Carotenoid isomers subcritical chromatographic test for C18 HPLC column classification

A test has been developed at LETIAM (IUT Orsay), within the analytical chemistry laboratory, Paris Sud. that discriminates more than ten groups of reverse stationary phases, from an analysis of carotenoid pigments. Stationary phase properties can then be broadly differentiated between each other.
Major features can then be compared for over 160 commercial C18 silica based HPLC columns. From mainstay generation phases such as Licrosorb, Kromasil & Partisil to more current developments, such as Uptisphere and Zorbax.

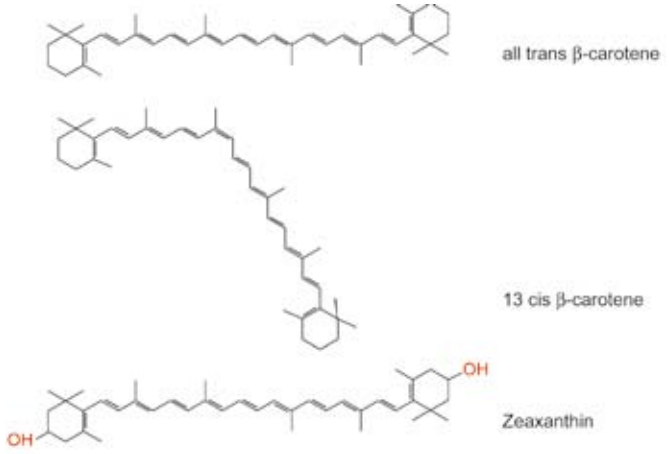
The test demonstrates, in a single analysis, three primary features of the stationary phase.

- Hydrophobicity, overall phase retention capacity, reliant on bonding rate and specific surface area.
- Steric selectivity, the ability to separate molecules which carry differing 3D structures (isomers).
- Accessibility to residual silanols, relevant for basic compounds.

Contrary to chemometric approaches, this test presents, simply & concisely, through two successive diagrams (highlighted on the following page), the classification of stationary phases that exhibit similar chromatographic behavior relative to phases with different chemical properties.
This test is performed in subcritical fluid chromatography, a rapid and effective technique offering an inherent value. The important eluting force of the modified subcritical fluid (carbon dioxide & methanol mixture) and its weak viscosity, lead to a reduced molecular kinetic dispersion (narrow peaks) and rapid analysis from high mobile phase flow rate capabilities. Results obtained with this methodology are correlated with those obtained using hydro-organic mobile phases.

Test conditions :

1. Injection of a15 µl diluted mixture of partially isomerizated beta-carotene, containing all-trans, 9-cis, 13-cis (main isomer cis), 15-cis, and zeaxanthin (di-hydroxylated beta-carotene)



2. The selected mobile phase is a mixture of methanol-carbon dioxide (15 : 85, v/v), at 25°C, outlet pressure 15MPa, flow rate : 3ml /min & detection wavelength at 440 nm.

HPLC method development

Results

Stationary phase classification is achieved in a two step process. Step one provides a primary classification through shape recognition and accessibility to polar sites, defining groups in which the stationary phase are placed. Step two examines the impact of hydrophobic characteristics, of a given phase, for a specified group.

Step 1:
Primary classification is established from the comparison of the selectivity value of shape recognition & polar accessibility, for each stationary phase.

Chart placement
a. X-axis
The selectivity, between the 13-cis isomer (bent form) and the all-trans (linear form) of the beta-carotene, is plotted on X-axis.
The cis/trans isomer separation of the beta-carotene is achieved through a 3D discrimination or shape recognition, by the stationary phases of the linear molecule vs the bent molecule. The variation in the tri-dimensional geometry of the two molecules directly results in differing interactions within the stationary phase.

A comparison of the results obtained through these studies, with other recognized tests of known stationary phases (such as NIST 869), provides a comprehensive organization of phases analyzed :

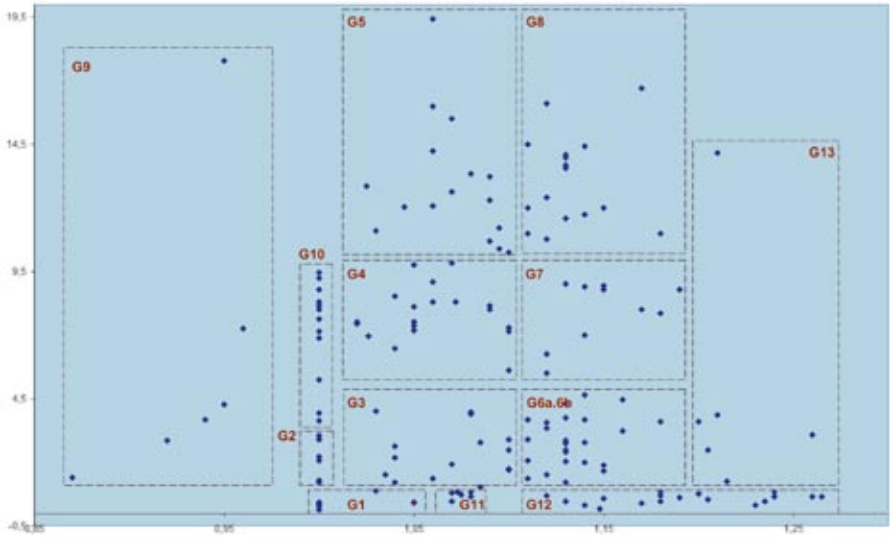
- Monomeric phases (mono-layer bonded silica) with low bonding density for cis/trans selectivity from 1 to 1.1
- Monomeric phases with high bonding density or partially polymeric (polymolecular layers bonded silica) from 1.1 to 1.2
- Polymeric phases above 1.2

Monomeric phases can be manufactured through bonding mono, di or tri-functional silanes upon a silica without water content. Polymeric phases are obtained from tri-functional silanes containing trace water. Typically, polymeric phases show a capacity for the separation of PAH (Polycyclic Aromatic Hydrocarbons) and rigid isomers of different stereochemistries.

b. Y-axis
Selectivity plotted on the Y-axis is given by the selectivity of all trans beta-carotene and zeaxanthin. Polar interactions are exhibited between hydroxyls of the zeaxanthin and the residual silanols of the stationary phase and /or additional accessible polar sites eg. (silane for polyfunctional phases, amide, urea, ether-oxide, quaternary ammonium or carbamates for polar embedded phases also called shielded). The higher the selectivity value, the lower this type of interaction is exhibited within the stationary phase. Therefore the less polar groups contained within the phase, the weaker the retention of zeaxanthin.

This selectivity ranges from 0.3 for phases exhibiting polar linked groups, to almost 20 for the most protected phases.
Polar site accessibility is significant from 0.3 to 1, moderate from 1 to 5, low from 5 to 10, and very low above 10. Increased selectivity is, in the majority of instances, relative to multiple end-capping treatments (single or combined), providing an indication of residual silanol coverage. Other non end-capped phases, with high bonding density, display high selectivity suggesting a reduced accessibility to the polar sites available on the stationary phase.

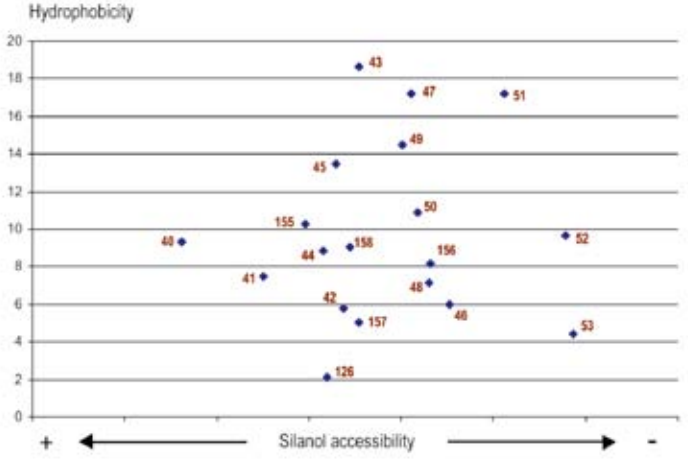
HPLC method development



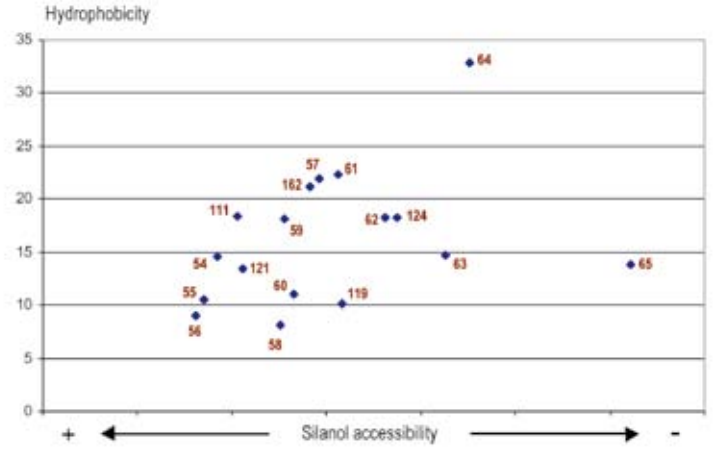
(The hydrophobicity value cannot be represented in this 2-D diagram)

Step 2 :
The first step groups columns that will achieve similar separations. Further classification for each group is achieved (4 5,7 & 8 are displayed) from further examination of silanol accessibility relative to hydrophobicity. For each group a separation will be similar but analysis times will differ .This provides a further indication for the optimization of a method, for a given separation.

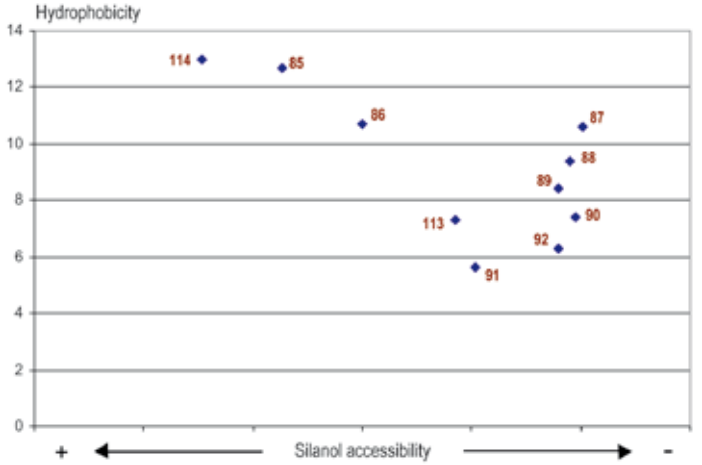
Group 4



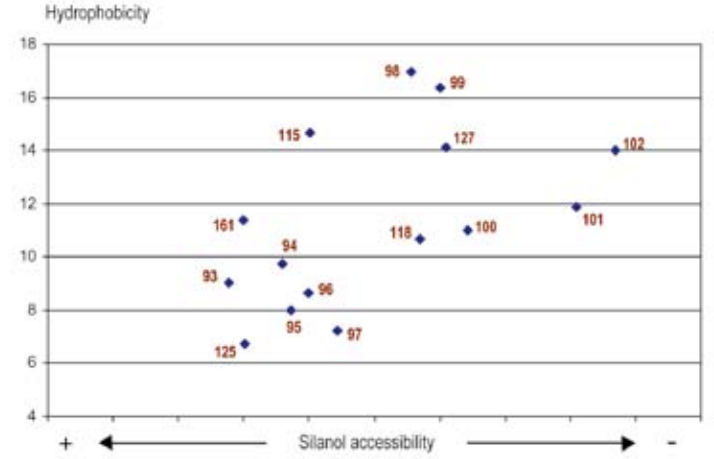
Group 5



Group 7



Group 8



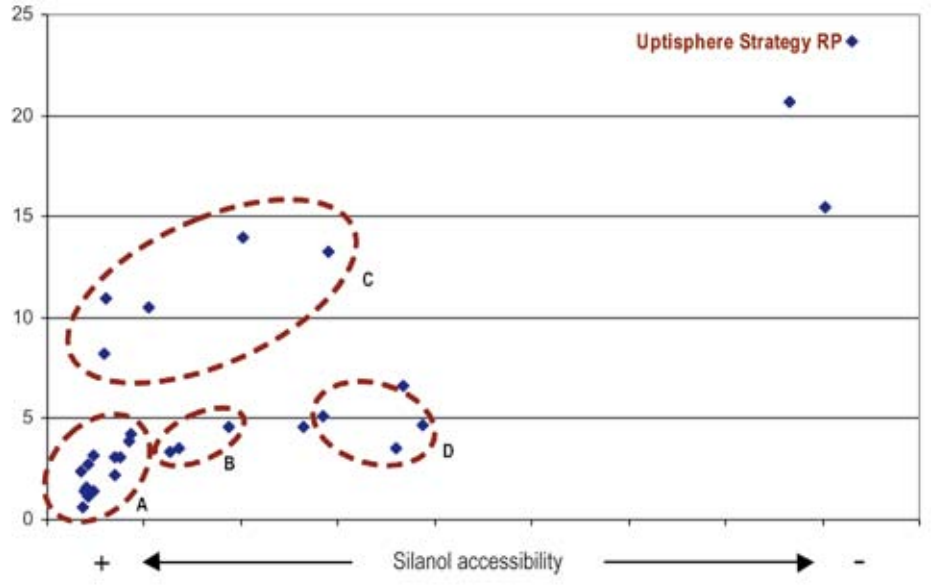
Test consideration for polar linked alkyl & polar end-capped phases

Polar linked alkyl and polar end-capped phases are designed to analyze basic compounds. The polar linked alkyl phase achieves sharp peak definition whilst the polar end-capped phase can be used with a 100% aqueous mobile phase. A major difference between polar linked alkyl and hydrophilic end-capped phases is a weaker hydrophobicity of the polar embedded phases. The hydrophobicity of the hydrophilic end-capped or sulfonamide phases compares to classical C18.
In supercritical chromatography, the lack of water within the mobile phase allows for the polar linked groups and the hydrophilic end-capping to display strong interaction with hydroxyl groups of the zeaxanthin analyte. The strength of these interactions can therefore be evaluated.

Selectivity less than 1 on the X axis indicates an inversion of retention between zeaxanthin and the β-carotene, this is a direct result of strong interaction of the two hydroxyls contained within the zeaxanthin molecule. Amide chemistry display a stronger interaction and subsequent peak inversion than the interaction shown by carbamate functionality. These are subsequently stronger than phases carrying ether chemisty.
Polar linked phases interact with water of a hydro-organic mobile phase forming a hydrophilic barrier, stopping basic compounds accessing the residual silanols.

Step 2 representation of hydrophobicity /silanol accessibility places phases in distinct groups. Amide phases form a cluster (group A). Group B displays Carbamate phases. Group C classifies two polar end-capped and the sulfonamide phase. Finally, group D covers ether phase technology.

A few phases exhibit distinctive characteristics. Uptisphere® Strategy™ RP is one of these unique phases.



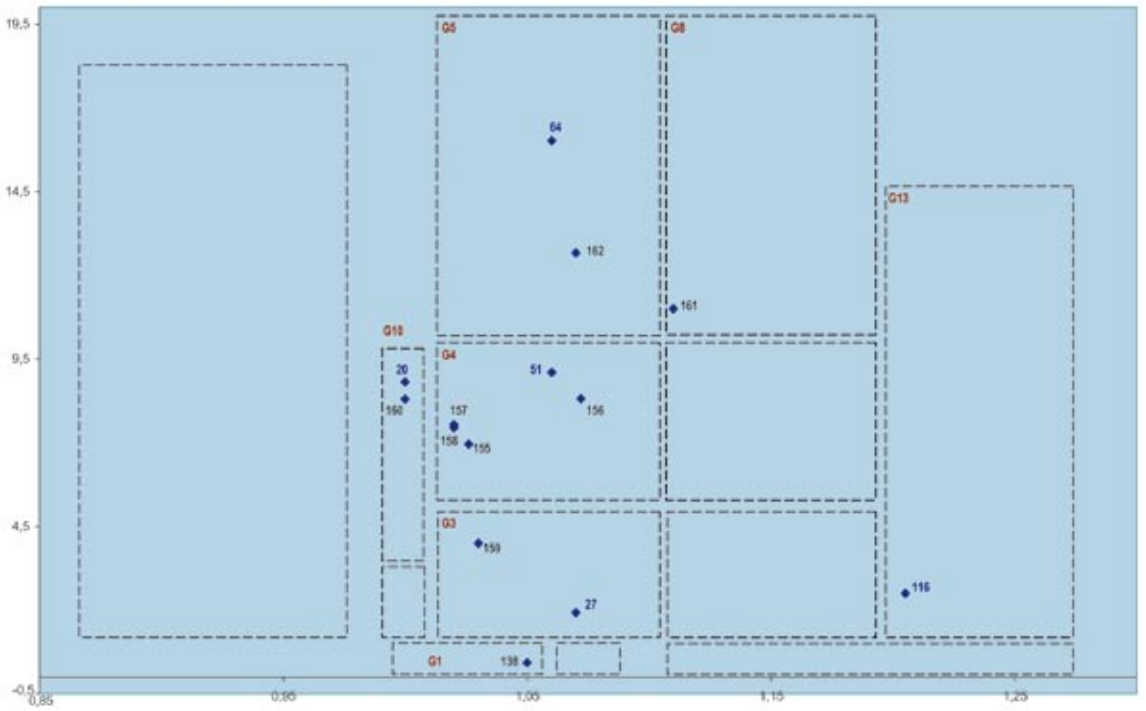
HPLC method development

Upti-select™ Kit

A tool to achieve consistent, reliable method development through a simple 2-step process

Interchim's C18 stationary phases have been developed to reflect the criteria outlined by the E. Lesellier et al. Tchapla test. Uptisphere® and Uptisphere® Strategy™ are the brand names for Interchim's proprietary HPLC technology, our stationary phases fall into strategic areas of the primary groups within the classification.

Place	Stationary Phase	Group
20	Uptisphere HDO	G10
27	Uptisphere NEC	G3
51	Uptisphere ODB	G4
64	Uptisphere HSC	G5
116	Uptisphere TF	G13
138	Uptisphere PLP	G1
155	IUptisphere BioP II	G4
156	Uptisphere BioP I	G4
157	Uptisphere WOD	G4
158	Uptisphere WRP	G4
159	Strategy Pro	G3
160	Strategy RP	G10
161	Strategy C18-2	G8
162	Strategy C18-3	G5



The Interchim Upti-select™ kit contains a range of 5 proprietary columns, across the classification, for the initiation of column selection for analytical method development.

Upti-select™ kit :

- Provides a method to predict and select the optimal column for analysis
- Eliminates column selection uncertainty
- Enhances the process toward column optimization
- Reduces development costs

HPLC method development

Step 1 :
Inject sample for analysis and the proprietary kit reference mixture onto the Uptisphere® 5 µm ODB packed column.

Determine :
- The retention time of a Reference Peak (RP) from the analysis sample.
- The retention time of the five probes from the Reference Mixture (RM) - [calibrate the system]

Choose a retention factor for the reference peak (RP) : K'_{RP}
An Excel spread sheet indicates the percentage of methanol and /or acetonitrile required, within the mobile phase, to reach the expected retention factor K'_{RP} on each of the five columns.

Step 2 :
Prepare the mobile phase according to step 1. Inject the sample of interest onto the five columns (including ODB column for verification). Choose the column highlighting the most suitable separation. Start column optimization.

Upti-select™ Kit and Upti-select™ Kit Pack components

Upti-select™ kit consists of 5 columns featuring identical dimensions and including all necessary hardware. i.e.

- A Reference column packed with the Uptisphere® 5 µm ODB
- Four additional columns with complementary selectivity based upon classification chart. e.g
  - Uptisphere® 5 µm HDO
  - Uptisphere® 5 µm NEC
  - Uptisphere® 5 µm HSC
  - Uptisphere® 5 µm TF
- Column connectors, (# CH953820)

Upti-select™ kit Pack also includes:

- Proprietary Reference Mixture (RM) containing five probes for test calibration
- CD-ROM (includes : excel spread sheet for automated organic percentage calculation).
- Instruction booklet

Description		Sizes	P/N
Upti-select Kit™		50 x 1.0 mm	S64060
Upti-select Kit™		50 x 2.0 mm	S57950
Upti-select Kit™		150 x 2.0 mm	T07670
Upti-select Kit™		250 x 2.0 mm	T80410
Upti-select Kit™		50 x 3.0 mm	S64050
Upti-select Kit™		150 x 3.0 mm	T77680
Upti-select Kit™		50 x 4.6 mm	S57930
Upti-select Kit™		100 x 4.6 mm	S64100
Upti-select Kit™		150 x 4.6 mm	S57920
Upti-select Kit™		250 x 4.6 mm	S57900
Upti-select Kit™	Pack	50 x 1.0 mm	BB2280
Upti-select Kit™	Pack	50 x 2.0 mm	BB2270
Upti-select Kit™	Pack	150 x 2.0 mm	BB2260
Upti-select Kit™	Pack	250 x 2.0 mm	BB2250
Upti-select Kit™	Pack	50 x 3.0 mm	BB2240
Upti-select Kit™	Pack	150 x 3.0 mm	BB2230
Upti-select Kit™	Pack	50 x 4.6 mm	BB2210
Upti-select Kit™	Pack	100 x 4.6 mm	BB2200
Upti-select Kit™	Pack	150 x 4.6 mm	BB2190
Upti-select Kit™	Pack	250 x 4.6 mm	BB2140
Reference Mixture (RM)	5 ml		BB2290
Upti-select Kit™		Upgrade	BB2310
(includes : Reference Mixture (RM) + CD-rom + instruction booklet)			

HPLC method development

Upti-select™ Kit methodology example : Anti-UV agents

Step 1 :

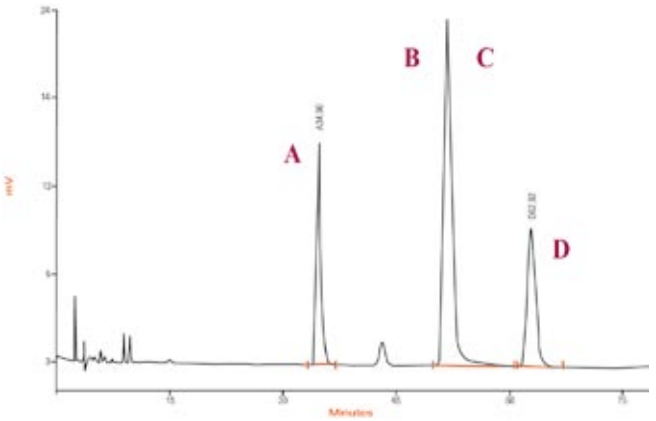
a) The anti-UV agent sample contained the following components :

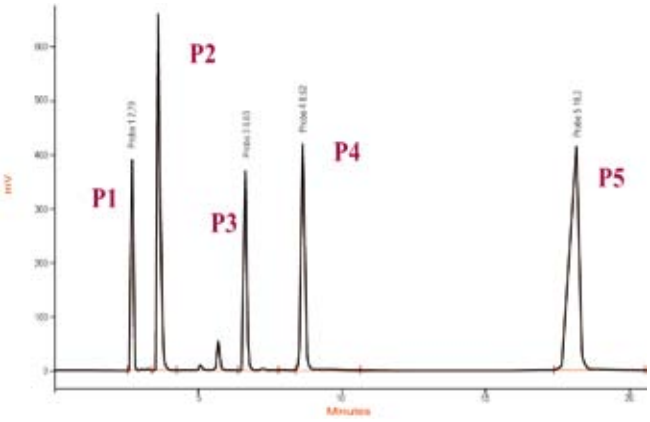
A : 2-Ethylhexyl-2-Cyano-3.3-Diphenylacrylate
B : 2-Ethylhexyl-3-[4-Methoxyphenyl]-2-Propenoate
C : 4-t-Butyl-4'-Methoxy-Dibenzoyl-Methane
D : 2-Ethylhexyl Salicylate

The sample was injected onto the Uptisphere® 5 µm ODB, 250 x 4.6 mm.
Conditions : MeOH-H_2O (80/20) - 1ml/mn - 25°C - UV : 238 nm.

Peaks B and C co-eluted and the analysis time was more than 60 mn
Peak D was thus chosen as the reference peak (RP)

b) The Reference Mixture (RM) was injected under the same conditions onto the same column.



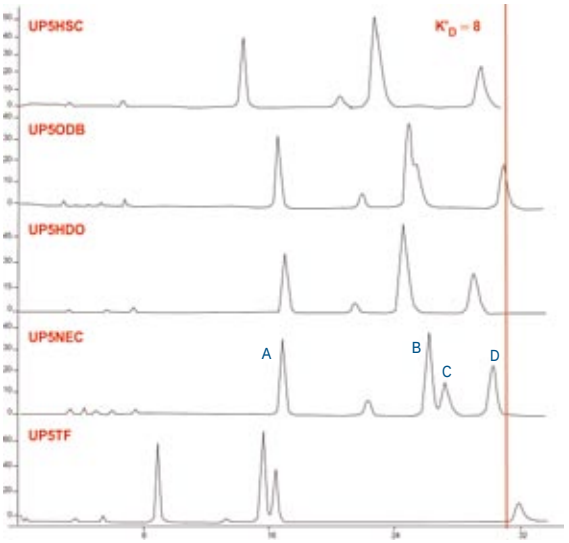


The retention of peak D and the retention time of the five probes (RM) were introduced into the excel spread sheet.
The retention factor of peak D was fixed at 8.
The calculation table provided the percentage values of methanol and acetonitrile for each mobile phase for each column to achieve K'_{RP}.

Step 2 :
The mobile phase were prepared according to Step 1. The sample of interest was injected onto the 5 kit columns according to the fixed conditions to obtain K'_{RP} = 8 for peak D.

HPLC method development

Chromatograms w/Methanol



Observations

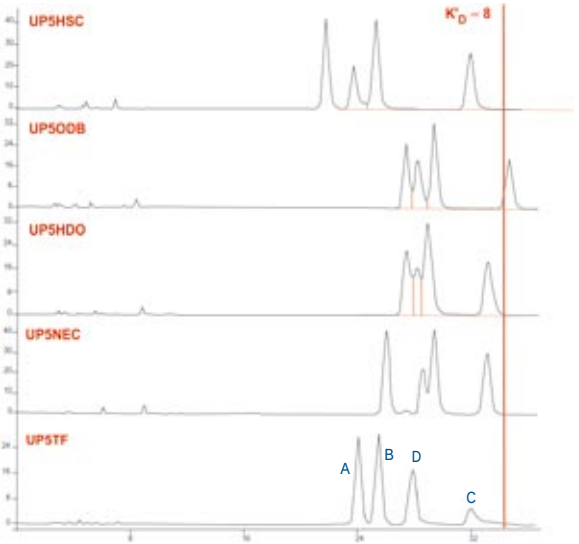
Results show K'_{RP} very close to 8 for each of the five Uptisphere® columns.
[This verifies our log P calculation and column transposition equations are relevant]

- The A, B, C, D elution order is preserved on the HSC, ODB, HDO, and NEC columns.
- C and D are inverted on the TF column.
- A better separation is observed on Uptisphere® NEC and TF.
- The B,C pair achieve a better separation when the column features higher silanol accessibility.
- Co-elution occurs on the ODB, HDO, and NEC, columns.

Conclusion

Uptisphere® 5 µm TF and a methanol phase was identified as the most appropriate column for this application.

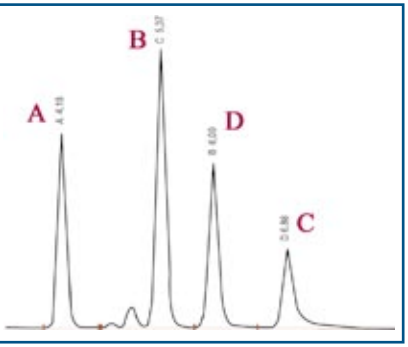
Chromatograms w/Acetonitrile

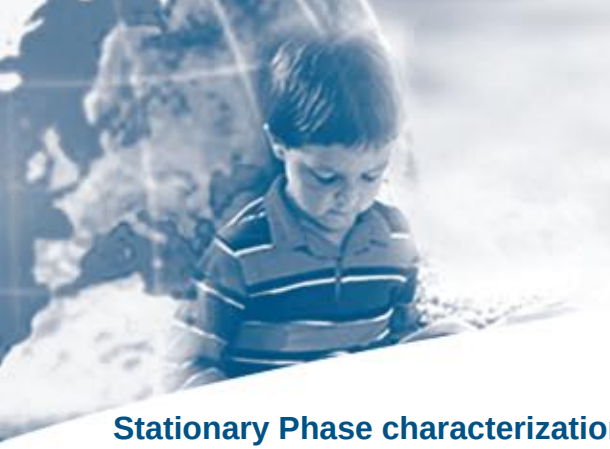


Final validated method

Separation was optimized using Interchim's Rodeo™ concept.

A running time of 6.88 mm was established for the last peak and a resolution of 3.02 for the critical pair (B, D).





HPLC method development

Stationary Phase characterization for basic compound analysis

The Laboratory of Pharmaceutical Analytical Chemistry – University of Geneva, Switzerland (LCAP) has established a global reputation for separative method development. Their mandate is to evaluate the fundamental aspects that influence the current drives in HPLC.

'Hot topics' such as monolithic sorbents, sub-2 micron particles and high temperature HPLC (T°> 60°C) are some of their recent considerations and further information on these studies are available upon request.

LCAP has developed and established chromatographic tests since 1993 to classify silica based HPLC columns for basic compound separation. Such separations have historically been a complicated process using traditional bonded silica based HPLC sorbent generally due to potential ion exchange interactions that can occur within the conventional pH range. For nearly two decades, manufacturers have attempted to overcome problems associateed with basic compound separation by offering new, chemically modified, chromatographic phases. LCAP's current evaluations revolve around the analysis of a mixture containing 7 basic complementary compounds, that allow the rapid discrimination of phase behavior in working conditions (hydro organic buffered mobile phases at pH 3 and 7) .

LCAP basic compound test

Chemometric tools such as Principal Components Analysis (PCA) or Hierarchical Classification Ascendant (HCA), are used to visualize and subsequently classify column performance relative to similar behavior patterns. Column function is classified, within the LCAP test, by the hydrophobic power and the residual interaction with basic compounds. The classification obtained with the LCAP test is correlated with recognized tests published in the scientific literature. LCAP utilizes compounds similar in structure to those found in the pharmaceutical industry and applies mobile phase conditions used in this environment. This establishes the LCAP test as providing relevant information for column suitability for basic compound analysis and discriminates chromatographic sorbents with similar properties.

A number of stationary phases from different manufacturers were tested with the 7 compound test mix at pH 3 & 7.

Retention, efficiency, asymmetry values were measured and the phases studied were represented according to their similarity with PCA and HCA chemometric tools.

Phases were plotted, within a 2 dimensional setting, relative to residual silanol activity at pH : 7 for the principal components within the mix.

HCA computation subsequently groups stationary phases displaying similar chromatographic behavior whilst retaining the “non visible” information within the PCA representation.

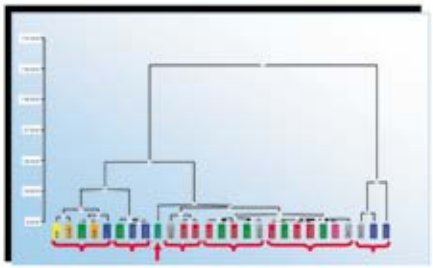
Hierarchical Classification Ascendant (HCA)

Column	Bonding	Chemistry	Manufacturer
Zorbax	C18	Bidentate	Agilent
Discovery RP Amide	C16	Polar	Supelco
Nautilus	C18	Polar	Macherey Nagel
Supelcosil ABZ+	C16	Polar	Supelco
Stability BS C23	C16	Polar	CIL-Cluzeau
Performance	C18	Monolithic	Merck
SpeedRod	C18	Monolithic	Merck
Xterra	C18	Polar (Hybrid)	Waters
Nucleosil HD	C18	High density	Macherey Nagel
Eclipse XDB	C18	High density	Agilent
Luna	C18	High density	Phenomenex
Nucleodur	C18	C18 (ultra pure)	Macherey Nagel
Acclaim	C18	C18	Dionex
Nucléosil AB	C18	C18	Macherey Nagel
Nucléodur Pyramid	C18	C18 endcapped Hydr	Macherey Nagel
Symmetry Shield	C18	Polar	Waters
Uptisphere ODB	C18	C18 endcapped	Interchim
Uptisphere TF	C18	C18 endcapped polyf.	Interchim
Uptisphere HDO	C18	C18 mixed-endcapped	Interchim
Uptisphere HSC	C18	C18 endcapped	Interchim
Uptisphere NEC	C18	C18 non-endcapped	Interchim

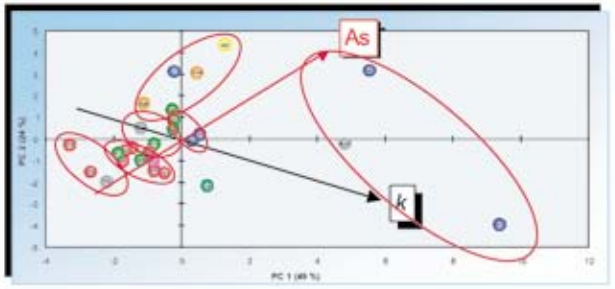
HPLC method development

Silanol activity

Ascending Hierarchical Classification



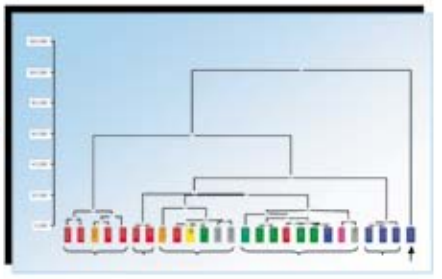
6 main components



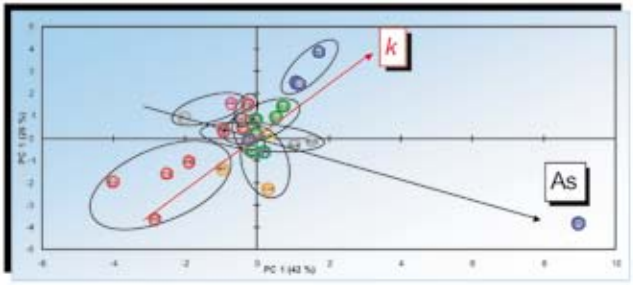
ACP 2D - AHC
95 % variability

Hydrophobic properties

Ascending Hierarchical Classification



6 main components



ACP 2D - AHC
95 % variability

HPLC Method Optimization- RODEO™

Method Development - to - Method Optimization

Interchim's general guide to selecting the most appropriate stationary phase can be represented as follows :

- For samples primarily containing basic compounds : initiate evaluation using the 3 column Upti-select basic compound development kit.

- Sample containing a mixture of basic, neutral and acidic compounds.
  Intitiate testing with the broad application Uptisphere Strategy column (5 µm C18-2, 250 x 4.6 mm), conditions : ACN - H_2O (70/30) & (30/70). If unsuccessful, apply Upti-select Kit™ methodology with standardized phase diversity and/ or apply other selectivities such as cyano, amino, ion exchange.

The appropriate column can then be optimized. Interchim have established the RODEO™ concept highlighted below to address column optimization.

The RODEO™ concept

The RODEO™ concept provides a novel approach for HPLC method optimization. It takes advantage of the ability to effectively couple multiple Uptisphere HPLC columns together with couplers from the Uptisphere hardware range (see Uptisphere Modulo-Cart hardware details later in this HPLC section).

The chromatograms on the following page demonstrate, and additional studies have shown, that when working under the same analytical conditions and evaluating at the same stage of analysis, pressure (P), efficiency (N), asymmetry (As), resolution (Rs) and retention factor (k') are extremely similar between a Modulo-Cart Quick Seal 250mm column and five Modulo-Cart Quick Seal 50 mm columns coupled together irrespective of the column internal diameter.

RODEO™ therefore provides a very simple procedure for the optimization of required column dimension, mobile phase, and appropriate temperature & is discussed further on the following pages.

Modulo-cart Quick seal 250 mm

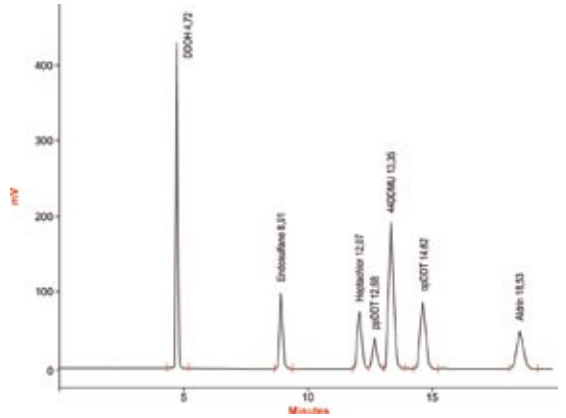
Rodeo™ 5 x 50 mm

[Modulo-Cart Quick seal technology exhibits 15% increased efficiency compared to other HPLC hardware. It is a real hand-tight fitting and the carbon doped PEEK on stainless steel guarantees long column lifetime].

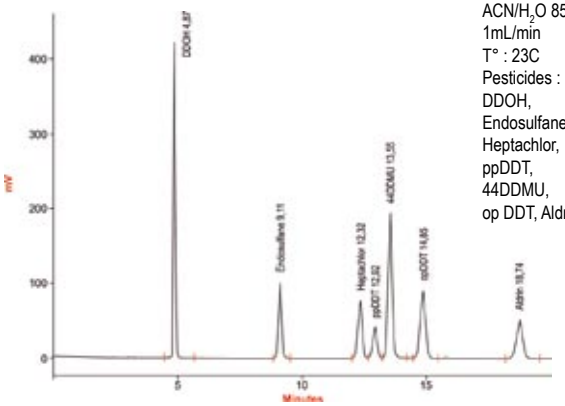
HPLC Method Optimization - RODEO™

Exploring the difference between a 250 mm Modulo-cart column and five 50 mm columns coupled together with Modulo-cart couplers.

Quick seal UP5HDO 250 x 4.6 mm



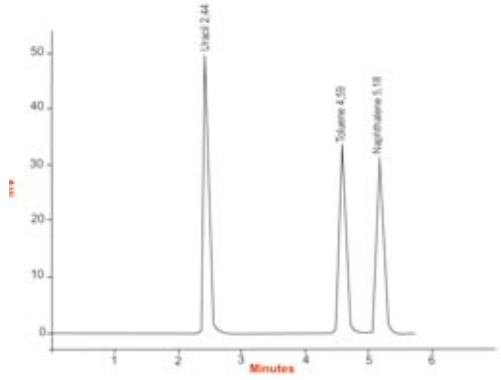
Rodeo™ **UP5HDO (5 x 50) x 4.6 mm**



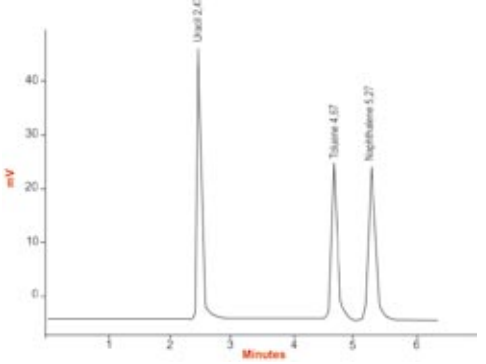
ACN/H_2O 85/15
1mL/min
T° : 23C
Pesticides :
DDOH,
Endosulfane,
Heptachlor,
ppDDT,
44DDMU,
op DDT, Aldrin.

Dimension	to	k'	P	As	N	Rs
250 mm	2.35	6.88	61	1.17	21148	8.58
5 x 50 mm	2.4	6.8	63	0.99	22574	8.56

Quick seal UP5TF 250 x 2.1 mm



Rodeo™ **UP5TF (5 x 50) x 2.1 mm**



ACN/H_2O 70/30
0.2mL/min -
T° : 23C
Test interne :
Uracil, toluene,
naphtalene.

Dimension	to	k'	P	As	N	Rs
250 mm	2.44	1.12	92	1.18	15875	3.7
5 x 50 mm	2.47	1.13	107	1.2	16200	3.9

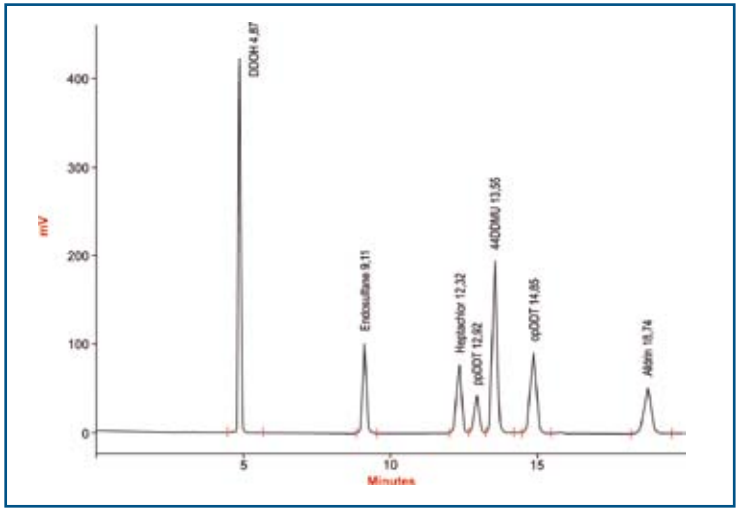
HPLC Method Optimization - RODEO™

RODEO™ column length optimization

The selection of a HPLC stationary phase is typically determined in the laboratory through the initial evaluation of a 5 µm, 250 mm column. The Rodeo™ concept provides a means to quickly select the correct column length and reach the required optimisation. Optimization sets to reduce analysis time whilst retaining sufficient resolution for peaks displaying the poorest separations. The principal objective is to shorten run time, re-equilibration time, and identify the simplest compatible mobile phase. A way in reaching this objective is to adopt the appropriate column length.

Example 1 displays this process for Rodeo™ [partical size reduction is then considered as a subsequent step]. The three subsequent chromatograms show the affect when a column is removed (de-coupled) for the same separation. In this instance it shows that by decreasing the column length by 40%, analysis time is reduced by 42.5% whilst sufficient resolution is retained for the critical pair separation.

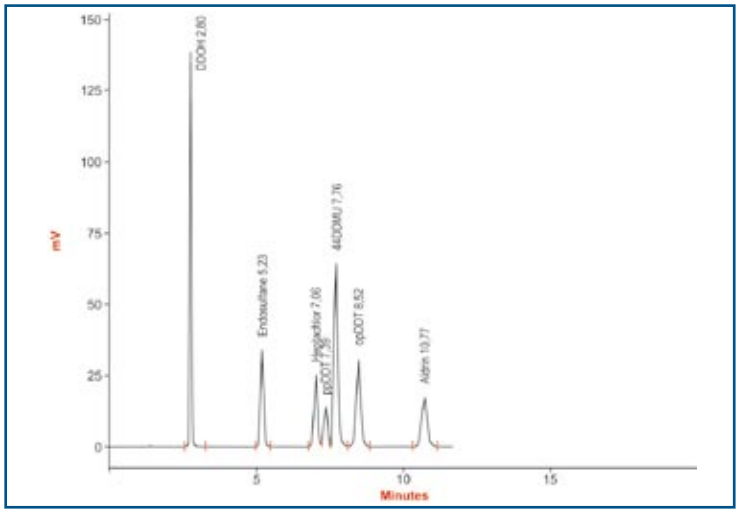
Rodeo™ **UP5HDO (5 x 50) x 4.6 mm**



Name	Tr	As	N	Rs
DDOH	4.87	1,08	23050	0
Endosulfane	9.11	1.01	22608	22.93
Heptachlor	12.32	1.01	23561	11.39
ppDDT	12.91	0.99	23692	1.81
44DDMU	13.55	1	24027	1.84
op DDT	14.85	0.99	22455	3.5
Aldrin	18.74	0.99	22574	8.68

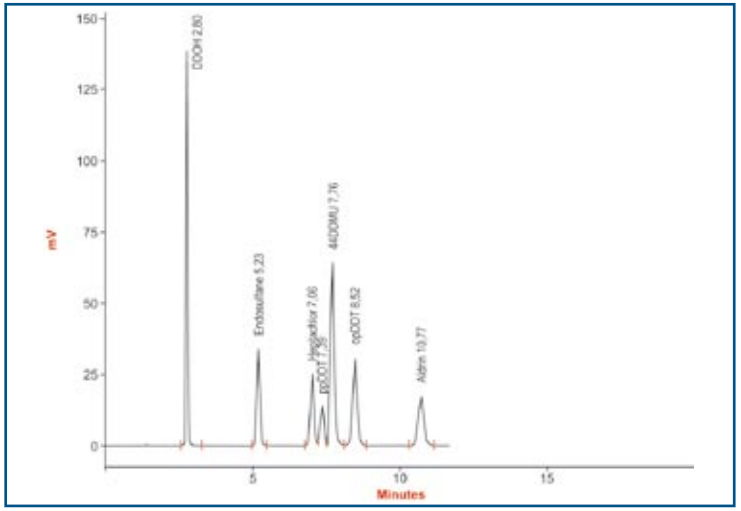
HPLC Method Optimization - RODEO™

Rodeo™ **UP5HDO (4 x 50) x 4.6 mm**



Name	Tr	As	N	Rs
DDOH	3.85	1.09	17499	0
Endosulfane	7.23	1.03	18122	20.43
Heptachlor	9.78	1.02	17585	10
ppDDT	10.27	1	17624	1.62
44DDMU	10.8	1.02	18611	1.7
op DDT	11.84	1	17732	3.08
Aldrin	14.95	1	17986	7.76

Rodeo™ **UP5HDO (3 x 50) x 4.6 mm**



Name	Tr	As	N	Rs
DDOH	2.8	1.08	12906	0
Endosulfane	5.23	1.02	13392	17.44
Heptachlor	7.06	1.04	13824	8.69
ppDDT	7.39	1.01	15796	1.37
44DDMU	7.76	1.01	13864	1.48
op DDT	8.52	1.01	13345	2.73
Aldrin	10.77	0.99	13398	6.75

HPLC Method Optimization - RODEO™

Mobile phase & RODEO™ column length optimization

Example 2. UP5HDO 50 x 4.6 mm :

The following studies show that an optimization step through a modification of mobile phase will have less impact than compared to optimization through selection of the appropriate column length. It is particularly significant when eluting conditions are fixed or predetermined.

fig1. Exhibits a separation of a mixture of pesticides from an Uptisphere 5 µm C18-HDO, 50 x 4.6 mm column. The mobile phase set as ACN /H_2O (95/5) leads to Heptachlor, 44DDMU & opDDT colelution.

fig2. Shows results when we increase the percentage of water within the mobile phase. All peaks are separated with a run time of 7.33 min

fig3. Shows results when we increase the length of the column. Peaks exhibit a sufficient resolution (> 1.5) and the final run time is 5.14 min

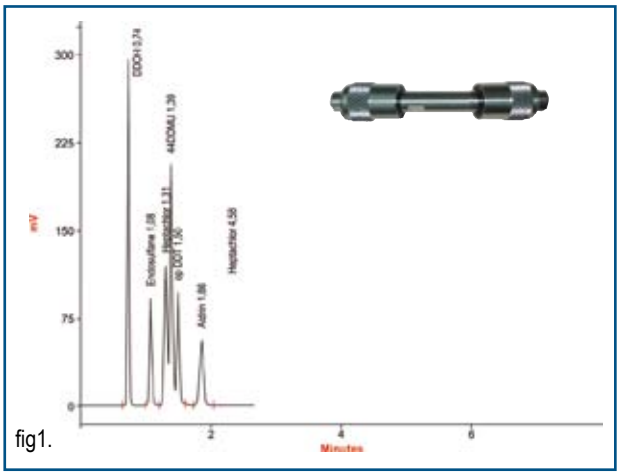
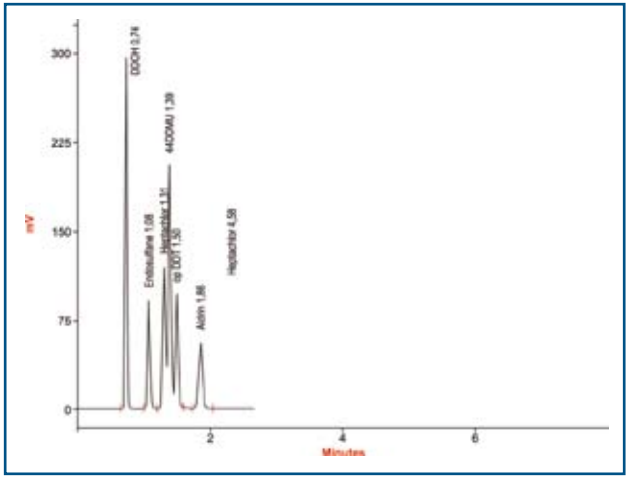


fig1.

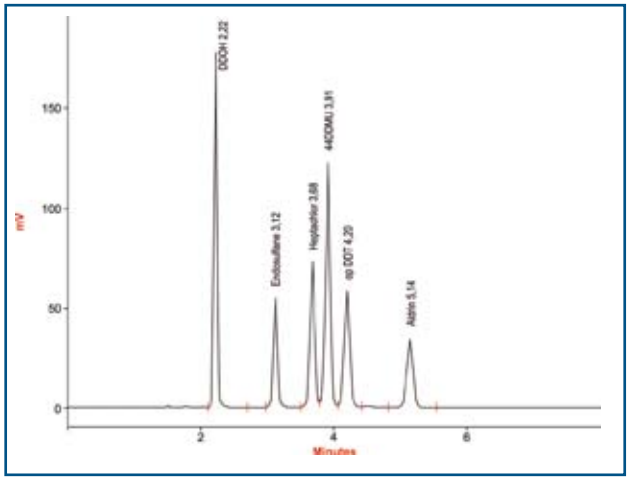
fig2. UP5HDO 50 x 4.6 mm : ACN /H_2O (75/25)



Name	Tr	As	N	Rs
DDOH	1.29	1.07	4509	0
Endosulfane	3.06	1.04	4929	14.05
Heptachlor	4.58	1.04	4728	6.91
44DDMU	5.19	0.94	3899	2.04
op DDT	5.79	1.01	4699	1.8
Aldrin	7.33	1	4764	4.03

HPLC Method Optimization - RODEO™

fig3. UP5HDO **3 x 50mm (coupled) x 4.6 mm** : ACN /H_2O (95/5)



Name	Tr	As	N	Rs
DDOH	2.22	1.05	12845	0
Endosulfane	3.12	1.01	11895	9.27
Heptachlor	3.68	1.01	12631	4.57
44DDMU	3.9	1	12050	1.67
op DDT	4.2	0.96	11682	1.97
Aldrin	5.14	0.98	12712	5.58

Description	Dimension	P/N
RODEO™ Strategy 5 C18-2	5 x 50 x 4.6 mm	US1740
RODEO™ Uptisphere 5 ODB	5 x 50 x 4.6 mm	BZ9130
RODEO™ Uptisphere 5 HDO	5 x 50 x 4.6 mm	BZ9150
RODEO™ Uptisphere 5 NEC	5 x 50 x 4.6 mm	BZ9170
RODEO™ Uptisphere 5 HSC	5 x 50 x 4.6 mm	BZ9210
RODEO™ Uptisphere 5 TF	5 x 50 x 4.6 mm	BZ9190
RODEO™ Strategy 5 C18-2	5 x 50 x 2.0 mm	US1750
RODEO™ Uptisphere 5 ODB	5 x 50 x 2.0 mm	BZ9140
RODEO™ Uptisphere 5 HDO	5 x 50 x 2.0 mm	BZ9160
RODEO™ Uptisphere 5 NEC	5 x 50 x 2.0 mm	BZ9180
RODEO™ Uptisphere 5 HSC	5 x 50 x 2.0 mm	BZ9220
RODEO™ Uptisphere 5 TF	5 x 50 x 2.0 mm	BZ9200

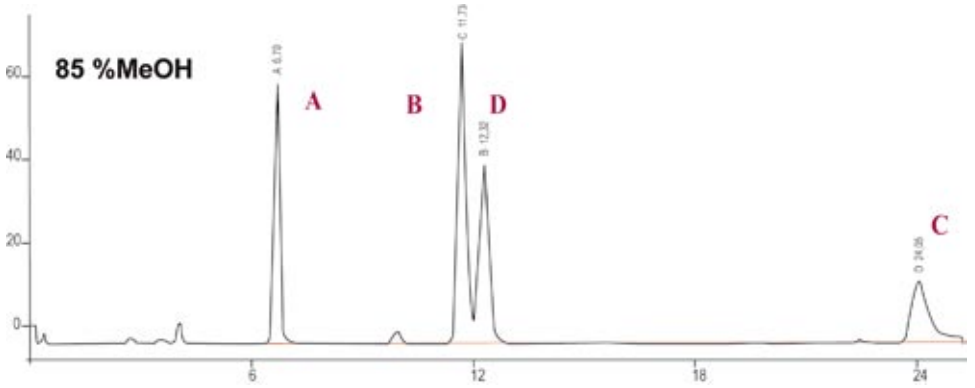
Further considerations

Mobile phase, temperature and pH optimization are explored below through an optimization of the anti-UV agent separation originally presented earlier in the method development section utilizing the Upti-select Kit™. In this example Uptisphere® TF was identified as the preferred column. Final peak retention time was 24 mn with 1.35 of resolution for the critical pair (B,D).

[It is also worth paying attention to simple HPLC system checks for your application type. i.e. dead volumes such as tubings, fittings etc ; check the detection cell volume, the injection loop volume and adapt the detector response time].

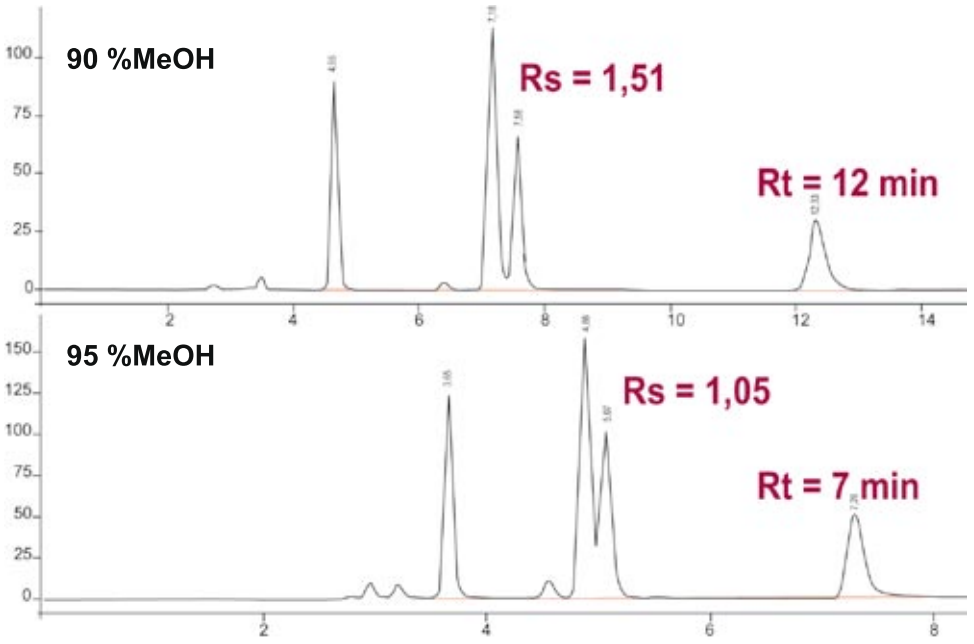
A modification of the organic concentration and temperature, finally achieves a run time of 6.88 min with a resolution of 3.02 for the critical pair (fig1).

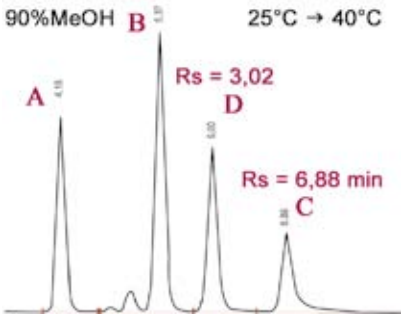
Original Separation of anti-UV agent by Uptisphere® TF



Followed by a subsequent variation in % methanol in the mobile phase...

HPLC Method Optimization





and a final variation in temperature (fig1.) ...

HPLC Method Optimization - OSIRIS™

OSIRIS™ - Software for HPLC method development & optimization

OSIRIS™ software is designed for the development and optimization of the analysis conditions in liquid chromatography and can generate a robust method from just a few preliminary analysis. OSIRIS™ allows, within isocratic or gradient elution mode, to optimize the conditions of elution (mobile phase content, temperature, pH). The latest version 4.0 of the software provides a new easy to use interface and a step by step guide through the optimization process.

OSIRIS™ provides efficient, accurate optimization through management of the analytical variables i.e.

- Mobile phase composition (isocratic, binary, ternary or quaternary, linear and multi-linear gradients), pH and /or temperature
- Multidimensional optimization ; isocratic composition (binary or ternary) / temperature - binary isocratic composition /pH - gradient /temperature - gradient /pH

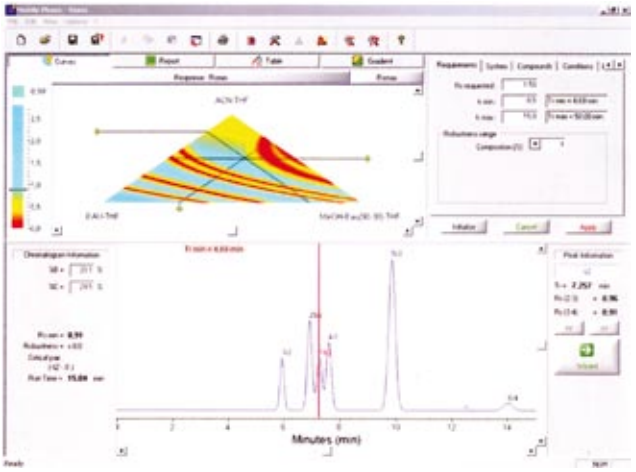
Optimize

OSIRIS™ takes account of your main criteria and enhances method and subsequent performance.

Establish your own requirement criteria in terms of separation quality (resolution), analysis time, and/or analysis condition robustness and identify the sensitivity of method to small variations in working parameters. OSIRIS™ also allows you to focus on the separation of specific compounds within a mixture.

Validate

OSIRIS™ is an excellent tool for validation and transfer of HPLC method taking account of the analytical variables. Physical properties of columns can also be studied with this software. The OSIRIS™ simulation table provides a means to compare chromatograms in different conditions of analysis.



Description	P/N
OSIRIS™ , HPLC optimization software	CC9260

Additional Uptipshere kits

Method Development

The kit consists of 3 HPLC columns from the Uptipshere® and Uptisphere® Strategy™ range that adhere to your considered requirements. Column phases need to be specified on the order.

Description	Micron	Dimension	P/N
Method development kit	3 µm	150 x 4.6 mm	DEV031546
Method development kit	5 µm	250 x 4.6 mm	DEV052546
Method development kit	3 µm	150 x 3.0 mm	DEV031530
Method development kit	5 µm	250 x 3.0 mm	DEV052530
Method development kit	3 µm	150 x 2.0 mm	DEV031520
Method development kit	5 µm	250 x 2.0 mm	DEV052520

Method Validation

The kit consists of 3 HPLC columns with the same dimensions from the Uptipshere® and Uptisphere® Strategy™ range that adhere to your considered requirements. Column phases need to be specified on the order. Every column is manufactured with a different stationary phase batch.

Description	Micron	Dimension	P/N
Method validation kit	3 µm	150 x 4.6 mm	VAL031546
Method validation kit	5 µm	250 x 4.6 mm	VAL052546

Analytical to Preparative Development

The kit consists of your chosen preparative column and an analytical column of same length with 4.6 mm internal diameter packed with the same phase batch. This kit is manufactured upon request. Contact your local distributor or Interchim's technical center for further information.