphylaxis but not as growth promoters. The most common fluoroquinolones in use are now available as Analytical Standard grade (Table 1):

Ordering information		
Product Name	Pack Size	Prod. No.
Dimension		
Ciprofloxacin	100 mg	33434
Danofloxacin	100mg	33700
Enrofloxacin	100mg	33699
Sarafloxacin-hydrochloride	100mg	33497

Ciprofloxacin

Ciprofloxacin Figure 1 is a synthetic broad-spectrum antimicrobial agent for intravenous (I.V.) administration. It is used to treat bacterial infections in many different parts of the body and it is approved for the inhaled form of anthrax after an individual has been exposed. Ciprofloxacin is a faint to light yellow crystalline powder. It is soluble in dilute (0.1N) hydrochloric acid and is practically insoluble in water and ethanol.

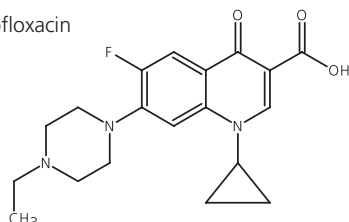
Figure 1. Ciprofloxacin



Enrofloxacin

Enrofloxacin was introduced in the US for food animal use in 1996 (Figure 2). Ever since then it has proven time and again to be a valuable tool for fighting disease in poultry and helped to ensure a safe food supply. Only available by veterinary prescription, enrofloxacin is used exclusively for therapeutic purposes. Administered as a short term therapy, is used in instances of life threatening disease of cattle or poultry flocks. Enrofloxacin is a light yellowish to yellow powder, slightly soluble in water. Structurally, it is related to the human-approved drug ciprofloxacin.

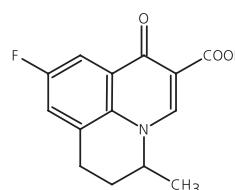
Figure 2. Enrofloxacin



Sarafloxacin

Sarafloxacin is an antibiotic drug used to prevent early death in poultry caused by Escherichia coli infection (Figure 3). It has been proposed for use in drinking water of poultry to treat bacterial infection and in fish feed to treat diseases such as furunculosis, vibriosis and enteric redmouth. Sarafloxacin hydrochloride is an off-white or yellowish crystalline powder. It is insoluble in water or in ethanol, soluble in sodium hydroxide solution.

Figure 3. Sarafloxacin

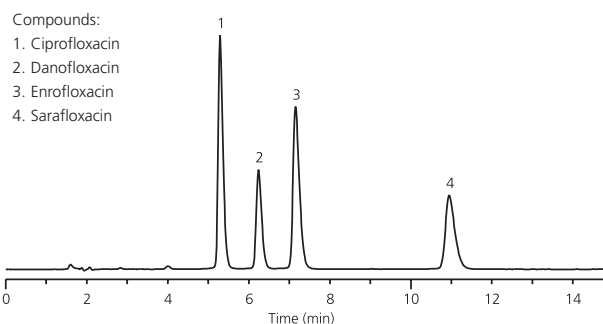


Other Nitrofuran Standards by Fluka:

Soon, you will be able to find new Nitrofuran Standards in our catalogue. In the near future, in addition to the normal working standards AOZ (Cat. No 33347), AMOZ (Cat. No. 33349), AHD (Cat. No 33656), and SCA (Cat. No 33656), you will also find available the 2-Nitrobenzaldehyde derivatized 2-NP-AOZ, 2-NP-AMOZ, 2-NP-AHD and 2-NP-SCA and the isotope-marked AOZ-D4, AMOZ-D5, 13C, 15N-SCA.

Fluoroquinolone Antibiotics on a C18 Discovery™ column.

Conditions
 Column: Discovery™ C18, 15cm x 4.6mm, 5µm
 Cat. No.: 504955
 Mobile Phase: 85:15 water (10mM ammonia acetate, pH 3.0 with acetic acid):acetonitrile
 Temperature: 35°C
 Flow Rate: 1.0ml/min
 Detection: UV, 254nm
 Injection Volume: 10µl
 Sample: 50µg/ml each in mobile phase



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