

Analysis - HPLC - Interchim Technology

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.
Initiate testing with the broad application Uptisphere Strategy column (5 μ m C18-2, 250 x 4.6 mm), conditions : ACN - H₂O (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



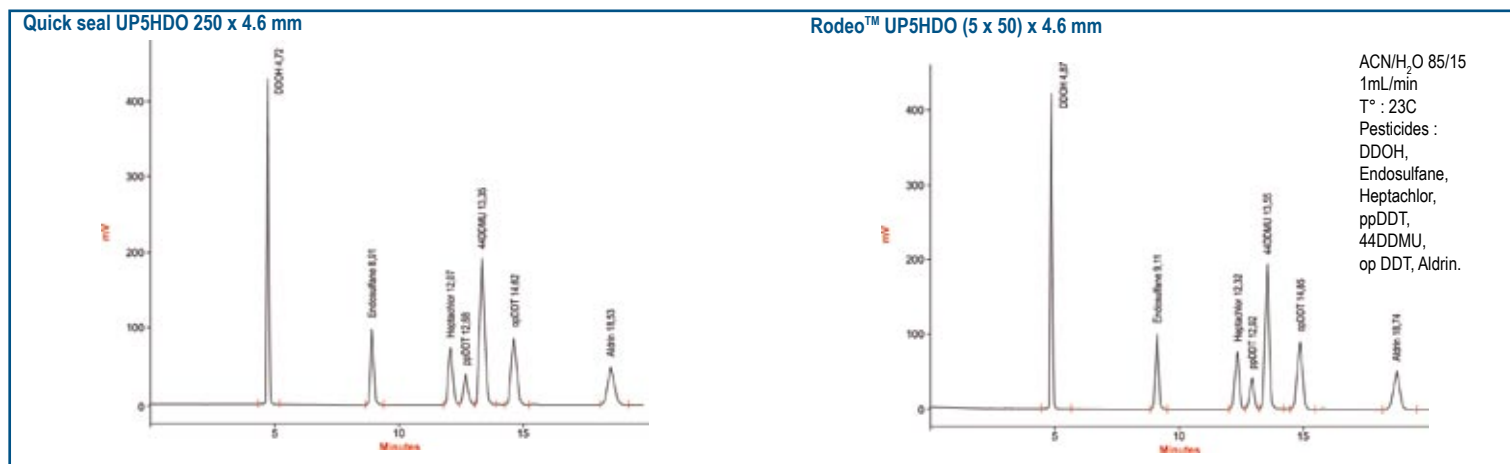
[Modulo-Cart Quick seal technology exhibits 15% increased efficiency compared to other HPLC hardware. It is a real hand-tight fitting and the carbon doped PEEK on stainless steel guarantees long column lifetime].



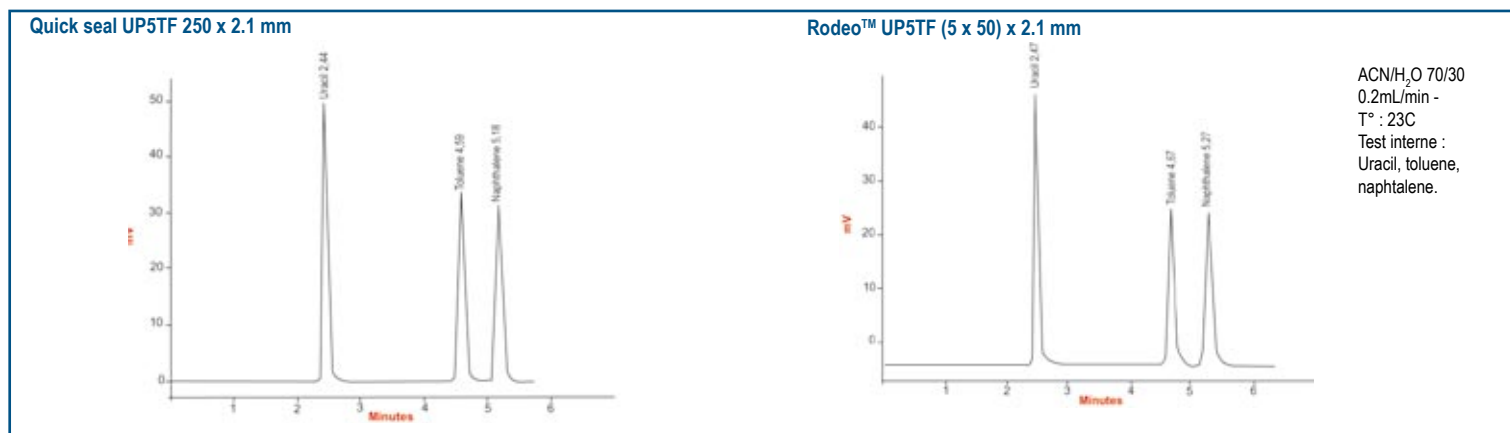
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Exploring the difference between a 250 mm Modulo-cart column and five 50 mm columns coupled together with Modulo-cart couplers.



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



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

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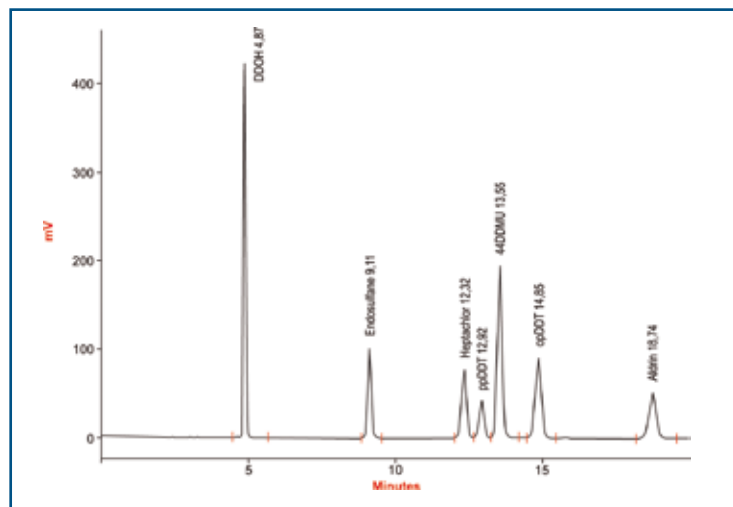
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RODEO™ column length optimization

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™ [partial 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

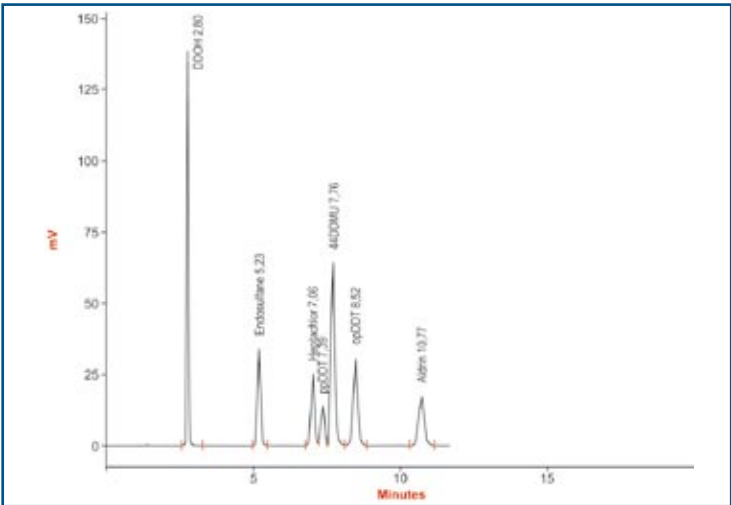


Name	Tr	As	N	Rs
DDOH	4.87	1.08	23050	0
Endosulfane	9.11	1.01	22608	22.93
Heptachlor	12.32	1.01	23561	11.39
ppDDT	12.91	0.99	23692	1.81
44DDMU	13.55	1	24027	1.84
op DDT	14.85	0.99	22455	3.5
Aldrin	18.74	0.99	22574	8.68

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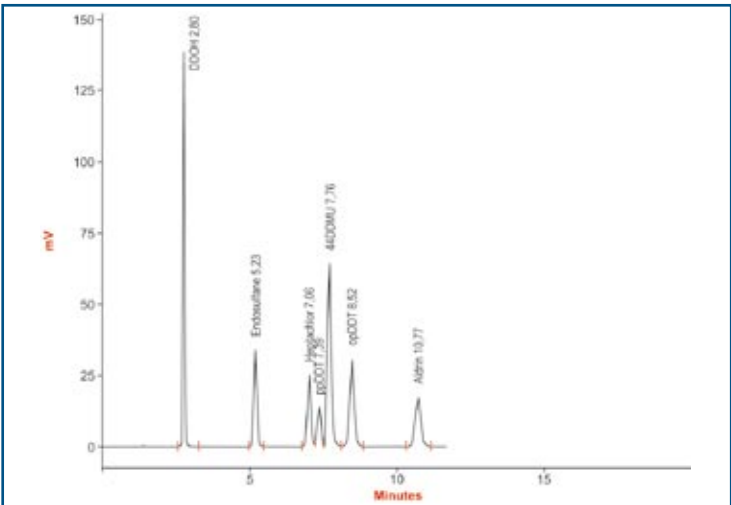
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Rodeo™ UP5HDO (4 x 50) x 4.6 mm



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

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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 /H₂O (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

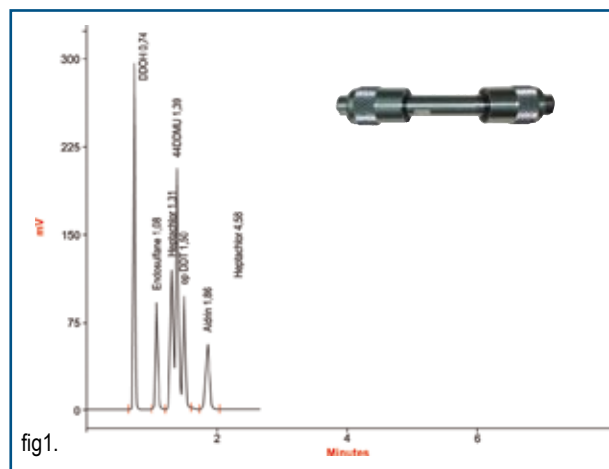
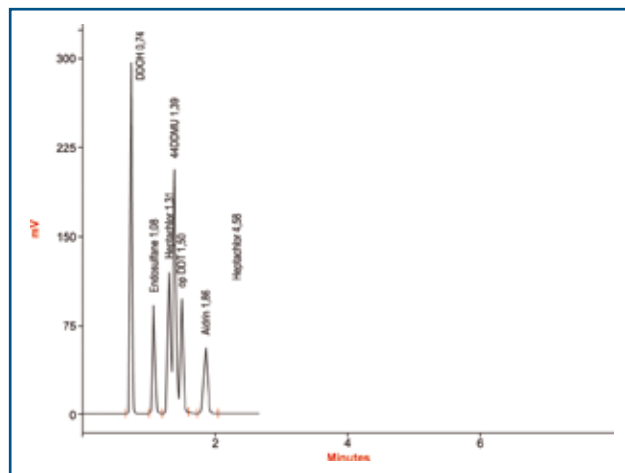


fig2. UP5HDO 50 x 4.6 mm : ACN /H₂O (75/25)

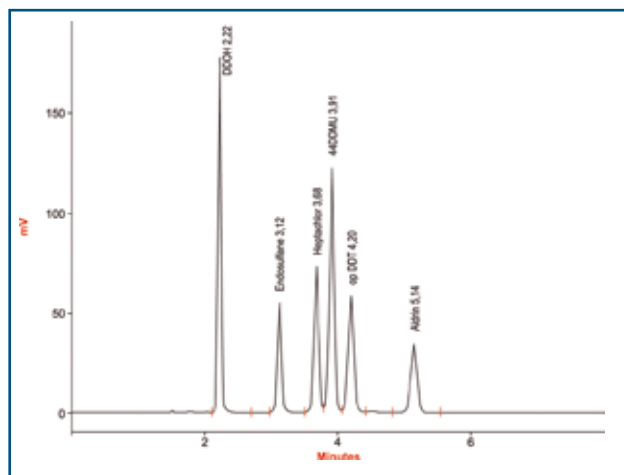


Name	Tr	As	N	Rs
DDOH	1.29	1.07	4509	0
Endosulfane	3.06	1.04	4929	14.05
Heptachlor	4.58	1.04	4728	6.91
44DDMU	5.19	0.94	3899	2.04
op DDT	5.79	1.01	4699	1.8
Aldrin	7.33	1	4764	4.03

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fig3. UP5HDO 3 x 50mm (coupled) x 4.6 mm : ACN /H₂O (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

