

Ibuprofen and Impurities Method Transfer from HPLC to U-HPLC

The modified European Pharmacopeia (EP) HPLC method for ibuprofen and related substances has been transferred to U-HPLC, reducing analysis time while maintaining chromatographic integrity.

Sub-2 μ m particle packed columns offer advantages over the more traditional columns packed with 3 and 5 μ m particles by providing shorter analysis times, improvements in resolving power, sensitivity and peak capacity. When transferring methods from HPLC to U-HPLC if both particle size and column dimensions are also reduced, significant savings in analysis time and solvent consumption can be achieved. However, care must be taken to ensure operating flow rate, gradient profiles and injection volumes are scaled appropriately to obtain an equivalent or superior separation. This application note illustrates the savings that can be made when transferring a method (adapted from the EP) from a 150 x 4.6 mm 5 μ m column to a 50 x 2.1 mm column packed with 1.9 μ m particles.

Experimental Conditions

Original HPLC Method

Instrument:	Accela™ U-HPLC system	
Column:	Hypersil GOLD™ 5 μ m, 150 x 4.6 mm	
Part Number:	25005-154630	
Mobile Phase:	A: 0.05% H ₃ PO ₄ in H ₂ O/ACN (66:34) B: ACN	
Gradient:	Time (min)	% B
	0	0
	25	0
	55	85
	70	85
Flow Rate:	1.0 mL/min	
Injection Volume:	10 μ L	
Detection:	UV at 214 nm (0.1 s rise time; 20 Hz)	
Temperature:	30 °C	

Fast U-HPLC Method

Instrument:	Accela U-HPLC system	
Column:	Hypersil GOLD 1.9 μ m, 50 x 2.1 mm	
Part Number:	25002-052130	
Mobile Phase:	A: 0.05% H ₃ PO ₄ in H ₂ O/ACN (66:34) B: ACN	
Gradient:	Time (min)	% B
	0	0
	3.2	0
	7.1	85
	8.9	85
Flow Rate:	0.55 mL/min	
Injection Volume:	0.7 μ L	
Detection:	UV at 214 nm (0.1 s rise time; 20 Hz)	
Temperature:	30 °C	

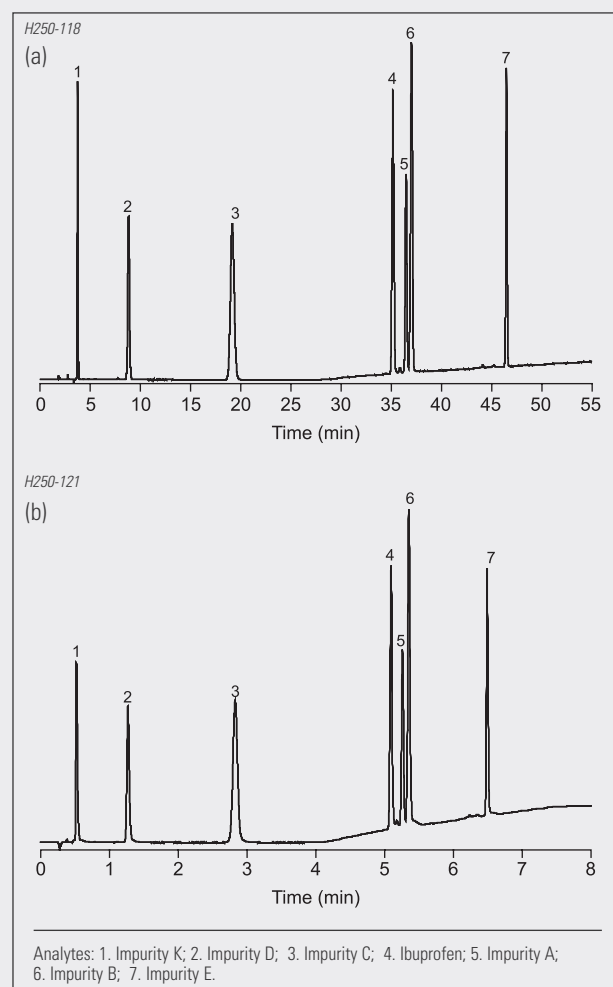


Figure 1: Chromatograms obtained with the original HPLC and geometrically scaled U-HPLC method.

Results and Discussion

The method was transferred geometrically to the 50 x 2.1 mm, 1.9 μ m column by scaling the flow rate, injection volume and gradient profile to give an equivalent separation in terms of chromatographic efficiency. The chromatographic profiles obtained for the original HPLC method (a) and the geometrically scaled U-HPLC method (b) are shown in the figure above. The resolution (USP) of peaks 5 and 6 is maintained (1.6) while analysis time is reduced by approximately seven-fold (last peak elutes at 46.5 min and 6.5 min, respectively). If column re-equilibration time between runs is taken into consideration then an eight-fold reduction in analysis time and a fourteen-fold reduction in solvent consumption were observed with the U-HPLC method.

References

1. L. Pereira et al, Poster presented at Pittcon 2007, Chicago (reference P020371_E 02/07)

Porous Graphitic Carbon for the LC/MS Analysis of Hydrophilic Peptides

The advantages of using porous graphitic carbon (PGC) in the LC/MS analysis of di-, tetra- and penta-peptides containing polar and basic terminal amino acid residues are demonstrated.

Small hydrophilic peptides are not retained and, therefore, are often found in the flow-through fraction from a C18 LC column, the type of stationary phase most commonly used for the separation of proteolytic digests of proteins. The analysis of the flow-through fraction requires either a stationary phase that can retain the peptides away from the solvent front, where the biological salts and buffers elute, or a sample clean-up step to remove the salts. PGC's retention mechanism involves a charge-induced interaction of the polar analyte with the polarizable surface of the graphite.² PGC is an ideal stationary phase ideal to retain and resolve very polar, hydrophilic molecules, which are normally not retained under reversed-phase LC using typical MS compatible mobile phases. In this application note it is demonstrated how PGC columns increase capacity factors over alkyl-silica columns for small hydrophilic peptides.

Experimental Conditions

Instrument:	Surveyor™ and LCQ™ Deca
Columns:	Hypercarb™ 5 μm, 50 x 2.1 mm Hypersil GOLD 5 μm, 100 x 2.1 mm
Part Numbers:	35005-052130 and 25005-102130
Mobile Phase:	A: H ₂ O + 0.1% Formic acid B: ACN + 0.1% Formic acid
Gradient:	5 to 100% B in 10 min
Flow Rate:	0.2 mL/min
Injection Volume:	10 μL
Detection:	+ ESI
Temperature:	30 °C

Results and Discussion

In the figure the retention of a di-, tetra- and a penta-peptide is compared on the alkyl-silica phase and on PGC. On the alkyl-silica phase, typically used in the separation of proteolytic digests, RGES elutes at the solvent front, closely followed by DSDPR. The basic (Arg) and alcohol (Ser) terminal residues make these short peptides hydrophilic and difficult to retain under conventional reversed-phase LC/MS conditions. On the PGC column these short peptides are well retained away from the solvent front. PGC provides higher retention (capacity factor) and different selectivity.

References

1. E. T. Chin, D. I. Papac, *Anal. Biochem.* 273 (1999) pp 179–185
2. P. Ross, *LCGC Europe*, May 2000

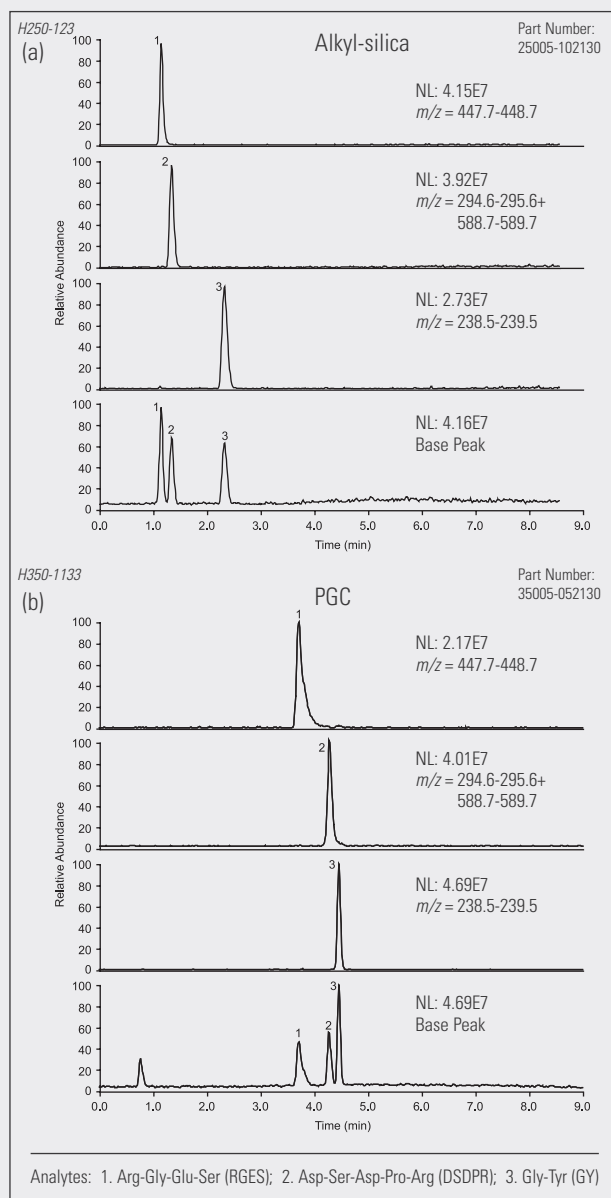


Figure 1: Comparison of the retention of 3 hydrophilic peptides on alkyl-silica and porous graphitic carbon. PGC provides higher retention and different selectivity.

On-Line Sample Clean-Up for the LC/MS Analysis of Small Molecules in Serum

On-line sample clean-up utilizing SEC for the removal of matrix interferences from biological samples reduces the total time required for analysis and has the added advantage of being easily automated.

Drug discovery/detection commonly focuses on analytes which are present in biological matrices. Direct injection of such samples onto LC and LC/MS systems is problematic as analytes of interest can be 'lost' in the concentrated matrix peak, plus instrument and column contamination readily occurs. Traditionally, solid phase extraction (SPE) has been used for sample pre-treatment and clean-up, but this can be time consuming and adds an additional stage to the analytical method.

The ability to automate biological assays, minimize the number of steps and reduce overall analysis time is becoming increasingly important. On-line sample clean-up utilizing size exclusion chromatography (SEC) fulfills these criteria. On-line SEC is a two-dimensional approach in which serum is injected directly onto a small pore size exclusion column and then the analytes, once separated from the matrix, are eluted onto an analytical column. On-line SEC reduces sample preparation time, minimizes potential analyte loss and results in increased productivity and higher sample throughput.¹

Experimental Conditions

Instrument: Surveyor™/LCQ™ Deca LC/MS		
Columns:	BioBasic™ SEC-60 5 μm, 150 x 2.1 mm* Hypersil GOLD™ 3 μm, 50 x 2.1 mm	
Part Numbers:	73305-152130 and 25003-052130	
Mobile Phase:	A: 10 mM ammonium formate, pH 3.0 B: MeOH	
Gradient:	Time (min)	% B
	0*	0
	0.75	0
	0.80	13
	7.0	13
	7.10	100
	10.10	100
	10.20	13
	13.20	13
Flow Rate:	500 μL/min	
Injection Volume:	2 μL	
Detection:	+ ESI	
Temperature:	45 °C	

* SEC column equilibrated with 100% buffer for 2 min before start of gradient, eluent diverted to waste.

Results and Discussion

To determine the time to divert the flow to waste after sample injection, a comparison of the injections of serum alone and serum dosed with the analyte was performed. Figure 1a shows the resultant UV trace for blank serum. Lamivudine (the least retained of the analytes) was found to elute at ~ 0.8 minutes, therefore the switch valve was set to direct flow to the analytical column at 0.75 minutes. The major serum peak elutes earlier at ~ 0.5 minutes and, therefore, is prevented from contaminating the analytical column.

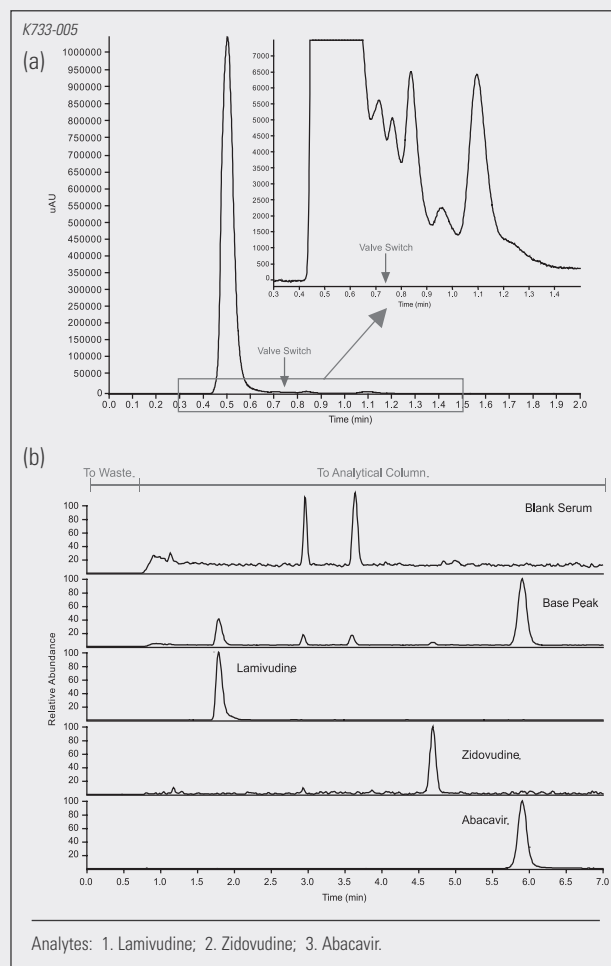


Figure 1: (a) Blank serum SEC extraction to determine valve switch time. (b) On-Line SEC Extraction of Anti-Retrovirals from Serum

The separation of the drugs from serum with on-line SEC teamed with isocratic analytical separation is depicted in Figure 1b. The percentage recoveries achieved were greater than 100, 89 and 79% for abacavir, lamivudine and zidovudine, respectively. During the course of experimentation, over 110 runs were performed with no significant deterioration in column performance observed, illustrating that the methodology is robust and reproducible.

References

1. C.E. Blythe et al, Poster presented at HPLC 2007, Ghent, Belgium. (Reference P020389_E06/07)

SPE-LC/ESI/MS Method for the Analysis of Polar Micro Pollutants in Environmental Waters

An SPE-LC/ESI/MS method for the analysis of polar degradation products of atrazine allows their determination in environmental waters at the 0.1 µg/L level.

Increasingly, environmental protection legislation designates maximum levels permitted for micro-pollutants in water below the µg/L level. These detection levels require sample preparation and pre-concentration because they are too dilute. In solid phase extraction (SPE), the analytes are extracted provided they are retained by the sorbent and not eluted too rapidly by the water in the sample. However, trace analysis of very polar pollutants is still a challenge, since traditional C18-silica and PS-DVB materials do not adequately retain these compounds.¹ Porous graphitic carbon (PGC, Hypercarb) retains highly polar and water-soluble compounds and is useful for the trace-level determination of polar water pollutants.

This application presents a SPE procedure and LC/ESI/MS method using Hypercarb for the analysis of polar degradation products of atrazine (ammeline - ANE, ammelide - ADE, atrazin-desethyl-desisopropyl - DEIA, atrazin-desethyl - DEA, atrazin-desisopropyl - DIA, Cyanuric acid - Cya) that allow their detection in water at the 0.1 µg/L level.

Experimental Conditions

SPE

Compounds:	ANE, ADE, DEIA, DEA, DIA, Cyanuric acid
Phase:	HyperSep™ Hypercarb™
Part Number:	60106-402
Volume:	6 mL
Bed Weight:	500 mg
Conditioning:	10 mL MeOH followed by 10 mL H ₂ O, vacuum at 3 mm Hg
Application:	500 mL, vacuum at 10 mm Hg
Elution:	6 mL (MeOH/THF, 1:1) + 0.1% TFA (stand for 1 min, vacuum at 3 mm Hg), 6 mL (MeOH/THF, 1:1) + 0.1% TFA (vacuum at 3 mm Hg).

The sample was dried under nitrogen and re-dissolved in 1 mL of H₂O.

LC/ESI/MS

Column:	Hypercarb 5 µm, 100 x 2.1 mm
Part Number:	35005-102130
Instruments:	Surveyor™ HPLC and LCQ™ Deca MS
Mobile Phase:	A: H ₂ O + 0.1% Formic acid B: ACN + 0.1% Formic acid
Gradient:	10 to 100% B in 10 min
Flow Rate:	0.2 mL/min
Injection Volume:	10 µL
Detection:	+ ESI (SIM MS ([M + H] ⁺) for ANE, ADE, DEIA, DEA, DIA; - ESI ([M - H] ⁻) for cyanuric acid
Temperature:	68 °C

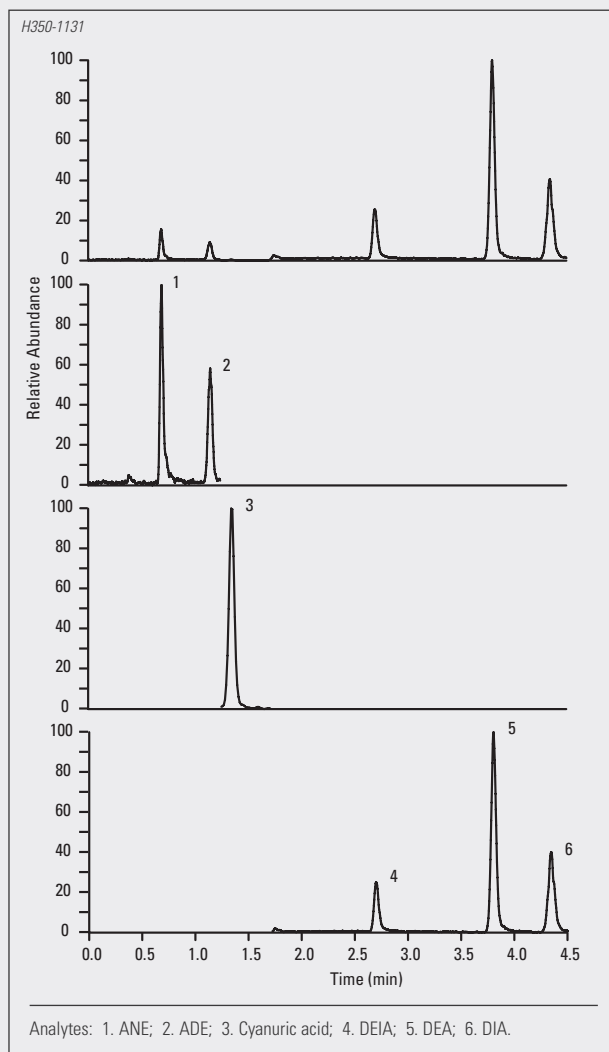


Figure 1: LC/ESI/MS trace for standard solution containing the six pollutants.

Results and Discussion

A typical trace for the separation in LC/ESI/MS is shown in Figure 1. Average recoveries for a pre-concentrated, 500 mL of water spiked with 0.1 µg/L each of the six polar degradation compounds ranged between 75 and 100%. These results demonstrate that the use of PGC in SPE to extract very polar pollutants from water will allow the loading of high volumes of water when target analyte pre-concentration is required to detect trace pollutants. Also PGC as a stationary phase for the LC separation retains very polar molecules and allows the use of weakly buffered mobile phases ideal for sensitive MS detection.

References

1. Pichon, L. Chen, S. Guenu, and M.C. Hennion, *J. Chromatogr. A*, 711, pp 257–267 (1995)

Rapid Analysis of Priority Phenolic Pollutants with Hypersil GOLD 1.9 μ m and U-HPLC

Transferring a method for the analysis of priority phenolic pollutants using Hypersil GOLD columns to U-HPLC significantly increases analysis speed and sensitivity

Phenolic compounds are of environmental importance due to their relatively high toxicity at low levels and their presence in environmental waters and organic matter, following degradation of a range of industrial products such as pesticides and herbicides.

Reverse-phase liquid chromatography (RP-LC) has been shown to effectively separate and detect a range of phenolic compounds at low ppb levels, following various extraction methods. Such methods provide a realistic alternative to traditional analytical approaches using gas chromatography (GC), which tend to be hindered by lengthy sample preparation/analysis times and difficulty in derivitization of certain phenols. We now demonstrate the effect of using Hypersil GOLD and 1.9 μ m particles on the separation and analysis speed of a number of priority phenols cited within the US Environmental Protection Agency (EPA) and European Union (EU) lists of priority pollutants.^{1,2}

Experimental Conditions

Standard HPLC Conditions

Instrument:	Surveyor™ LC
Column:	Hypersil GOLD™ 5 μ m, 150 x 2.1 mm
Part Number:	25005-152130
Mobile Phase:	A: H ₂ O + 0.1% Acetic acid B: MeOH + 0.1% Acetic acid
Gradient:	5% B (Hold for 1.5 mins) to 95% B on 19.5 mins (Hold for 1.5 mins)
Flow Rate:	0.6 mL/min
Injection Volume:	10 μ L
Detection:	UV diode array (270-320 nm)
Temperature:	60 °C

U-HPLC Conditions

Instrument:	Accela™ High Speed LC
Column:	Hypersil GOLD 1.9 μ m, 100 x 2.1 mm
Part Number:	25002-102130
Mobile Phase:	A: H ₂ O + 0.1% acetic acid B: MeOH + 0.1% acetic acid
Gradient:	5% B (Hold for 0.6 mins) to 95%B on 7.8 mins (Hold for 0.6 mins)
Flow Rate:	1.0 mL/min
Injection Volume:	1 μ L
Detection:	UV diode array (270-320 nm)
Temperature:	60 °C

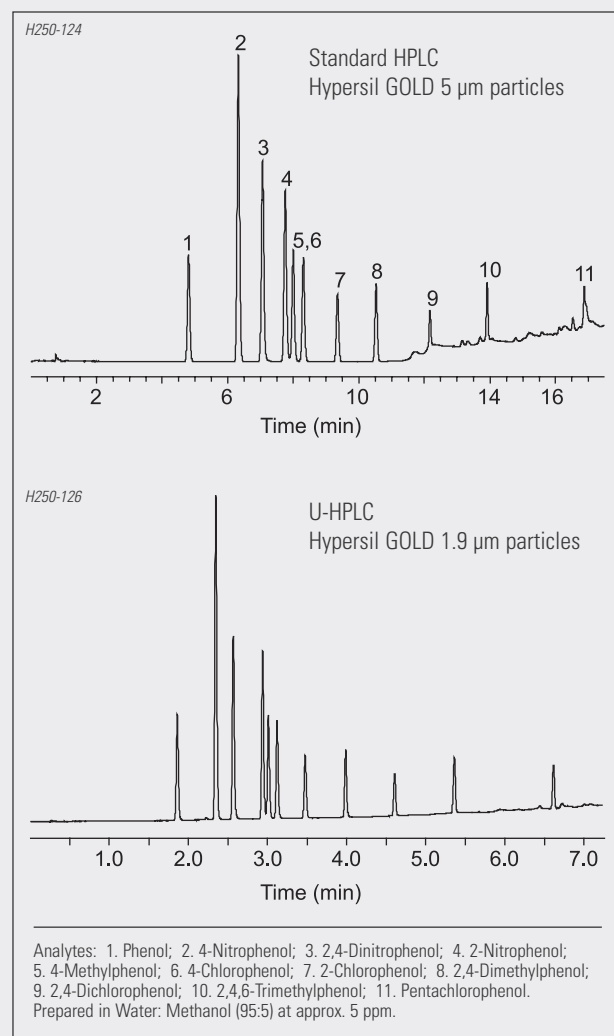


Figure 1: Separation of eleven priority phenolic pollutants. Analysis time is reduced from 17 to 7 minutes.

Results and Discussion

A number of priority phenols can be successfully separated using Hypersil GOLD columns. Method transfer from 5 μ m to 1.9 μ m particles and shorter column lengths allows significantly faster analysis times (a reduction of approximately three times in this example), while maintaining excellent resolution and reducing peak widths, resulting in increased peak heights and improved sensitivity.

References

1. N. Jones et al Poster presented at HPLC 2007, Ghent, Belgium (Reference: PO20390_E06/07).
2. US Environmental Protection Agency Method 625, 1984.

Nandrolone Analysis

As steroid use becomes more complex, doping control agencies need a highly sensitive, discriminatory tool to detect these drugs. Hypersil GOLD columns provide symmetrical peak shapes leading to enhanced sensitivity and separation of structurally similar steroids.

The use of anabolic and androgenic steroids by athletes help them to increase muscle mass and improve athletic performance. Steroid use is banned in competitive sport, and doping control laboratories regularly test competitors at all levels, both at competitions and in training sessions, to ensure they are not gaining an unfair advantage over others by chemically assisting their performance.

At large sporting events, the number of samples submitted is very large. Both the testing laboratories and sports governing bodies are under a great deal of pressure to detect banned drugs, such as nandrolone, as quickly and efficiently as possible. Since many steroids do not remain in the body in an unchanged format, the testing laboratories also need to detect analytes resulting from the degradation of the original parent drug, or produced by the action of the parent drug on other biological analytes. These secondary analytes are often structurally similar to the parent drug.

High sample throughput of structurally similar compounds requires a column chemistry that can easily resolve individual analytes and generate sharp, symmetrical peaks for ultimate quantification confidence. An important requirement of the analytical method is its sensitivity. Athletes who do not want to be caught taking performance-enhancing drugs will often cease taking the drugs for a period of time before competition to allow the drug to leave their bodies. Often, trace levels of the drug will still be present after this time. Using a column that offers enhanced resolution and sensitivity provides greater opportunity for drug detection.

Experimental Conditions

Column:	Hypersil GOLD™ 5 µm, 150 x 4.6 mm
Part Number:	25005-154630
Mobile Phase:	A: H ₂ O B: ACN
Isocratic:	43:57
Flow Rate:	1.0 mL/min
Detection:	UV at 254 nm
Temperature:	25 °C

Results and Discussion

The chromatogram shown in Figure 1 demonstrates the fast analysis of steroids. The run time of less than 5 minutes using isocratic conditions allows rapid sample turnaround and helps reduce overall analysis cost. Hypersil GOLD's ultra-pure base silica and state-of-the-art bonding generate highly symmetrical peaks, and allow structurally similar analytes to be positively differentiated from each other, decreasing the risk of inaccurate peak identification, and increasing peak height, both of which improve the confidence in the method.

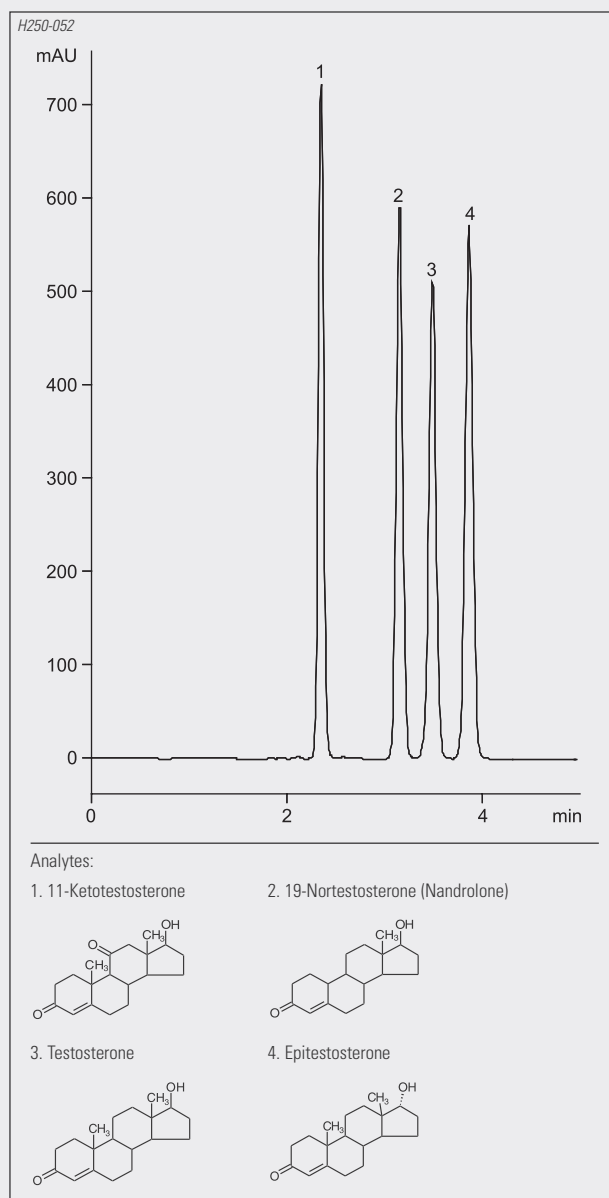


Figure 1: Separation of four testosterone derivatives, including nandrolone, in less than 5 minutes.

HPLC Analysis of FOX-7 using a Porous Graphitic Carbon Column

The strong retention properties of porous graphitic carbon (PGC) for very polar analytes were used to retain and analyse FOX-7 (2,2-dinitro-ethene-1,1-diamine) with HPLC.

The explosive FOX-7 is an extremely polar compound which is very difficult to retain in conventional reversed phase conditions without the addition of an ion pair reagent. When using a C18 column under standard conditions FOX-7 elutes with the solvent front.¹

PGC is a material that provides strong retention of very polar compounds. The retention mechanism involves a charge-induced interaction of the polar analyte with the polarizable surface of the graphite.² This so-called “polar retention effect on graphite” (PREG) can be controlled by the use of electronic modifiers, which compete with the polar analytes to the surface of the graphite reducing retention.³ In this application note the retention of FOX-7 is demonstrated on PGC using a mobile phase containing an electronic modifier, either TFA or ammonia.

Experimental Conditions

Column:	Hypercarb™ 5 µm, 100 x 3 mm
Part Number:	35005-103030
Mobile Phase:	A1: H ₂ O + 1% TFA A2: H ₂ O + 1% NH ₃ B1: ACN + 1% TFA B2: ACN/ H ₂ O/ NH ₃ (96:3:1)
Gradient:	Time (min) % B (B1 or B2) 0 0 3 0 7 100 24 100
Flow Rate:	0.8 mL/min
Injection Volume:	10 µL
Detection:	UV at 278 or 272 nm

Results and Discussion

Due to the PREG, FOX-7 did not elute with an acceptable retention even when a strong organic eluent such as acetonitrile was used. When 1% trifluoroacetic acid (TFA) was added to the mobile phase, as a competitive electronic modifier, the polar retention was reduced and the analyte eluted at 14 minutes with good peak shape (Figure 1a). The suppression of polar interaction was also possible using a basic modifier, ammonia (NH₃) in the mobile phase, in which case the retention time was 11 minutes (Figure 1b). This methodology was successfully applied to the analysis of possible by-products in the synthesis of FOX-7.¹

References

1. E. Holmgren, P. Goede, N. Latypov, C. Crescenzi, H. Carlsson, Poster 2001
2. P. Ross, LCGC Europe, May 2000
3. J. Knox, P. Ross, *Advances in Chromatography*, 1997, vol. 37, pp 120–161

(Data courtesy of C. Crescenzi and H. Carlsson, Univ. of Stockholm, Sweden)

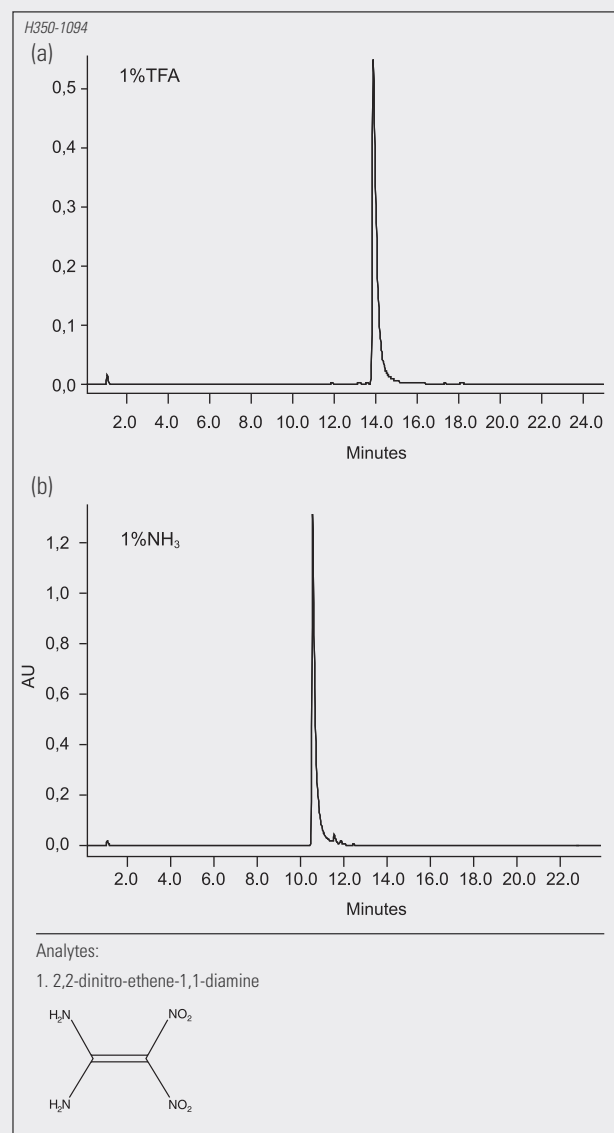


Figure 1: Retention of FOX-7 on PGC using an acidic (TFA) or basic (NH₃) electronic modifier.

Analysis of Aromatic Amines Produced by Degradation of Azo Dyes

Hypersil GOLD columns generate highly symmetrical, narrow peaks to provide the ultimate sensitivity and quantification required for the accurate analysis of closely related, highly basic analytes.

Sixty-five percent of the organic colorants in use around the world are azo dyes.¹ They provide intense, bright colors that are stable and highly cost-effective due to the simple manufacturing methods. Their manufacture is also environmentally friendly as it takes place in water, so there is little solvent disposal or recycling required afterwards. The majority of azo dyes are not considered dangerous; only about 5% are considered potentially hazardous because, under reduction conditions, they degrade to produce aromatic amines, a small number of which are classified as being carcinogenic or potentially carcinogenic to humans.

New legislation by the European Commission to protect public health proposes that an amendment to Council Directive 76/769/EEC, "Marketing and Use of Azo Colorants" be made to restrict the use of azo dyes.^{2,3} In order to protect human health, those azo dyes, which can break down under reductive conditions to release any of a group of defined aromatic amines, are prohibited from being used in those consumer goods that are considered to have regular skin contact (such as wristwatch straps, bed linen, textiles, toys etc). The EU Directive 2002/61/EC lists 22 aromatic amines which are banned and thus need to be monitored. The detectable amount of any aromatic amine should not exceed 30ppm in the finished articles or in the dyed parts thereof. Thus, sensitive and accurate analytical methods are required to monitor these aromatic amines.

The challenge for the chromatographic analysis of aromatic amines is that these are basic compounds with closely related structures, occurring at low levels. Older type A and B, L1 bonded-phase silica column chemistries have been used in the past to assay highly basic amines but have required the addition of a competitive amine in the mobile phase to obtain symmetrical peaks. Today, the accurate HPLC quantification of these amines requires an analytical column that can easily replace older L1-type columns in analytical methods without the requirement for excessive method revalidation and one that can distinguish between structurally similar compounds and provide highly symmetrical peaks.

Experimental Conditions

Column:	Hypersil GOLD™ 3 μm, 150 x 2.1 mm
Part Number:	25003-152130
Mobile Phase:	A: 25 mM Ammonium acetate, pH 5 B: ACN
Gradient:	20 to 100% B in 10 min
Flow Rate:	0.2 mL/min
Detection:	UV at 254 nm
Temperature:	40 °C

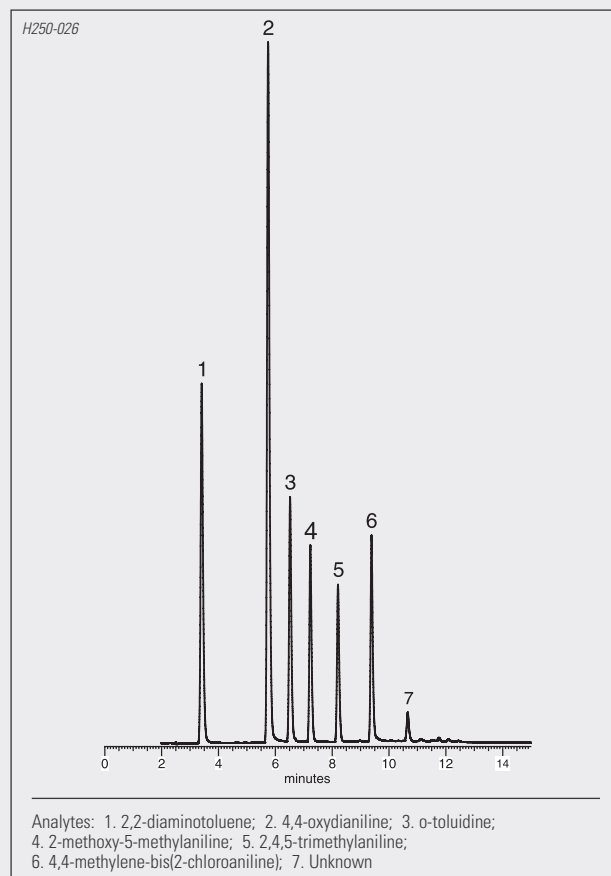


Figure 1: Separation of six aromatic amines. Symmetrical peaks are obtained with a low concentration of a volatile buffer and a mobile phase gradient.

Results and Discussion

The chromatogram in Figure 1 demonstrates that the ultra-pure base silica and state of the art bonding used for Hypersil GOLD media generate highly symmetrical peaks and allow very basic substances to be positively differentiated from each other, increasing the confidence in analytical results and decreasing the risk to the consumer.

References

1. Faversham House Group Ltd, 2002. *Edie News Article*
2. *Official Journal Of The European Union L 243, 11/09/2002, p 15-18*
3. *Official Journal Of The European Union 57, 25/02/2004, p 4-5*

Characterization of High Boiling Components in Petroleum Products using Hypersil GOLD and LC/MS

A fast and robust characterization of non- and semi-volatile components found in petroleum products is achieved, using a Hypersil GOLD 1.9 μm column and LC-MS.

The characterization of petroleum products is usually carried out using GC and GC/MS due to the high volatility of a number of the components. However, a significant portion of these products are high boiling or non/semi volatile and are difficult to analyze using GC/MS due to volatilization or molecular weight limitations. LC/MS does not suffer from such problems. Traditionally, however, such separations have been marred by lengthy analysis times and hydrocarbon contamination of the MS interface. Now, using Hypersil GOLD 1.9 μm particle columns, together with a patented 'cone wash' integrated into the Surveyor MSQ Plus ESI source, a fast, uninterrupted and robust analysis is possible.

Experimental Conditions

Instruments

LC:	Surveyor Plus™
MS:	Surveyor MSQ Plus™

LC Conditions

Column:	Hypersil GOLD™ 1.9 μm, 50 x 2.1 mm
Part Number:	25002-052130
Mobile Phase:	A: H ₂ O + 0.1% Formic acid B: MeOH + 0.1% Formic acid
Gradient:	80% B to 100% B over 9 mins (Hold for 6 mins), re-equilibration for 2 mins
Flow Rate:	0.5 mL/min
Injection Volume:	10 μL
Temperature:	50 °C

MS Conditions

Mode:	+ ESI
Probe Temp.:	550 °C
Cone Voltage:	75 V
Cone Wash:	MeOH, 200 μL/min

Samples

Fuel System Cleaning Solutions, available commercially.

Dilutions:	10 μL of sample into 2 mL Hexane, followed by a 100 μL aliquot into 1 mL Hexane.
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Results and Discussion

LC/MS analysis enables analysis of non- and semi-volatiles in petroleum products over a wide mass range, including polymer as well as low mass components. Use of Hypersil GOLD 1.9 μm particles provides excellent resolution and efficiency for the sensitive analysis of petroleum products as well as enabling fast analysis times. The MSQ Plus cone wash removes non-polar hydrocarbon components, eliminating system contamination and increasing reproducibility and method robustness.

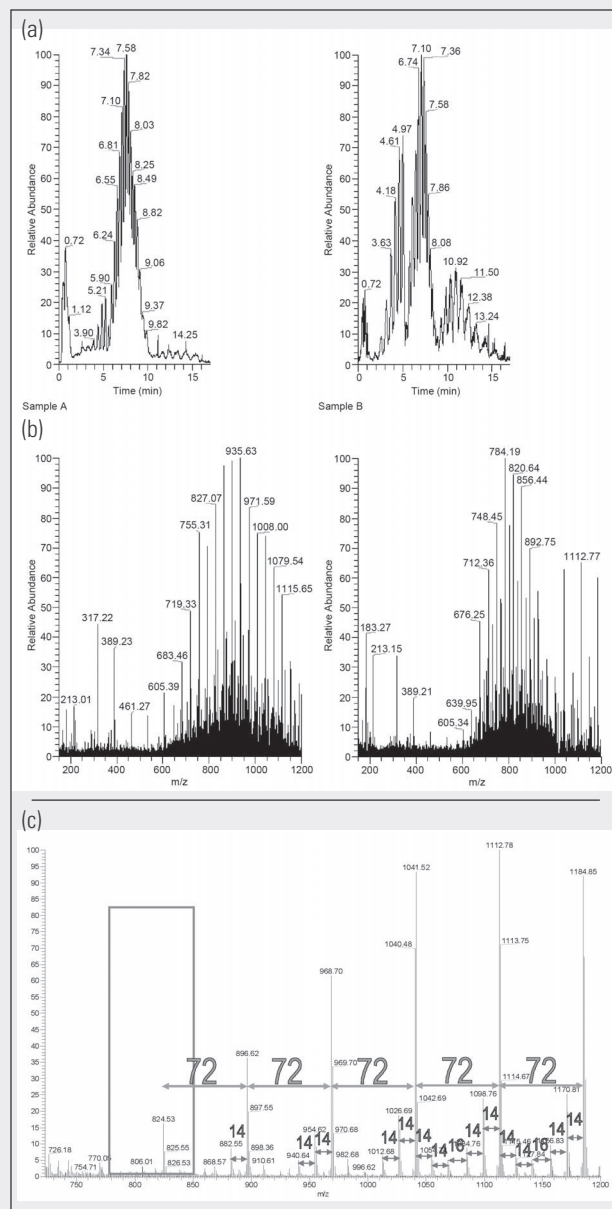


Figure 1: Total Ion Chromatograms (a) and averaged mass spectra (3 to 15 min) for 2 samples (b). Example of a polymeric distribution series (c).

References

1. J. Huang et al. Poster presented at ASMS 2006. (Reference T018P_ASMS06).

Acrylamide in Cooked Food

The excellent retention, peak shape and reproducibility of Hypercarb columns allows accurate measurement of acrylamide in a wide variety of foods.

In April 2002, a research group at the Swedish National Food Administration published a paper in *The Analyst* demonstrating the presence of acrylamide at elevated levels in cooked food.¹ Acrylamide is a suspected carcinogen and although no link has been proven between acrylamide and cancer in humans, the World Health Organization has described its presence in food as “a major concern”.

Acrylamide is formed naturally in foods with high starch content during cooking, with elevated concentrations of acrylamide detected in french fries, potato chips, breads and processed cereals, particularly after cooking using a microwave. Consequently, several food agencies and proficiency testing schemes are recommending more research to determine ways to minimize its concentration in food. These findings attracted worldwide attention, increasing demand for fast, sensitive analytical methods.

Traditional methods of analysis for acrylamide involve sample derivatization followed by gas chromatography. This application note reports a simple and accurate method for the analysis of acrylamide in cooked food using the polar retention capacity of Hypercarb columns for a faster LC/MS technique.

Acrylamide has a high degree of polarity and shows limited capacity to interact with silica-based hydrophobic (C18) bonded phases. Such silica based columns show poor quantitation at the picogram level, as acrylamide elutes with the column void volume and is difficult to distinguish from other polar sample constituents.

Hypercarb (100% porous graphitic carbon) has a polarizable surface that is able to form charge induced dipole interactions with polar molecules which have a permanent dipole. This dipole-dipole interaction results in strong retention of polar analytes, which are not retained on silica-based phases. This capability to create charge induced interactions gives Hypercarb far greater retention for polar compounds than silica-based phases.

Experimental Conditions

Column:	Hypercarb™ 5 µm 50 x 2.1 mm
Part Number:	35005-052130
Mobile Phase:	H ₂ O
Gradient:	Isocratic
Injection Volume:	10 µL
Flow Rate:	0.4 mL/min
Detection:	+ ESI SIM ([M + H] ⁺ , m/z = 72)

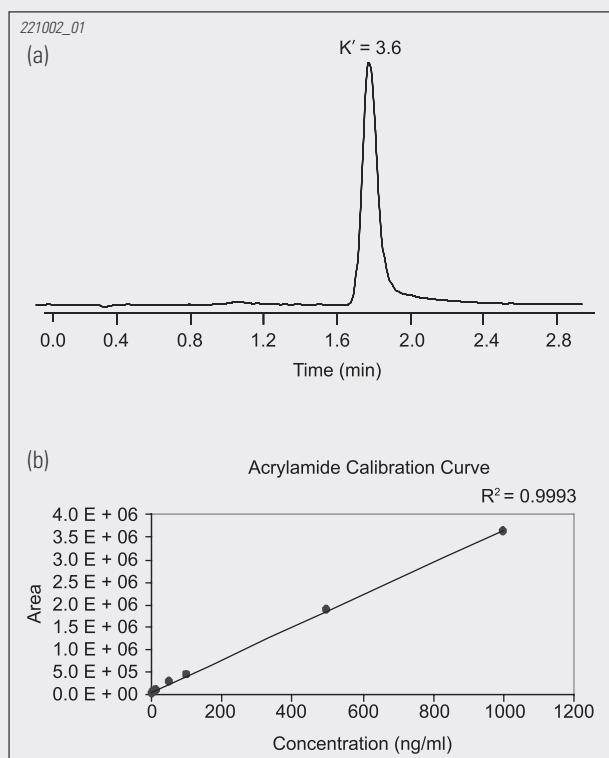


Figure 1: Retention of acrylamide on Hypercarb and linearity range. The method gives a linear response enabling accurate quantitation.

Results and Discussion

This method gives a retention time of 1.8 minutes for acrylamide with $k' = 3.6$, which gives adequate retention beyond the column void volume for accurate quantification (Figure 1a). This is highlighted by the linearity of response for the method over a concentration range 1 to 1000 ng/mL, as shown in Figure 1b.

References

1. J. Rosén & K-E. Hellenäs *The Analyst*, 2002 127 pp 880–882

Analysis of Water-Soluble Vitamins on a Polar Endcapped C18 Stationary Phase

Water-soluble vitamins are retained and resolved using a Hypersil GOLD aQ column and mobile phase conditions which are ideal for sensitive detection using positive ESI.

Vitamins are a group of chemically unrelated compounds which occur in small amounts in biological materials. Because of their nutritional importance, it is necessary to have sensitive and robust analytical methods to determine vitamins in food materials and dietary supplements.

HPLC is commonly used for the determination of vitamins, but the analysis of water-soluble vitamins by reversed phase HPLC (RP-LC) can be challenging since these compounds have very different chemical structures and therefore exhibit different retention behaviors. The smaller, more polar vitamins such as thiamine show little retention in RP-LC, whereas the more hydrophobic vitamins such as riboflavin are well retained. Gradient conditions are necessary to elute all analytes within a reasonable analysis time. Moreover, polar vitamins are difficult to retain on typical reversed phase columns unless high concentrations of non-volatile buffers or even ion pairing reagents are used. These mobile phase conditions are not amenable to high sensitivity LC/MS methods.

The use of a polar endcapped C18 phase has the advantage of retaining the polar water-soluble vitamins sufficiently to allow for accurate quantification, even when acidic, volatile mobile phase additives are used. The polar functional group used to endcap Hypersil GOLD aQ media provides an interaction mechanism in addition to the dispersive interaction with the alkyl chain, by which polar compounds can be retained and resolved. Additionally, because this phase is built upon the technology of Hypersil GOLD™ silica, symmetrical and efficient peaks are obtained even with weakly buffered mobile phases.

Experimental Conditions

Column:	Hypersil GOLD aQ™, 5 μm, 150 x 4.6 mm
Part Number:	25305-154630
Mobile Phase:	A: H ₂ O + 0.1% Formic acid B: ACN + 0.1% Formic acid
Gradient:	4% B for 3 min then to 100% B by 12 min
Flow Rate:	1.0 mL/min
Detection:	UV at 266 nm
Temperature:	25 °C

Results and Discussion

The six water-soluble vitamins are retained and separated on a Hypersil GOLD aQ column using a gradient of water and acetonitrile with 0.1% formic acid, conditions which promote good ionization for sensitive detection in positive electrospray. Analysis is completed in less than 8 minutes and the peak shape is very symmetrical for all analytes. Transfer of this method to LC/MS will only require reduction of the column internal diameter to 2.1 mm and reduction of flow rate to 0.2 mL/min.

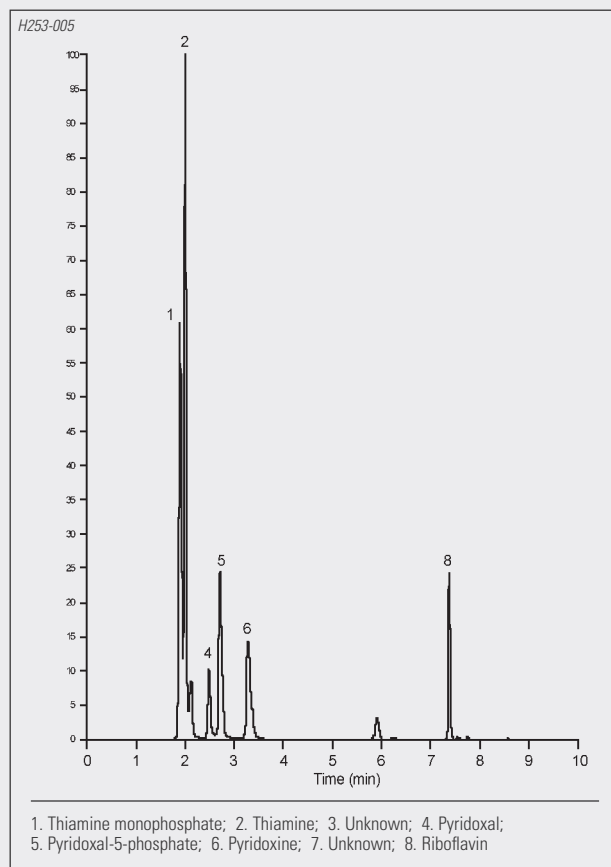


Figure 1: The analysis of water-soluble vitamins on a polar endcapped C18 phase illustrates the enhanced retention of polar compounds using Hypersil GOLD aQ columns.

Selectivity Screening in the HPLC Analysis of Catechins

The selectivity changes that can be obtained with alkyl chain, cyano and perfluorinated chemistries when using generic mobile phases are illustrated for a mixture of catechins. This approach for stationary phase screening can significantly reduce method development time.

In traditional Chinese medicine, green tea has been used as an astringent, cardiotonic, central nervous stimulant and a diuretic. Most of the healthful properties of green tea are attributed to the catechins, which are polyphenols with a flavonoid structure. The selectivity of three column chemistries for the high performance liquid chromatography (HPLC) separation of a mixture of catechins was evaluated.

Following the success of the Hypersil GOLD stationary phase for providing extremely symmetrical peaks, even for very basic analytes, additional chemistries have been developed using the same highly pure silica support and robust bonding process. These include cyano and perfluorinated chemistries. Hypersil GOLD CN offers a cyano chemistry with alternative selectivity for reversed phase and can also be used for normal phase separations. Hypersil GOLD PFP has a pentafluorophenyl ligand that provides extra selectivity for halogenated compounds. It also performs well for molecules which contain several nitro, hydroxyl, carboxyl or other polar groups and that may not be well retained or resolved on alkyl chain phases. The introduction of a fluorine group in the stationary phase causes significant changes in the solute-stationary phase interactions. The carbon-fluorine bond is more polar than the carbon-hydrogen bond, which explains the extra selectivity and retention observed for compounds containing halogens and polar functional groups.

Experimental Conditions

Columns:	Hypersil GOLD™ 5 µm, 150 x 4.6 mm Hypersil GOLD PFP 5 µm, 150 x 4.6 mm Hypersil GOLD CN 5 µm, 150 x 4.6 mm
Part Numbers:	25005-154630 25405-154630 25805-154630
Mobile Phase:	A: H ₂ O + 0.1% Formic acid B: ACN + 0.1% Formic acid
Gradient:	20 to 50% B in 15 min
Flow Rate:	1 mL/min
Detection:	UV at 280 nm
Temperature:	25 °C

Results and Discussion

The series of chromatograms shows the effect of changing the column chemistry on the separation of catechins when a generic mobile phase is used. The Hypersil GOLD PFP produces a change in elution order of analytes 2 and 3, compared with the alkyl chain phase. The extra retention of epigallocatechin gallate over epicatechin is thought to be caused by the additional substituted ring, which interacts with the phenyl ring on the perfluorinated stationary phase.

Similarly, these analytes also switch elution order on the Hypersil GOLD CN column. This column also shows another reversal of elution order between gallicocatechin gallate and epicatechin gallate. This is likely to be caused by the additional hydroxy group in gallicat-

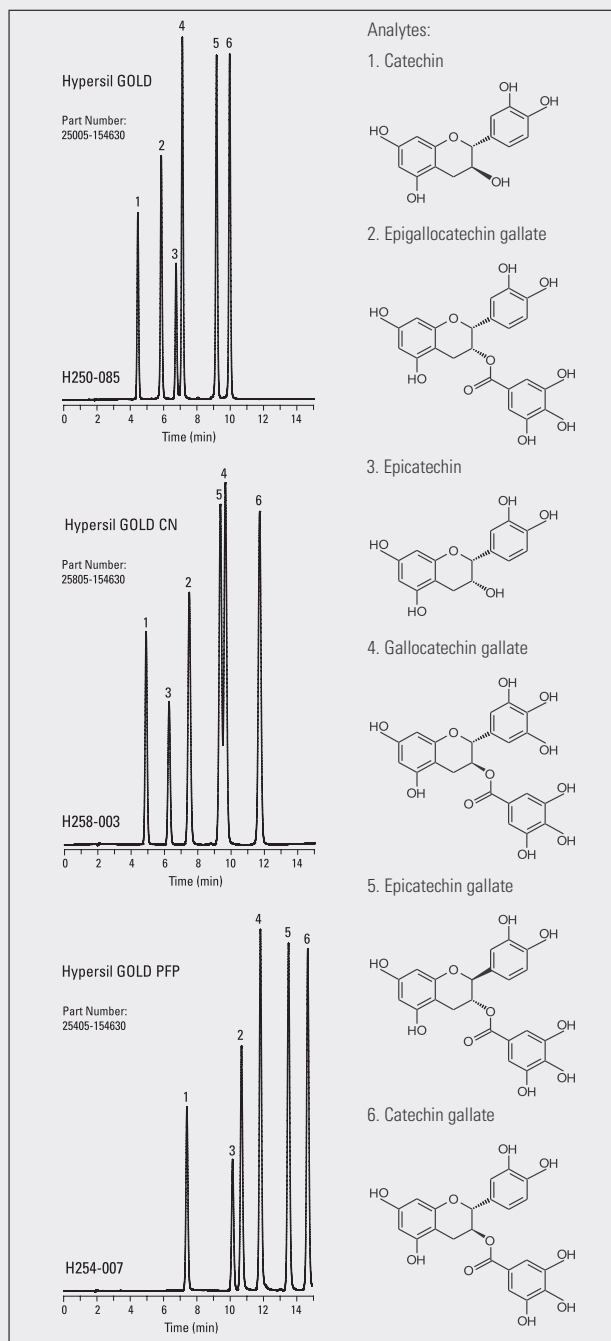


Figure 1: Separation of catechins on three column chemistries: C18 selectivity, perfluorinated phenyl, and cyano.

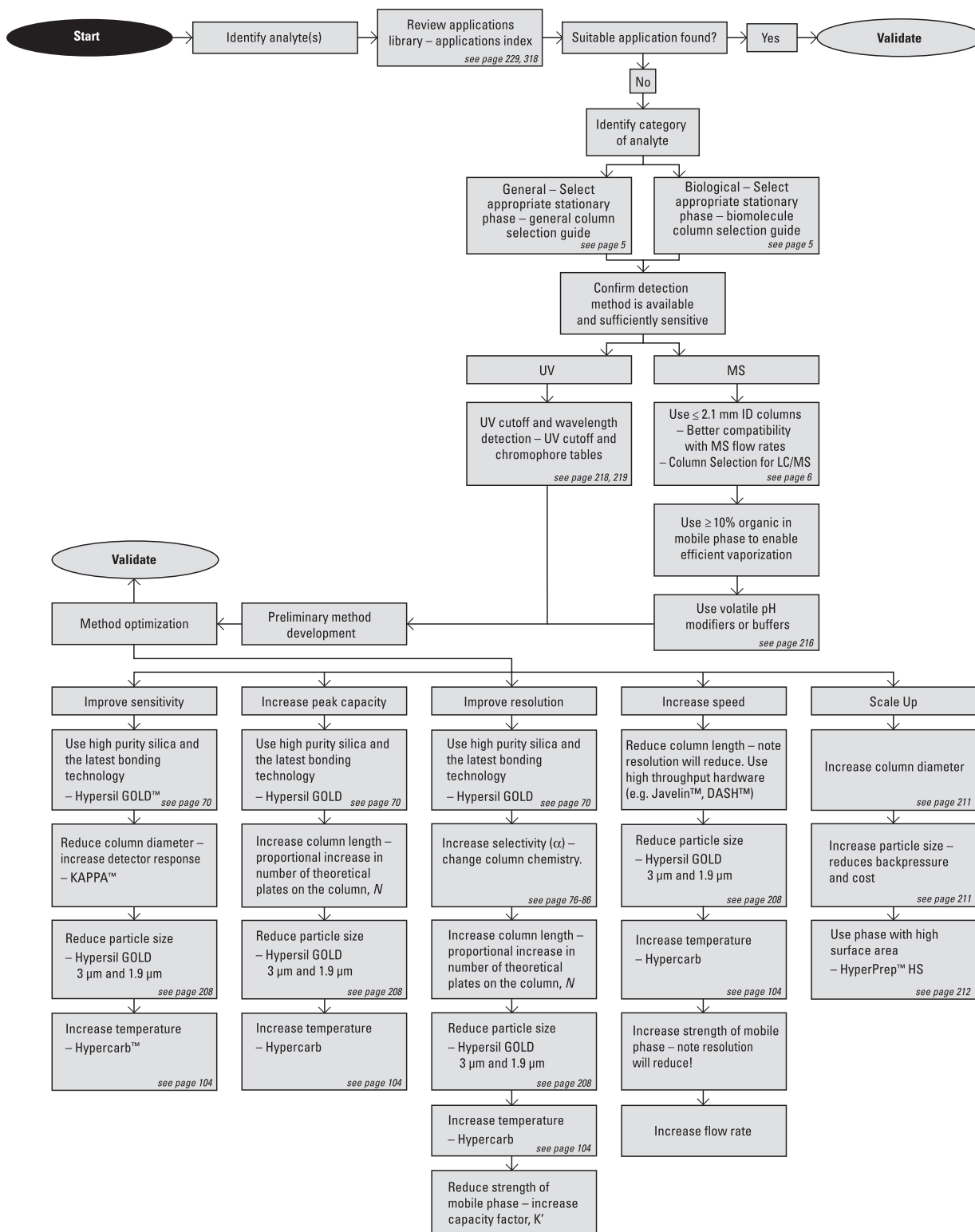
echin gallate. Screening different column chemistries with a generic mobile phase can significantly reduce method development time in reverse phase HPLC.

HPLC分析法の選択と最適化

HPLC Method Selection and Optimization

以下のフローチャートに、HPLC分析法の開発および最適化のステップを簡単にまとめました。個別のカラムの頁も参考にしてください。

HPLC Method Selection and Optimization Overview



Chromatography Resource Center ご紹介

新しくできたサーモエレクトロンのクロマトグラフィーリソースセンターには、クロマト分離に役立つ多数の技術情報があります。是非、そのホームページをご覧ください。

- 応用データライブラリには、HPLC、GC 及びSPE などの約400 件の測定データがあり、分析法開発の参考にできます。
- カラム選択ツールやクロスレファレンスツールを用いて最も適切なカラムを選ぶことができます。
- トラブルシューティングツールも有ります。
- Tips 及びFAQ が日常の問題解決に役立ちます。
- ポスター発表資料、応用ノート、技術ガイドなどの技術資料はダウンロードできます。あるいは、郵送で入手が可能です。

Chromatography Resource Center へは、下記のいずれかにアクセスしてください。

<http://www.thermo.com/columns>

ダイレクトアクセスの場合は、

<http://www.separatedbyexperience.com>

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Chromatography Resource Center.



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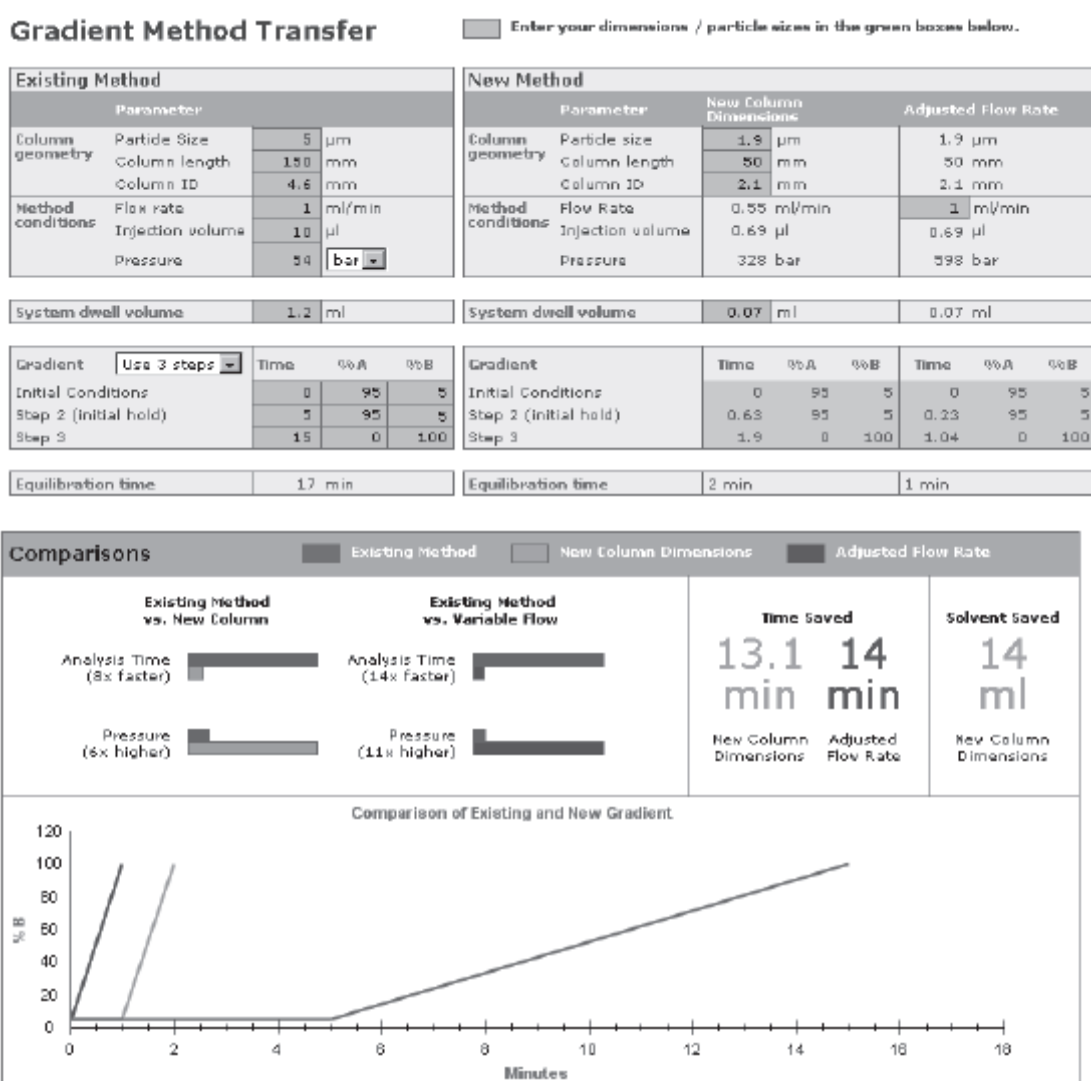
<http://www.separatedbyexperience.com/>

HPLC Method Development Calculator ご紹介

クロマトグラフィーリソースセンターの中にある HPLC Method Development Calculator は、 $3\mu\text{m}$ や $5\mu\text{m}$ 粒子径で作成した既存の分析法を、 $1.9\mu\text{m}$ 粒子径のカラムに変えるときに、 $1.9\mu\text{m}$ 粒子径における流速、試料注入量、分析時間、圧力、グラジエント条件、それぞれの値を WEB 上で自動計算してくれる大変便利なソフトです。

HPLC Method Development Calculator へは、[前頁で右端の Column Selectors の欄の HPLC Method Transfer Calculator をクリックしてください](#)

以下に、グラジエントとアイソクラチック条件での計算例を示します。



Isocratic Method Transfer

Enter your dimensions / particle sizes in the green boxes below.

Existing Method			New Method		
Parameter			Parameter	New Column Dimensions	Adjusted Flow Rate
Column geometry	Particle Size	5 μm	Particle size	1.9 μm	1.9 μm
	Column length	150 mm	Column length	50 mm	50 mm
	Column ID	4.6 mm	Column ID	2.1 mm	2.1 mm
Method conditions	Flow rate	1 ml/min	Flow Rate	0.55 ml/min	1 ml/min
	Injection volume	10 μl	Injection volume	0.69 μl	0.69 μl
	Analysis Time	30 min	Analysis Time	3.8 min	2.1 min
	Pressure	54 bar	Pressure	328 bar	598 bar

Comparisons			<input type="checkbox"/> Existing Method	<input type="checkbox"/> New Column Dimensions	<input type="checkbox"/> Adjusted Flow Rate
Existing Method vs. New Column		Existing Method vs. Variable Flow		Time Saved	
Analysis Time (8x faster)		Analysis Time (14x faster)		26.2 min	27.9 min
Pressure (6x higher)		Pressure (11x higher)		New Column Dimensions	Adjusted Flow Rate
				Solvent Saved	
				27.9 ml	
				New Column Dimensions	