

TheReporter

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When the Separation Doesn't Work on the First Try...

Supelco recommends starting a new GC analysis with a 30m x 0.25mm ID x 0.25µm, SPB-1, or SPB-5 capillary column. In combination with generic run conditions, these columns often yield acceptable separations on the first try. If the analytical objectives are not achieved on the first attempt, the next step is to improve the sample resolution. This article describes the approach of adjusting run conditions, operational parameters, and column dimensions to improve resolution.

Resolution Defined

Resolution is a measure of how well two compounds separate. It is expressed theoretically as a function of the three terms illustrated in Equation 1.

Equation 1: Resolution

$$R = \frac{k}{1+k} \frac{\alpha-1}{\alpha} \frac{n^{(1/2)}}{4}$$

Capacity Selectivity Efficiency

Capacity is expressed in the Resolution Equation as a compound's retention factor (k) divided by the retention factor plus one.

Selectivity is related to the separation factor (α), the relative retention time value calculated for two closely eluting peaks of interest. In Equation 1, it is expressed as the ratio of α minus one divided by α . Selectivity changes depending on the stationary phase.

Efficiency is related to the capillary column dimension and is influenced by the amount of sample placed on the column. It is expressed as the square root of the total theoretical plates available in the capillary column.

Because analytical run conditions and column dimensions directly impact these terms, any change to them affects resolution. Systematically adjusting these terms permits analysts to influence the separation. Analysts typically adjust analytical run conditions first, as this is the easiest change to affect. Only after exhausting changes to the run conditions will analysts modify column dimensions or stationary phase. These changes require removing and replacing the analytical column.

Modifying Run Conditions

The first adjustments when improving resolution are to the temperatures and linear velocity (LV). Temperature adjustments include the starting and final temperatures, hold times, and temperature program rates. Changes to temperature directly impact the Capacity term in Equation 1.



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Optimizing the system's LV can lead to dramatic results. LV's slower or faster than optimum will negatively impact the separation. The optimum LV is determined by measuring the column efficiency at different LV's. Changes impact the Efficiency term of Equation 1.

Changing Column Dimensions

When adjusting the analytical run conditions yields no further improvement in the separation, the analyst must consider changing the column. Increasing the column length increases the number of plates available for the separation, but the analysis time changes proportionate to the change in column length. In an isothermal analysis, for example, doubling column length doubles the analysis time.

Increased column efficiency will result from decreasing internal diameter. In general, the greater the efficiency of the column, the greater the resolution. However, there is a trade off. Sample capacity decreases with column ID. A 0.25mm ID column, for example, has a capacity of only 50-100 nanograms on-column.

Adjusting the stationary phase film thickness impacts the Capacity and Efficiency terms in Equation 1. The capacity of the columns increases because there is more phase available. As film thickness increases, however, analytes are retained longer but peaks broaden. Very thick film phases (>3.0µm) therefore yield proportionately lower efficiency comparative to thinner film columns run under similar analytical conditions.

Column Selectivity

The most significant impact to a separation is observed when changing the stationary phase.

There are multiple phase selectivities ranging from non-polar, such as SPB-1 or SPB-5, to a highly polar, such as SP-2380 or SP-2340. Specialty phases, such as cyclodextrin phases, are also available for unique applications.

Increasing phase polarity often results in an elution shift of sample components. This is because the interaction mechanisms between sample and stationary phase change with polarity. The SPB-5, for example, contains phenyl groups making it selective for aromatic compounds.

Changing polymer structure can significantly impact a separation. The polar, polyethylene glycol based phase chemistry in the SUPELCOWAX 10 yields a different separation when compared to the non-polar SPB-1. This is why Supelco recommends evaluation of a SUPELCOWAX 10 column when other adjustments to the initial column have failed.

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SUPELCO
a member of the Sigma-Aldrich family

OMI 4 Indicating Purifier



P000245

Jun-Air Oilless Air Compressors



P000198

LC/MS Nitrogen Generators



E000776

Therm-O-Ring Seals / Inlet Seals for Agilent/HP GC's



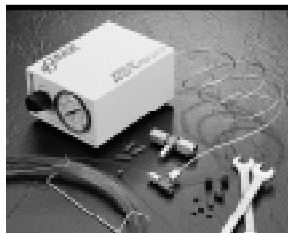
P000752

Micro-Flo 20 flowmeter



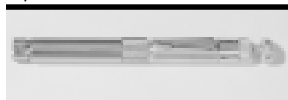
P000219

MS-NoVent



P000699

Septum Insertion Tool



P000258

NEW PRODUCTS

Gas Generation, Purification and Delivery

OMI-4 Indicating Purifier

The OMI-4 is a high capacity version of the popular OMI-2 indicating purifier that provides final polishing before the gas enters the GC. Install an OMI indicating purifier downstream from your primary gas purifying device to irreversibly and simultaneously remove oxygen, water vapor, CO, CO₂, most sulfur compounds, most halogen compounds, and other contaminants to less than 10ppb. OMI indicating purifiers provide visual assurance of gas quality and indicate when a purifier is completely spent, thus eliminating unnecessary and premature changes of gas purifiers.

☎ For more information, request T100848.

Jun-Air Oilless Air Compressors

Jun-Air is a leader in compressor technology with more than 30 years of product development. Jun-Air compressors are quiet and reliable. Highly efficient cooling enables you to continuously run the compressor. These compressors are an ideal source of compressed air for nitrogen and zero air generators available from Supelco.

☎ For more information, request T497077.

Nitrox LC/MS Nitrogen Generators from domnick hunter

Nitrox LC/MS nitrogen generators from domnick hunter are specially designed to meet the gas flow, purity, and pressure requirements of LC/MS. By providing a continuous supply of clean, dry, 99.5% pure nitrogen, they eliminate the need for expensive and labor intensive gas cylinders or high rental costs of bulk supply equipment. They are compatible with both atmospheric pressure chemical ionization (APCI) and electrospray ionization (ESI) interfaces.

☎ For more information, request T499204.

Sample Preparation and Introduction

Therm-O-Ring Seals

High pressure Therm-O-Ring inlet seals for Agilent inlet liners provide superior GC performance at temperatures as high as 375°C. Supelco's proprietary formulation yields O-rings that do not stick to the injection port or fragment during removal. These rings are a superior replacement for Viton O-rings and are available exclusively from Supelco.

☎ For more information, request T400003.

Inlet Seals for Agilent GC's

Low cost, replacement inlet seals for Agilent GC's from Supelco reduce the need for cleaning and reuse. Supelco metal selection yields a better inlet seal. Seals are available in stainless steel and gold plated versions. Precise, computerized machining reduces dimensional variation that can occur with other seals.

☎ For more information, request T400006.

Fittings and Accessories

Micro-Flo 20 Flowmeter

A more accurate and less time consuming method of measuring capillary flow, the Micro-Flo 20 electronic flow meter is designed to provide continuous, accurate linear velocity and volumetric flow of 20ml or less for helium and hydrogen. It is particularly useful for setting flows for methane-retaining columns or when using detectors that do not respond to methane. It also eliminates errors introduced when using nominal column lengths to calculate linear velocity.

☎ For more information, request T496002.

ms-NoVent

A new time saving accessory for GC/MS systems, the ms-NoVent allows capillary users to begin using the mass spec within minutes after changing columns. The ms-NoVent supplies the mass spec with carrier gas during column change, eliminating system pump down. It consists of a pressure switching valve, fused silica restrictor, and an external control module. Installation of Agilent, Varian, Shimadzu, and other systems is typically less than 30 minutes.

☎ For more information, request T400005.

Septum Insertion Tool

Change a septum quickly without cooling the injector thanks to Supelco's new patented septum insertion tool. This tool installs a septum into any GC injection port that uses 9.5 - 11mm diameter septa. The tool compresses the septa and makes insertion into the injector easier. It reduces the potential for burned fingers and contamination from finger oils or other sources.

☎ For more information, request T400004.

Q-MAX Air Sampling Pump

This new sampling pump combines an electronic laminar flow sensor in an easy to operate system. The pump provides constant flow as battery voltage changes during operation. An internal secondary standard calibrates the pump continuously so that primary standard calibration is needed only monthly.

☎ For more information, request T499076.

ASSET Air Sampling Solid Extraction Tubes

ASSET-32 tubes are a unique timesaving replacement for charcoal containing glass-sampling tubes. These polypropylene solid phase extraction tubes contain a single bed of adsorbent, secured by polypropylene frits. The open design allows analyte elution by gravity in only a few minutes, in contrast to removing and extracting tube adsorbent as with other sampling tubes. ASSET-32 tubes are also adaptable to automation and fully compatible with standard NIOSH and OSHA methods.

☎ For more information, request ASSET Packet T499071.



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All literature mentioned in this issue can be obtained from the website, www.sigma-aldrich.com/TheReporter, by completing the Literature Request section on the reply card, or by calling our Technical Service Department.

NEW PRODUCTS

Columns

Capillary Columns for Agilent 6850

Supelco makes it easy to purchase off-the-shelf capillary GC columns for the Agilent 6850! By referencing order code PRO100060, you can purchase any stock or custom Supelco capillary column wound on an authentic Agilent Technologies 6850 cage. To order, simply provide order code PRO100060 plus the stock capillary item number or custom column information. Supelco will coil the column onto a 6850 cage and ship the column within 24 hours.

☎ For more information, request T400051.

NEW APPLICATIONS

Bob Shirey SPME, *J. Chrom. Sci.*

A group of volatile analytes of molecular weight less than 90 and representing 11 organic classes are extracted using identical conditions with six different Solid-Phase MicroExtraction (SPME) fiber coatings. The amount of analyte extracted using the various fibers is shown. The effects of sample modifiers such as pH and ionic strength on the recovery of the analytes are presented. A comparison of headspace and immersion extraction techniques is shown.

☎ For more information, request DFU.

SEMINAR TRANSCRIPTS

The following is available via the web or by mail.

Bob Shirey SPME PittCon Paper

Presented at the 2000 Pittsburgh Conference, this seminar compares liquid and porous coated Solid Phase MicroExtraction (SPME) fibers. Fibers extract by adsorption or partitioning mechanisms depending on the material used to coat the fiber. In the presentation, different analyte classes are extracted using various fibers and the selectivity of coatings is discussed. Capacities, linear ranges and detection limits are presented. The presentation investigates the adsorption mechanism of the fibers based upon pore structure and determines the effects of multiple analytes competing for pore sites. Tips are given on fiber selection.

☎ For more information, request T400011.

GC PERFORMANCE TIP

Hydrocarbon Peak Shape – A Good System Indicator

A tailing hydrocarbon peak can sometimes indicate a bad column but more often than not, it indicates an installation problem. Hydrocarbons are reasonably inert and chromatograph easily. A tailing peak generally indicates dead volume somewhere in the system. This could be caused by a broken column, improper installation, a leaky connection, or problems with the make-up gas flow.

Sometimes hydrocarbon peaks front. When this happens, consider the injector split, linear velocity and column temperature. If the split is set too low, too much analyte will

NEW LITERATURE

Capillary Trouble Shooting Guide

The real task in correcting a problem with your capillary GC system is identifying the cause of the problem without wasting time. The systematic approach to troubleshooting described in this guide will enable you to solve many problems. This updated guide also contains suggestions for maintaining your system, including the column, at optimal performance levels. By following these recommendations, you can reduce repair costs and instrument down time.

☎ For more information, request T112853.

Petroleum Guide

This 48-page guide contains information about Supelco products and technology for separating hydrocarbons by chromatographic methods. The updated guide illustrates nearly one hundred different hydrocarbon separations including PONA, PIANO, and SIMDIS applications. Multiple capillary and HPLC products are included to help with your analytical hydrocarbon needs. The guide is recently updated to reflect current products and applications available at Supelco.

☎ For more information, request T100858.

Gas Generator Summary Form

This new form allows users to request literature either by application or by cylinder gas being replaced by a generator. Multiple applications are listed including Atomic Absorption, Atomic Thermal Desorbers, Autosamplers, CO₂ Analyzers, GC, FTIR, LC/MS, and NMR. By selecting the desired application or gas, Supelco will forward the corresponding literature for domnick hunter and Whatman / Packard gas generators.

☎ For more information, request T400058.

CUSTOMERS WIN AT PITTCON 2000!

Visitors to the Supelco booth at the recent Pittsburgh Conference in New Orleans were eligible to enter a drawing for either a new hydrogen generator or a capillary start up kit.

Congratulations to the winners!

Capillary Start Up Column Kit and Capillary Start Up Tool Kit
Steven Colgrove - Chemist, Exxon - Mobil

Packard Model 9150 Hydrogen Generator
Ed Layman - Chemist QA Lab, Colgate



Len Sidisky - R&D Manager,
Gas Separations Business Unit

reach the column, causing over-load. Similarly, operating at too low a temperature or too slow a linear velocity will effectively limit column capacity and peaks will front.

Either way, odd hydrocarbon peak shapes only occasionally indicate a bad column. In most instances, the problem behind the peak shape is installation or system related. You generally can eliminate the problem without purchasing a new column. At the first sign of poor peak shape, always thoroughly check the system for leaks, remove and reinstall the column, and take a close look at the conditions you are following.

S For more information request:
Bulletin 875: Supelco Capillary Column Selection Guide (T195875)
Bulletin 853: Capillary GC Troubleshooting Guide (T112853)
 You may also purchase *Analytical Gas Chromatography*, by Jennings: CAT. NO. Z22,143-0

Capillary Starter Column Kits
0.25mm ID Capillary Starter Kit:
 30m x 0.25mm ID, 0.25µm film fused silica columns, one each: PTE-5, SPB-50, SUPELCO WAX 10
 CAT. NO. 24142
0.53mm ID Capillary Starter Kit:
 30m x 0.53mm ID, 0.5µm film fused silica columns, one each: SPB-1, SUPELCO WAX 10 CAT. NO. 25377

Capillary Starter Tool Kit
 CAT. NO. 23639

CASE STUDY 2

When the Separation...

(continued from page 1)

When switching column phases, return to the separation conditions recommended for the first attempt (**Reporter 18.2**). Also, note the operating temperature range of the new phase. Polyethylene glycol stationary phases, for example, have different temperature limits than polysiloxane phases. Analysts must operate above the minimum temperature and limit the maximum temperature based on the phase used.

TABLE 1: Improving Resolution - A summary of various changes and discussion on their effect

Action	Resolution Equation Term	Ease of Change	Impact on Resolution	Other Considerations
Change Run Conditions	Capacity and Efficiency	Easy	Moderate	Impacts on the analysis time
Change Film Thickness	Capacity and Efficiency	Moderate – new column	Moderate	Change in peak shape and analysis time
Change Column Length	Efficiency	Moderate – new column	Major	Analysis time proportionate to change in length
Change Column ID	Efficiency and Capacity	Moderate – new column	Major	On-column sample capacity
Change Phase	Selectivity	Drastic – new column	Drastic	Shift in elution pattern

The GC Startup

Initial Separation

A new graduate student in analytical chemistry is asked to resolve xylene isomers in a BTEX sample using capillary GC. Baseline resolution of p- and m-xylene is required. Analysis on a 30m x 0.32mm ID x 0.5µm, methyl silicene column using a simple temperature program yields only a 5% resolution for the p- and m-xylene pair.

Changing Run Conditions

The student revises run conditions by lowering the starting temperature, slowing the temperature program rate, and changing the initial hold time. These changes improve resolution of p- and m-xylene to about 10%. After multiple GC runs, the student concludes that achieving baseline separation is not possible with the current column. They decide to increase column efficiency by requisitioning a 60m x 0.32mm ID x 0.5µm column of the same phase.

Increasing Efficiency

The student analyzes the xylene sample on the new column according to the run conditions used for earlier separations. Resolution of the p- and m-xylene isomers increases to only 20%. The longer column also yields a 75% increase in run time. While tolerable, the run time means taking longer than desired to improve peak resolution by modifying run conditions. The student decides to try a different stationary phase.

Changing Selectivity

Recalling that switching from nonpolar methylsilicone to a polar polyethylene glycol phase will have a significant impact on compound elution, the student orders a 30m x 0.25mm ID x 0.25µm, SUPELCO WAX 10 column. Run con-

Conclusion

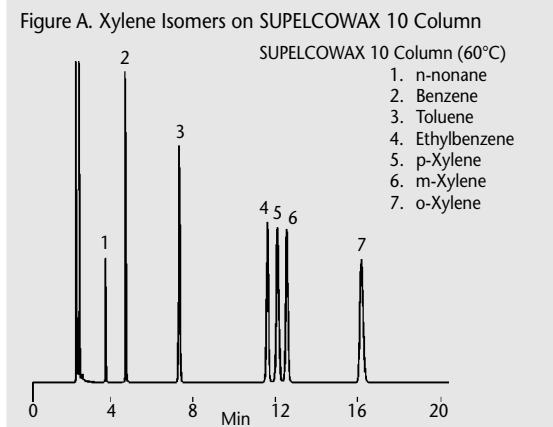
Analysts rarely accomplish analytical objectives with the very first separation. Improving the initial separation is a process of manipulating terms in the Resolution Equation. Analysts can improve a separation by altering analytical run conditions, changing column dimensions, and finally switching to a different stationary phase. The recommended change when switching stationary phase is from SPB-1 to SUPELCO WAX 10. This change will yield the most significant impact on elution patterns of the sample.

ditions are adjusted to reflect minimum and maximum temperature limits of the new phase but otherwise are identical to those used with the initial column. The first separation yields success. Within only two additional runs, the graduate student optimizes run conditions and quantitates the xylene isomers within the sample. The final separation is illustrated in the Figure A.

Conclusions

The columns in the initial separations are incapable of achieving baseline separation of p- and m-xylene isomers. Changing to the polyethylene glycol based, SUPELCO WAX 10 phase introduces hydrogen bonding and acid base interactions to the separation. These interactions, in combination with the dispersive interactions, permit baseline resolution of the isomers.

S For more information, request T494088.



Trademarks and Registered Trademarks:

Agilent - Agilent Technologies, Inc.
ASSET, OMI, SPB, SUPELCO WAX, Therm-O-Ring - Sigma Aldrich
domnick hunter - domnick hunter Limited
Jun-Air - Jun Air A/S
Nitrox - domnick hunter Limited
Packard - Hewlett Packard Company
PittCon - Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy
Shimadzu - Shimadzu Corporation
Viton - DuPont Dow Elastomers
Whatman - Whatman International Limited

Patents:

SPME - Technology licensed exclusively to Supelco. US patent #5,691,206; European patent #523092.

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