HALO®

PIONEERING FUSED-CORE® TECHNOLOGY SINCE 2005

A Committment to Innovation, Quality and Experience

advancedmaterialstechnology

DISCOVER MORE WITH FUSED-CORE®

Since our founding in 2005, Advanced Materials Technology has been focused on one mission – Improving the presentation of the sample to the detector. Using our novel Fused-Core® particle design, we have challenged conventional wisdom and engineered innovative solutions for the separations community. Our quality procedures and practices are integrated into every HALO® column delivered ensuring your success – every time.

AMT QUALITY POLICY

AMT is committed to providing world-class innovative products that uniquely fill the growing needs of small molecule and large molecule separation scientists. We take pride in delivering products that exceed customers' expectations on quality and delivery time and collaborate to break down any barriers that would prevent an exceptional customer experience. We continually strive to improve our organization to stay focused on safety, quality, and cost.

We embrace ISO 9001 standards in our work systems and daily work. We pledge to have dynamic leadership promoting culture of excellence embedded in every employee.

ASR American Systems Registrar

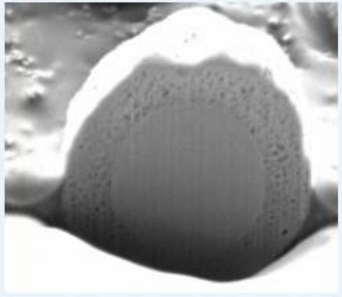
ANAB ANSI National Accreditation Board ACCREDITED ISO/IEC 17021-1 MANAGEMENT SYSTEMS CERTIFICATION BODY

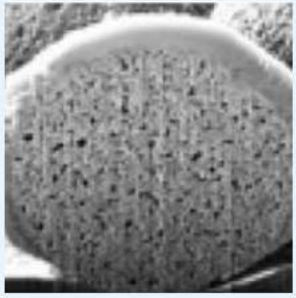
ISO 9001:2015 Certified QMS

INTRODUCTION

WHAT IS FUSED-CORE® TECHNOLOGY?

Sixty years ago, Horvath and Lipsky published their groundbreaking paper in which they described their pellicular particles which were made up of glass particles coated with a thin layer of ion- exchange resin. This paper inspired Jack Kirkland, then working for the DuPont Company, to develop the first superficially porous silica particles with solid cores and a porous crust (30 µm Zipax®, 1969). The introduction of superficially porous particles enabled liquid chromatography to begin its development into the major analytical tool it has become. This type of particle was soon displaced (early 1970s) by the development of small-particle, fully porous silica particles which dominated HPLC column technology thereafter. Superficially porous particles were reintroduced in 2006 as a major advance in HPLC particle technology when Kirkland, Langlois, and DeStefano, at Advanced Materials Technology, commercialized the first sub-3µm Fused-Core® silica particle under the brand name HALO®. This particle consists of a solid silica core surrounded by a thin porous shell that is heat-sintered to the core, hence the name Fused-Core®. Fused-Core® columns can be operated on both HPLC and UHPLC systems with superior performance.





ADVANTAGES OF FUSED-CORE® TECHNOLOGY

The advantage of superficially porous particles (SPPs) over fully porous particles (FPPs) is best shown by comparing scanning electron micrographs of cross-sections of the particles in the figure on previous page. The diffusion path for sample molecules into and out of the particles is shorter for the SPP due to the presence of the impermeable solid core leading to faster mass transfer. The classical van Deemter characterizations of FPP columns and a SPP column in the figure to the right demonstrates the effect of mobile phase velocity on column performance. Column performance is determined by measuring the width of peaks eluting from the column. This measurement of width can be converted into the number of theoretical plates (N).

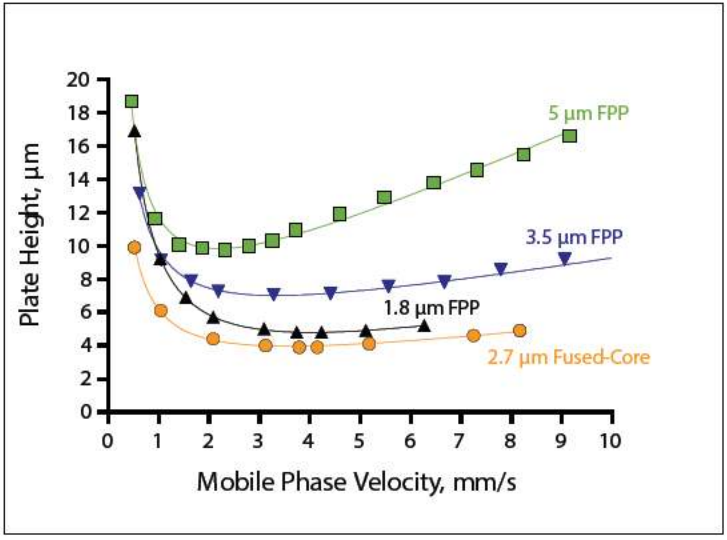
$$N = 5.54 (t_R/w_{1/2})^2$$

where t_R = peak retention time, $w_{1/2}$ = peak width at half height

The greater the number of theoretical plates the more resolving power in the column. The plate count in columns can then be converted to the Height Equivalent to a Theoretical Plate (H) by dividing the column length (in microns) by the number of theoretical plates. The Plate Height (H) is comprised of three terms that contribute to the van Deemter curve:

$$H = A + B/\mu + C\mu$$

where the A term is affected by how well the column is packed (eddy diffusion term), the B term is a measure of longitudinal diffusion and is positively affected by the presence of the solid core (25-30% smaller B-term for SPP), and C is the mass transfer term that is smaller for SPP resulting in a flatter curve as mobile phase velocity (μ) is increased. The 2.7 μm Fused-Core® column has a clear advantage as depicted in the van Deemter plot, outperforming even the sub-2-μm FPP column.



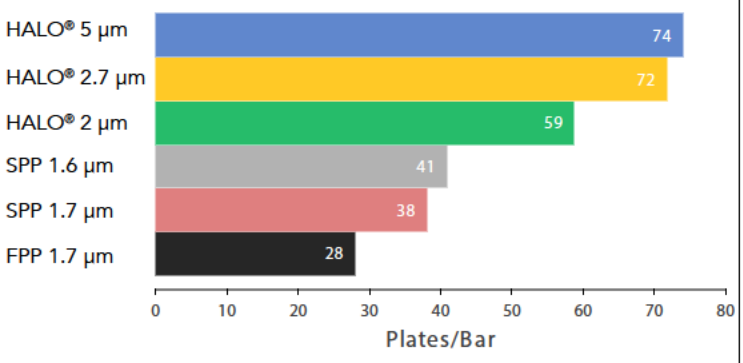
OTHER ADVANTAGES OF HALO®

PLATES/BAR, KINETIC PLOTS, PEAK CAPACITY

Another way to compare column performance between different types of columns is to measure the ratio of the number of theoretical plates and required back pressure (in bar) that are obtained under a fixed set of operating conditions (plates/bar). Example performance measurements for three HALO® and three competitor 2.1 x 50 mm SPP and FPP columns, run at 0.5 mL/min with 50/50 acetonitrile/water at 35°C are shown in the figure below.

All of the HALO® columns provide more theoretical plates per unit of pressure than any of the competitor sub-2-micron SPP and FPP columns.

Comparison of HALO® Particles to Sub-2-µm Particles using Ratio of Efficiency to Back Pressure

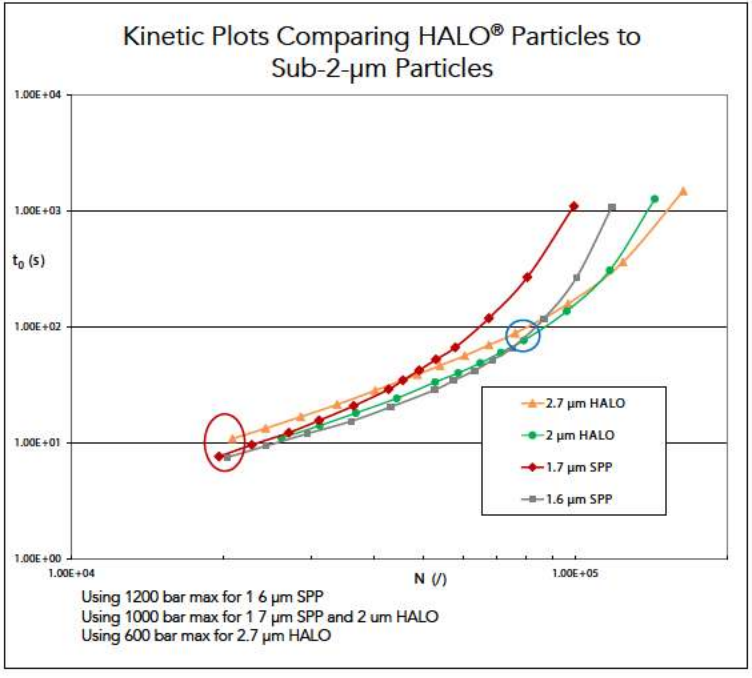


KINETIC PLOTS – HOLD UP TIME AND PRESSURE

Kinetic plots can aid the analyst to compare different columns packed with different particles at different column lengths by plotting the hold-up time as a function of the column efficiency (J. Sep. Sci. 2011, 34, 877–887). Practical decisions on the final dimensions are often limited to lengths between 3 – 15 cm. Generally, the shortest columns are ideal for high throughput analyses, and the longer length columns ideal for separations that prioritize separation efficiency and can afford longer analysis times/ larger hold up volume. The kinetic plot illustrates the same columns depicted in the previous figure (both axes in log scale). These plots can be interpreted a number of ways and are critical to demonstrate key factors important for method development.

One case scenario may be to aid particle size selection when developing an assay for speed – hence a small hold up time with the shortest column format must be selected. Looking at the graph (p. 6) in this specific region highlighted in red - The HALO® 2.7 µm particle in a shorter column length is a sensible column choice as it does not require the dedicated UHPLC system.

Another case scenario highlighted in blue – are for separations that can afford relatively longer analysis times: the HALO® 2 µm column in a longer length column format affords more plates at a longer length; the HALO® 2.7 µm column at a smaller column length is a sensible option at 600 bar.

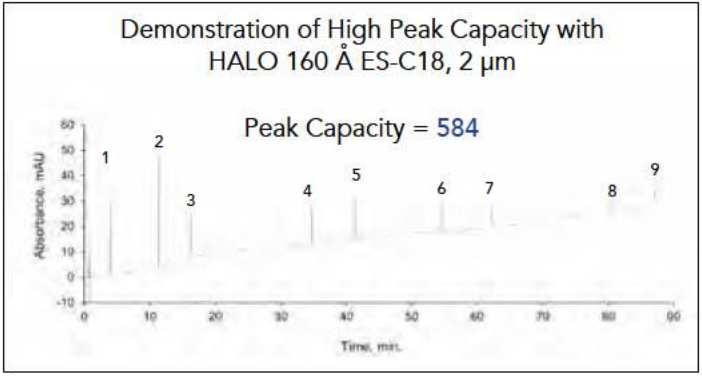


PEAK CAPACITY

Peak capacity is an important metric used to demonstrate the resolution power of the final separation strategy - tightly associated to the column(s) and how it is operated in the final method developed. It is calculated from the separation's chromatogram and the following equation:

$$Peak\ capacity = \frac{t_{R\,(last\ eluting\ peak)} - t_{R\,(first\ eluting\ peak)}}{W_{average}}$$

This number is particularly useful to gauge the separation performance of complex separations that utilize unidimensional LC (1DLC) and/or two-dimensional LC (2DLC) approaches. The following figure demonstrates the high peak capacity enabled by a HALO® column for peptide analysis.

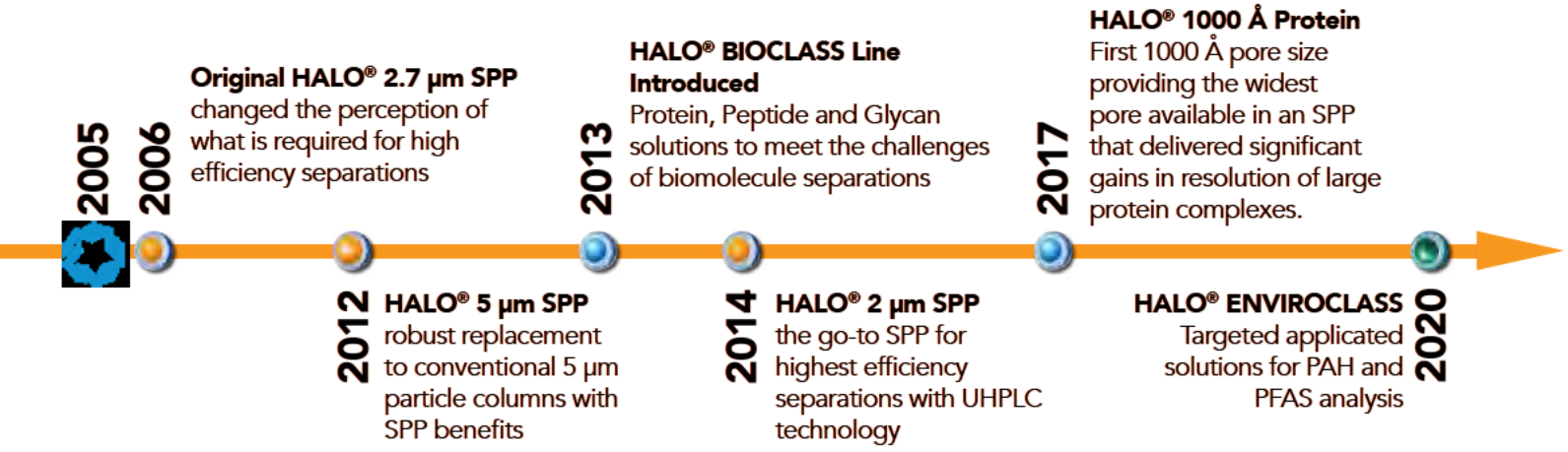


HALO® INNOVATION

What began in 2006 at AMT with the 2.7 µm HALO® particle that proved the possibility for high efficiency separations with conventional HPLC's has revolutionized the chromatography world! The innovation continues by forging a new path through manipulations of the particle morphology which has advanced the field of biopharmaceutical chromatographic separations.

The timeline below represents AMT's commitment to delivering innovative tools to enhance the separation resolution afforded by the column technology.

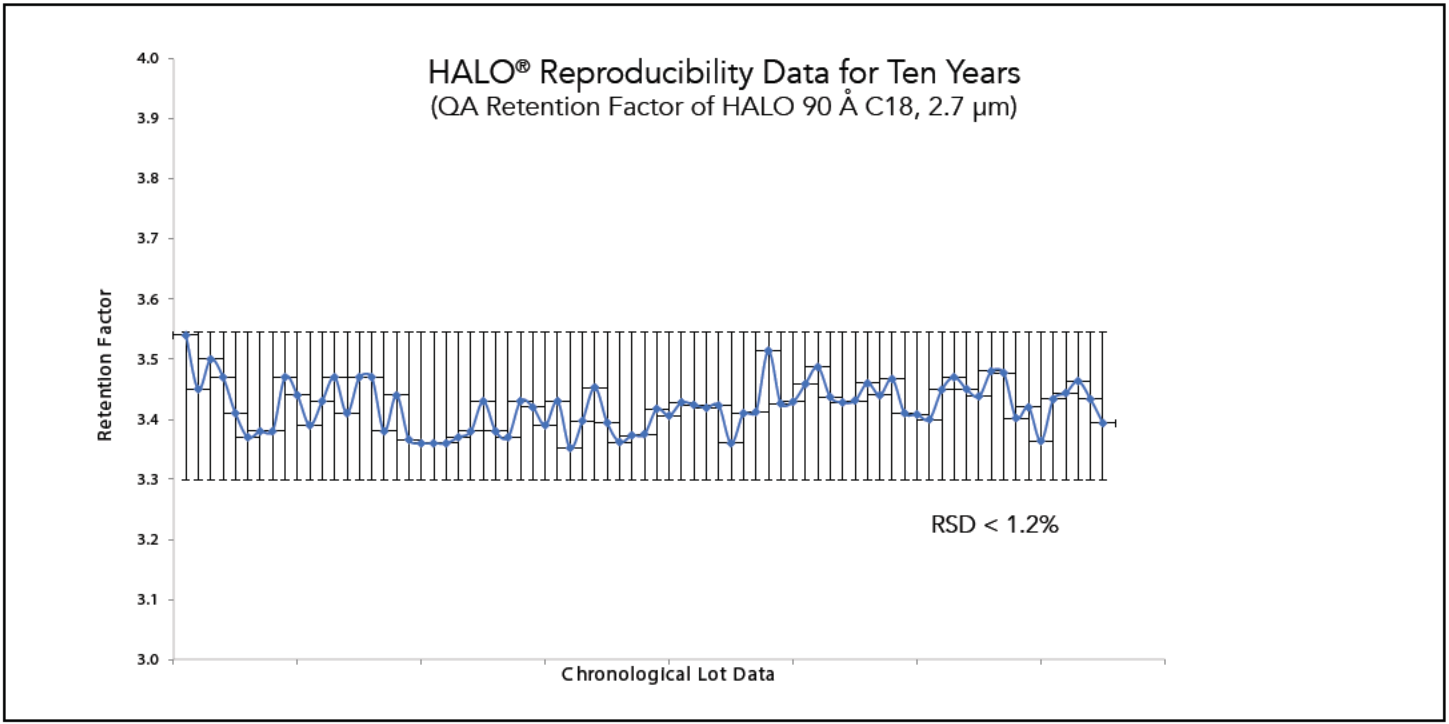
As separation demands evolve so does Advanced Materials Technology's industry leading innovation.



QUALITY

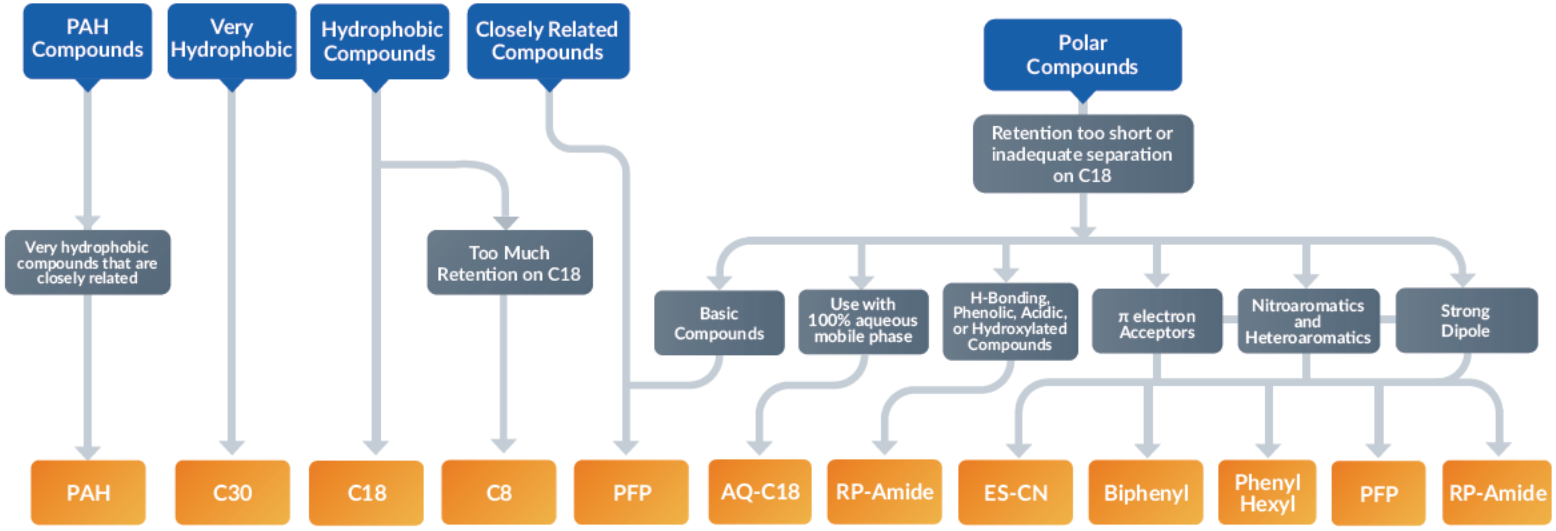
Along with the innovation that is demonstrated by HALO® products, the quality of HALO® is unsurpassed as demonstrated in the following figure, highlighting the tight control of the retention factor over a ten-year period with an RSD of less than 1.2%. Demonstrated with QA data from our original HALO 90 Å C18 provides:

Innovation You Can Trust and Performance You Can Rely On.



PRACTICAL GUIDE TO COLUMN SELECTION

In the following sections we highlight the use of two common models for column characterization to guide the analyst in comparing and selecting the stationary phase for separations. In the following figure we depict a global overview of our reversed phase chemistries.



HYDROPHOBIC SUBTRACTION MODEL (HSM)

In the Hydrophobic Subtraction Model, the phase parameters may be compared using an 'Fs value'. When two columns have similar values – they have comparable behavior; Fs>12 values represent orthogonal columns with very different reversed phase retention characteristics. The Fs values relative to HALO® C18 are listed in the following Table 1 along with the USP designations (**apps.usp.org/app/USPNF/columnsDB.html**). For more information on the Hydrophobic Subtraction Model, please refer to the **Guidebook on Reversed Phase Chemistries & Utilizing Selectivity for HPLC Separations** which can be found at **www.fused-core.com.**

Table 1.

Decreased Similarity to C18

Fs	Phase	USP type	H	S*	A	B	C (pH 2.8)	C (pH 7.0)
0	HALO C18	L1	1.100	0.040	0.000	-0.050	0.050	0.040
10.04	HALO C8	L7	0.910	0.020	-0.130	0.000	-0.010	0.180
12.07	HALO AQ-C18	L1	1.000	-0.036	0.099	-0.048	0.156	0.864
17.35	HALO Phenyl-Hexyl	L11	0.780	-0.090	-0.230	0.000	0.100	0.450
17.43	HALO C30	L62	0.938	-0.046	-0.140	0.023	0.170	0.350
22.78	HALO ES-CN	L10	0.566	-0.110	-0.344	0.021	0.126	1.150
26.76	HALO Biphenyl	L11	0.708	-0.183	-0.279	0.028	0.047	0.990
52.83	HALO RP-Amide	L60	0.850	0.080	-0.380	0.190	-0.410	0.310
94.45	HALO PFP	L43	0.702	-0.117	-0.073	-0.062	1.170	0.972

*Note HSM values listed are for 2 7 µm particle size.

REFERENCES

1. *The Hydrophobic-Subtraction Model for Reversed-Phase Liquid Chromatography: A Reprise, Sept. 01, 2016, John W. Dolan, Lloyd R. Snyder, LCGC North America, Volume 34, Issue 9, 730–741.*
2. *The hydrophobic-subtraction model of reversed-phase column selectivity, L.R. Snyder, J.W. Dolan, P.W. Carr, Journal of Chromatography A, 1060 (2004) 77–116.*

EUERBY-MODIFIED TANAKA PLOTS

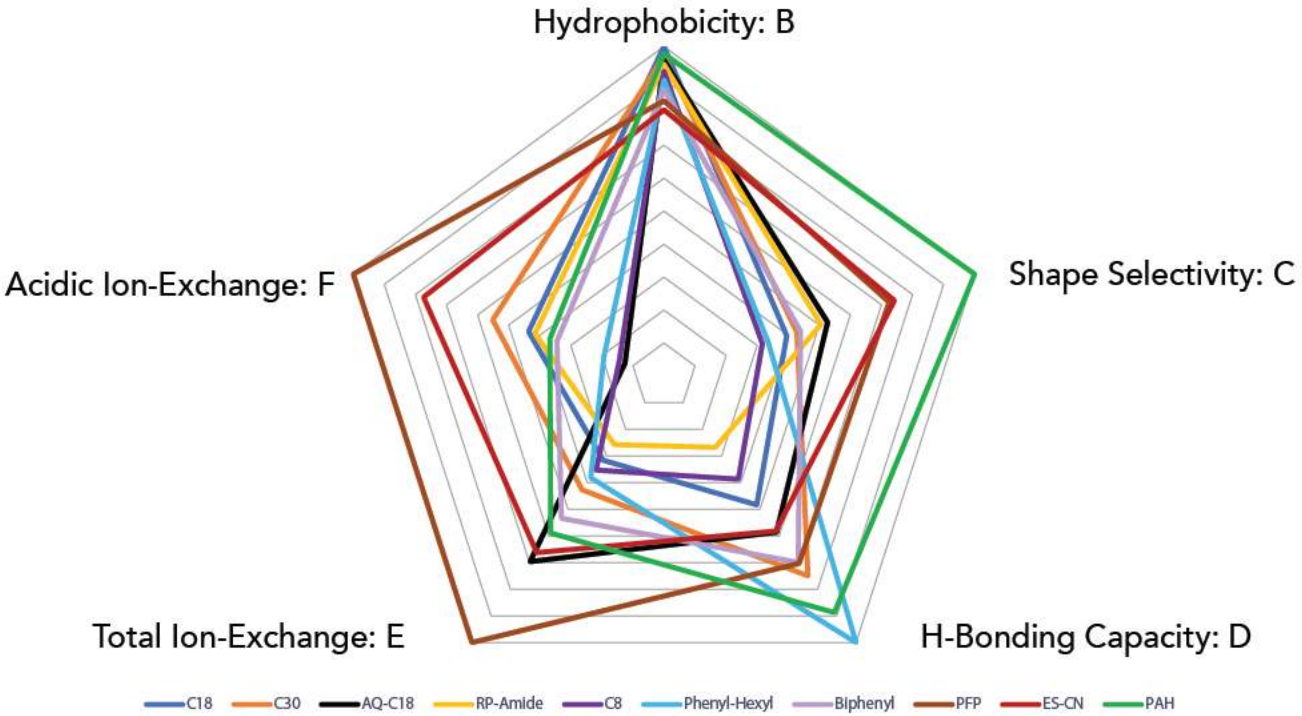
The Tanaka test was first developed to determine differences between C18 stationary phases. Six different parameters/ column properties (A,B,C,D,E,F) are determined based on the retention factors (k) of certain solute(s) and their respective selectivity factors under different isocratic and pH conditions.

Briefly, the parameters represented are:

A: amount of alkyl chains
B: hydrophobicity
C: shape selectivity
D: hydrogen bonding capacity
E: total ion-exchange capacity (pH>7)
F: acidic ion-exchange capacity (pH<3)

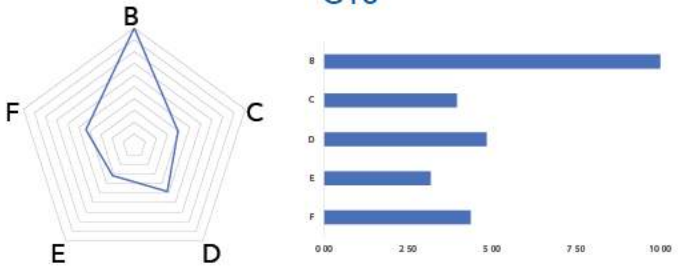
The Euerby-modified Tanaka plot is identical minus the A parameter, as the hydrophobicity parameter (B) entails this same information. The following radar plots visually compare ten of our HALO® columns for reversed phase separations, normalized at each axis for comparative purposes.

EUERBY-MODIFIED TANAKA PLOTS OF HALO® REVERSED PHASE SMALL MOLECULE CHEMISTRIES



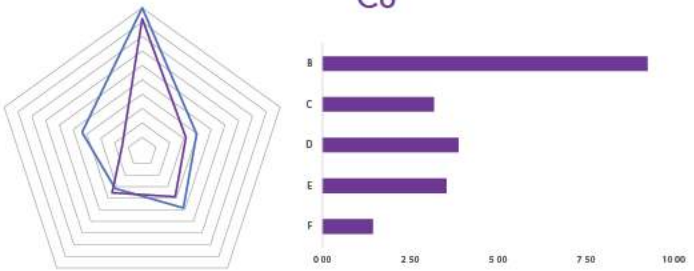
- Note: the reciprocal value was represented for elution order changes: (i) C8 – shape selectivity; (ii) Biphenyl – H- bonding capacity; and (iii) PFP – total ion-exchange.
- This global view of all the HALO® columns may be useful when trying to select a column based upon the five attributes.
- Closer inspection of each individual column's Euerby-modified Tanaka plots, key characteristics and applications are addressed in the following pages.

C18



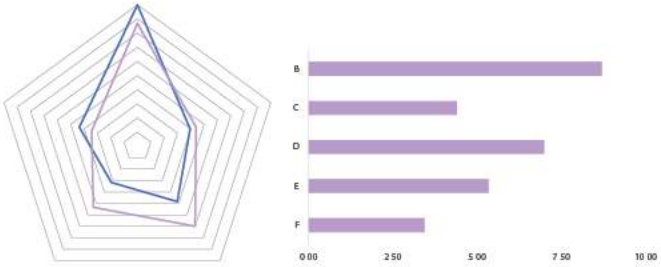
- Retention mainly governed by hydrophobicity, can offer other attributes but to a lesser extent.
- RPLC workhorse for majority of routine assays.
- Universal phase for acids, bases and neutral solutes.

C8



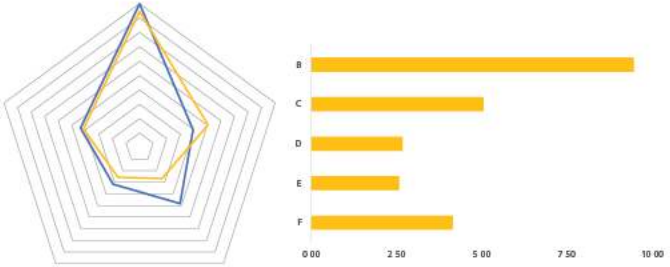
- Less hydrophobic compared to C18 and significant lower acidic ion exchange capabilities compared to C18. Shape selectivity value remained low and had an order elution change.
- Applications: cholesterol lowering pharmaceutical (statin) compounds, flavonoids, and lipid analysis of algal oil.

Biphenyl



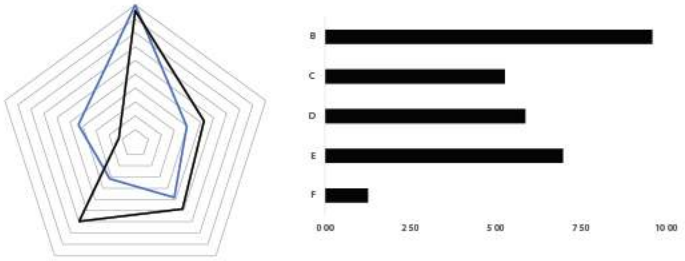
- H-bonding capacity value had an order elution change, compared to the other nine phases.
- Challenging separations that utilizes hydrogen bonding capacity, increased total ion exchange, pi-pi interactions, and relatively less hydrophobic characteristics and acidic ion exchange (reduced residual silanol interactions) compared to C18.
- Applications: opiates, polar and non-polar pesticides and via LC-MS.

RP-Amide



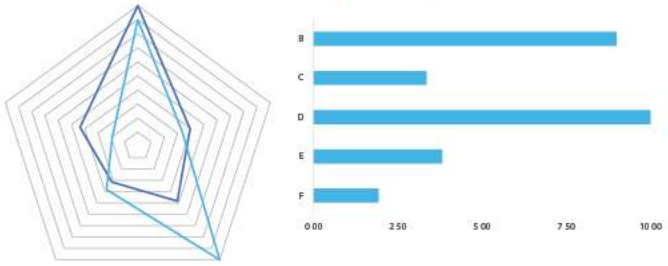
- Unique selectivity characteristics relative to C18 – increased shape selectivity and decreased hydrogen bonding capacity.
- Applications: antibiotics, cholesterol lowering drugs, phenolic acids in food and beverages, active ingredients in sunscreen, melatonin and related compounds.

AQ-C18



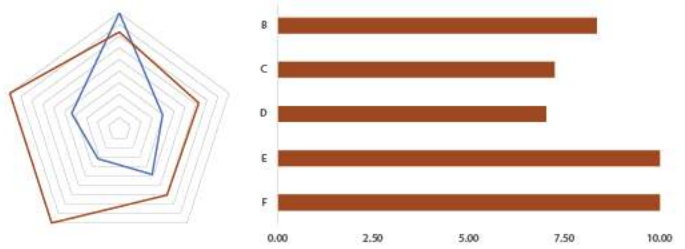
- Increased retention for polar analytes separated via RPLC and for total ion exchange capabilities.
- Offers 100% aqueous compatibility.
- Applications: polar pesticides, polar organic acids and separations that employ 100% water conditions e.g. pharmaceutical separation of nucleobases in 100% water 0.1%TFA <1.2 min.

Phenyl-Hexyl



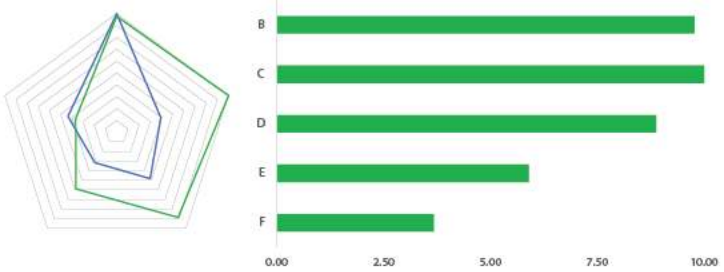
- Less hydrophobicity characteristics than C18.
- Phenyl moiety offers pi-pi interactions – with the highest H-bonding capacity compared to the other nine HALO® RP phases. Significantly decreased acidic ion exchange/residual silanol group interactions and slightly higher total ion exchange (pH>7) compared to C18.
- Applications: penicillins, anti-coagulants, and fluoroquinolone drugs.

PFP



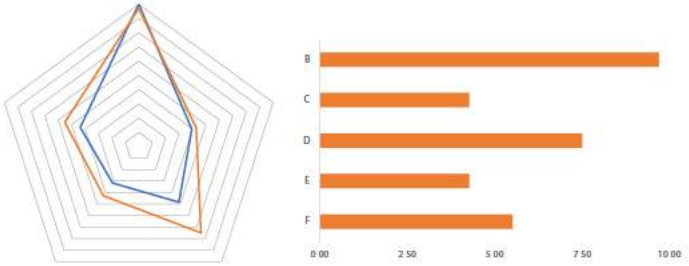
- Versatility to be employed in both RP and HILIC separations and unique characteristics in comparison to C18 phase - key to include in method development/column screening work.
- Highest ion exchange attributes and total ion exchange had an order elution change, compared to the other nine phases, shape selectivity and less hydrophobic relative to C18.
- Applications: basic drugs, mycotoxin screening, tranquilizers.

PAH



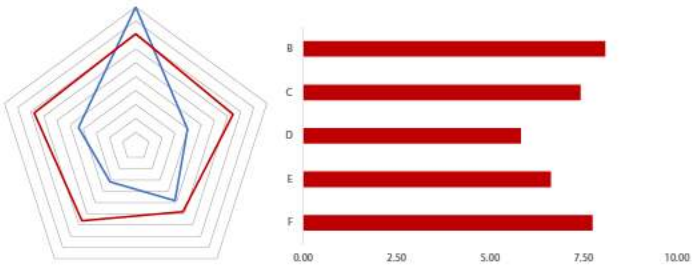
- Highest shape selectivity compared to all other nine HALO® columns.
- Increased total ion exchange in basic conditions, and vice versa for acidic conditions compared to C18. Slightly lower H-bonding capacity compared to the phenyl-hexyl phase.
- Applications associated to its tailor-designed moiety for high throughput analyses of 18 PAH contaminants.

C30



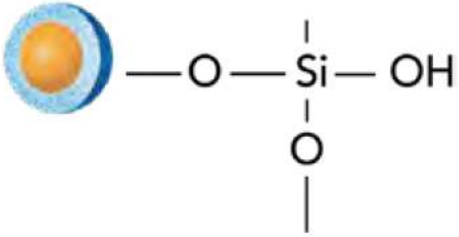
- Shape selectivity advantages compared to C18.
- Larger H-bonding capacity value may be associated to increased interactions with residual silanol groups, in turn – increased ion exchange capabilities in both acidic and basic conditions compared to C18.
- Applications: isomer separations, fat/water soluble vitamins, carotenoids, lipids, anti-inflammatory, anti-lymphatic and anti-allergy steroids.

ES-CN



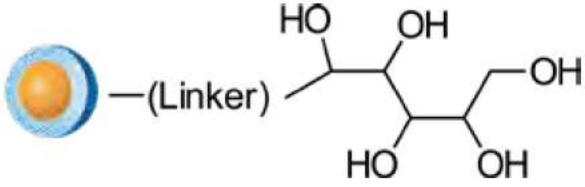
- Less hydrophobic, increased ion exchange capabilities, shape selectivity, lone pair of electrons of the phase's CN moiety provide unique RP interactions - compared to C18.
- Another must have tool for method development/column screening.
- Applications: ß-lactam antibiotics, NSAIDs and penicillins.

HILIC



- Ideal for polar analytes.
- Alternate mode to reversed phase modes.
- Can be used in HILIC and normal-phase modes.

Penta-HILIC



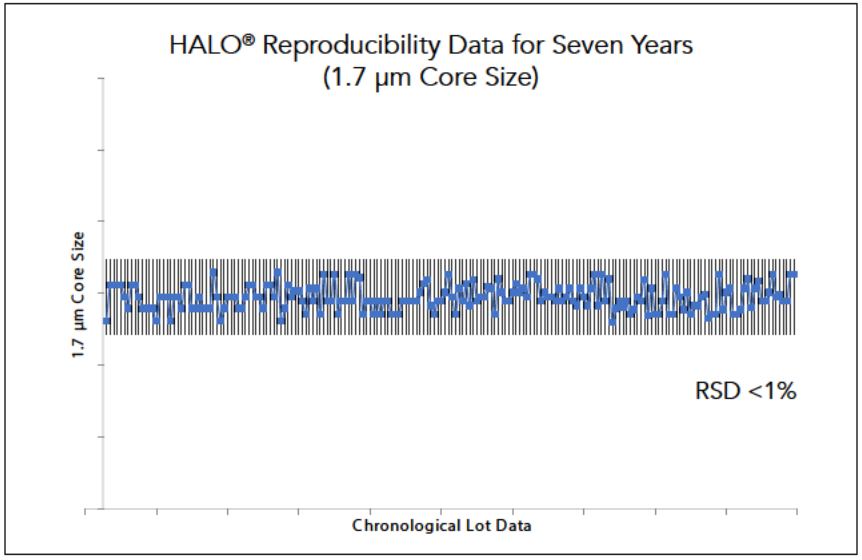
- Ideal for polar compounds poorly retained in RPLC.
- Alternate selectivity for HILIC mode.
- Excellent peak shape for basic compounds in HILIC mode.

HALO® ENSURING SUCCESS – QUALITY AND RELIABILITY

QUALITY

The quality of AMT products stems from its complete control over the entire manufacturing process – from the start of high quality raw materials to the final particle, quality controls and measurements are built into every process. Cores and shells are carefully controlled resulting in tight particle size distributions for optimized loading parameters that culminates to a highly efficient performance packed column. Rigorous quality assurance testing is completed for every lot. The following figure shows data for the solid silica core that is the foundation of Fused-Core® particles.

The tight control of particle size distribution shown in the following figure represents an impressive %RSD of less than 1% over the last five years highlighting the upmost quality in our manufacturing process associated to a narrow particle distribution. This result contributes greatly to our commitment for customers' long-term repeatability of separations utilizing AMT powered HPLC columns.



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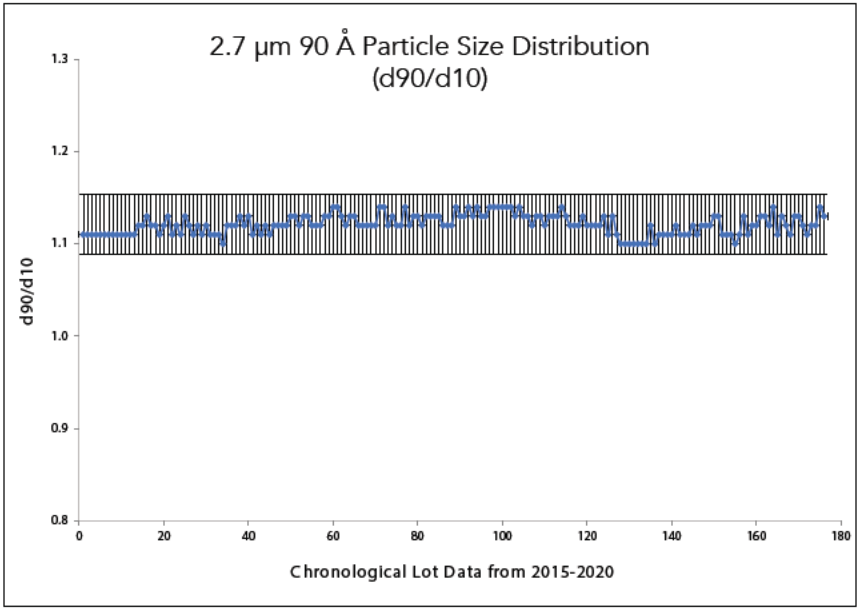
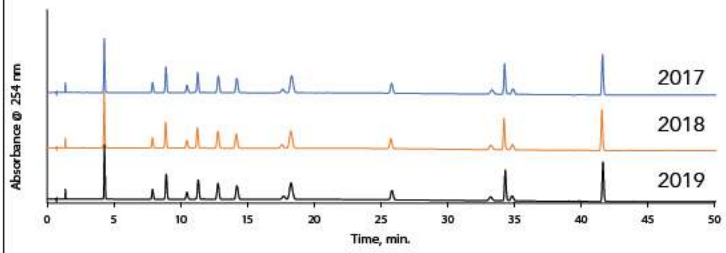


With such control over the entire manufacturing process, it is no surprise that the chromatography from HALO® columns is highly reproducible.

LOT TO LOT DATA

Repeatability of the separation profile attributes from different manufactured lots and different years represent AMT's commitment to deliver the highest quality products; specific applications for both small and large molecule separations are shown.

Lot to Lot Comparison of HALO® C18, 2.7 µm used in EP 9.4 for Paracetamol

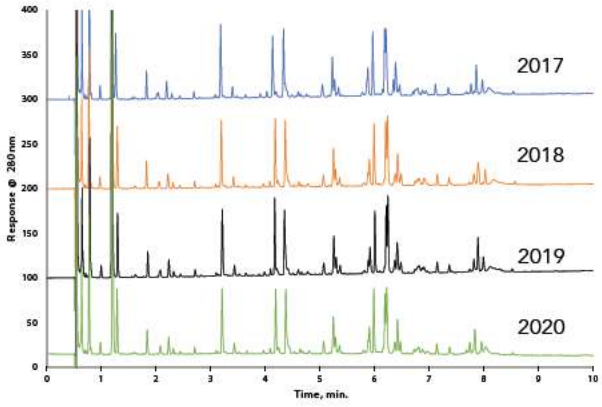


METHOD CONDITIONS

Mobile Phase A: pH 7 Phosphate buffer (prepared by dissolving 1.7g of potassium dihydrogen phosphate and 1.8g of dipotassium hydrogen phosphate in HPLC grade water and diluting to 1000mL with water

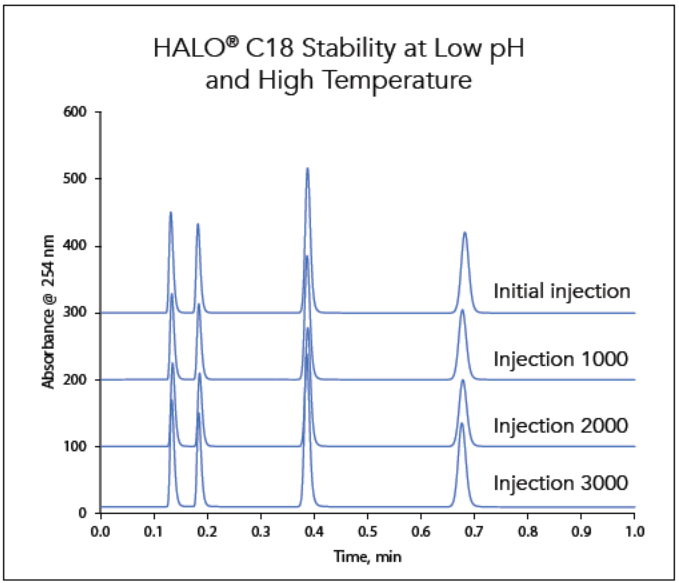
Mobile Phase B: Methanol

HALO® Peptide 160 Å ES-C18 Lot to Lot Comparison of an Adalimumab Digest



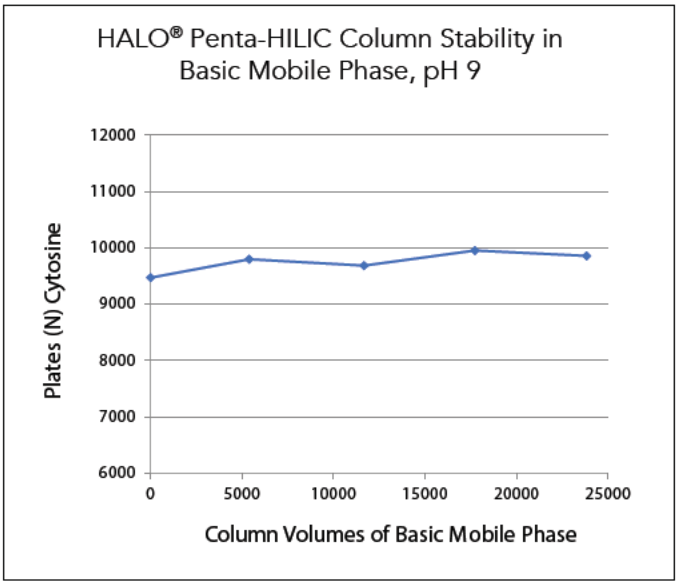
STABILITY

The longevity of our superficially porous technology was showcased at low pH and relatively high temperature for 3000 injections (approximately 23,000 column volumes); the results illustrated no significant changes.



HILIC STABILITY AND SEPARATION PERFORMANCE

Our quality transcends the limits of not only reversed phase chromatography product lines, but is also inclusive of high quality HILIC products. The stability of our Penta-HILIC column was tested at a challenging environment of high pH 9.0 and demonstrated superior performance (no loss in number of theoretical plates of cytosine) for over 20,000 column volumes of mobile phase consumption.



COMPANY PROFILE

Advanced Materials Technology founded in 2005 has been focused on one mission – Improving the presentation of the sample to the detector. Using our novel Fused-Core® particle design, we have challenged conventional wisdom and engineered innovative solutions for the separations community.

All company operations and functions are proudly located in Wilmington, Delaware, USA.

AMT invites a company culture of diversity, respect and pride in delivering quality products. We embrace ISO 9001 standards in our work systems and daily work. We pledge to have a dynamic leadership team which promotes our culture of excellence embedded in every employee.

Joseph J. DeStefano B.S., M.S., Ph.D.

Chairman of the Board, CSO, Co-Founder

Co-founder of AMT with over 50 years of experience in silica product development, research supervision, and business management with the DuPont Company, Rockland Technologies, Hewlett Packard where he was integral in the business development of the well-known Zorbax stationary phase, and Agilent Technologies. Previously, President and co-founder of Rockland Technologies.

Dr. DeStefano holds a B.S. degree in Chemistry from University of Connecticut and Ph.D. in Analytical Chemistry from University of Delaware. At AMT he is involved in strategic planning of new products, applications development and business relationships.

Timothy J. Langlois B.S.

President, Co-Founder

Active for 25 years in silica technology development, quality assurance, and manufacturing. Prior experience as a product engineer at Rodel (now DuPont Microelectronics). Later held R&D, operations and engineering positions at Hewlett-Packard (now Agilent Technologies). Mr. Langlois holds a B.S. degree in Chemical Engineering from Lehigh University.

Mr. Langlois was involved in the start-up of AMT and instrumental in the development of HALO®. As President he is involved in strategic business decisions for AMT including R&D, operations, business development and sales initiatives.

Dr. Joseph "Jack" Kirkland

Principal Scientist, Founding Vice President, R&D

Regarded as a founding father of HPLC Jack is highly renowned for his contributions in the field of chromatography and pioneering superficially porous particle technology with >160 publications, 32 patents and 8 books. Together with Lloyd Snyder, he educated more than 5000 people in the principles and practice of HPLC by means of the first American Chemical Society short course and was a major contributor to the hydrophobic subtraction model of reversed phase column selectivity used widely today. His research interests involved HPLC method development, field flow fractionation, silica chemistry and silane bonding reactions (i.e., novel HPLC columns).

His long list of awards is too long to include, but is highlighted by the 1972 American Chemical Society Award in Chromatography, the 1982 Torbern Bergman Medal in Analytical Chemistry from the Swedish Chemical Society, DuPont's Lavoisier Medal in 1997 (DuPont's highest technical award), the the A. J. P. Martin Chromatography Award Medal in 1997, and the first Uwe Neue Award in 2013 for achievements by an industrial scientist, an honorary D.Sc. degree by Emory University in 1974 and the 2014 LCGC Lifetime Achievement in Chromatography Award.

ADDITIONAL TECHNICAL RESOURCES

Application notes, guidebooks, white papers, and technical reports can be found on our website at:

www.fused-core.com

INNOVATION YOU CAN TRUST – PERFORMANCE YOU CAN RELY ON

HALO®

advancedmaterialstechnology

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