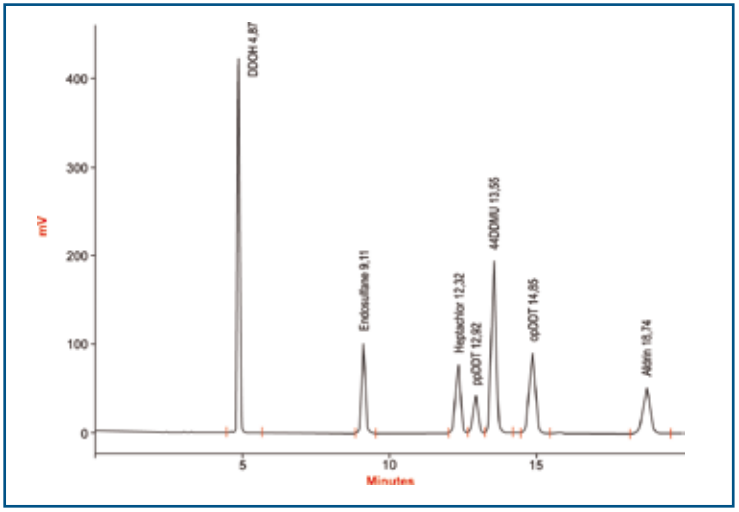
HPLC Method Optimization - RODEO™

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

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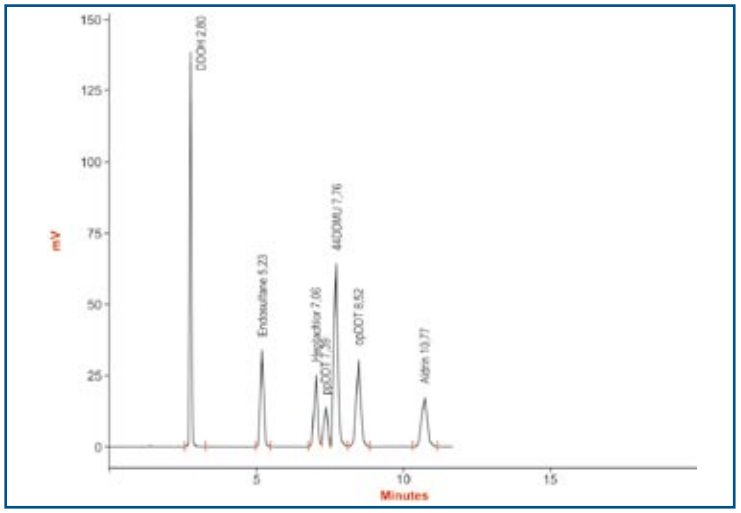
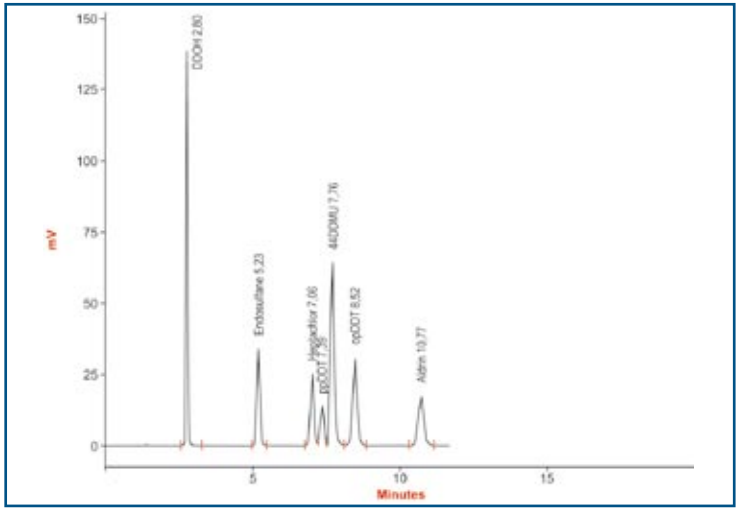
Rodeo™ UP5HDO (4 x 50) x 4.6 mm



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

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

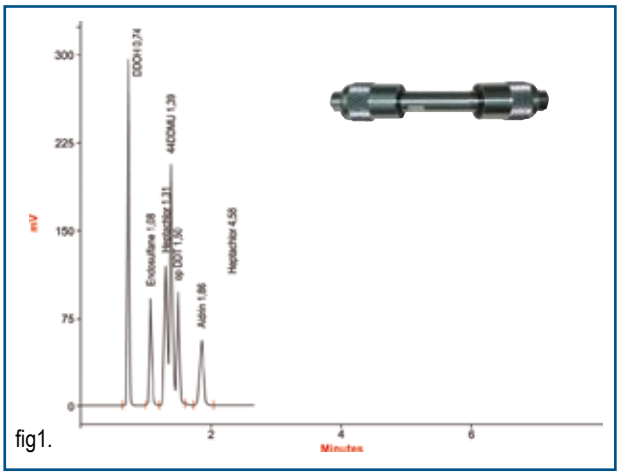
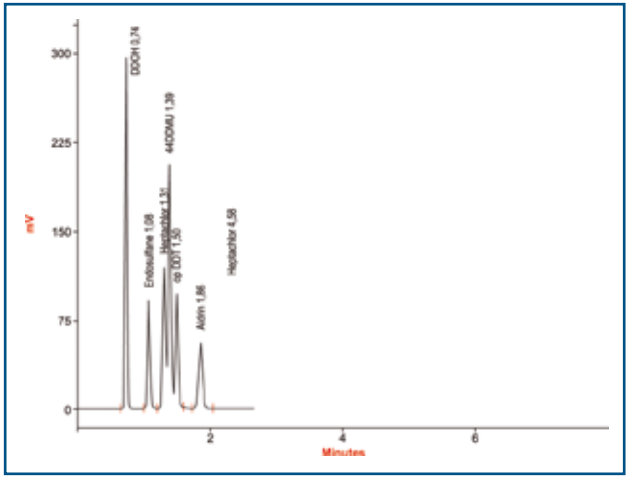


fig1.

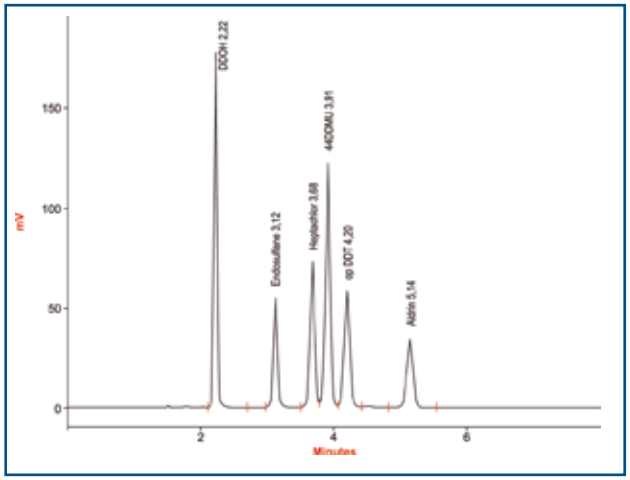
fig2. **UP5HDO 50 x 4.6 mm : ACN /H_2O (75/25)**



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**

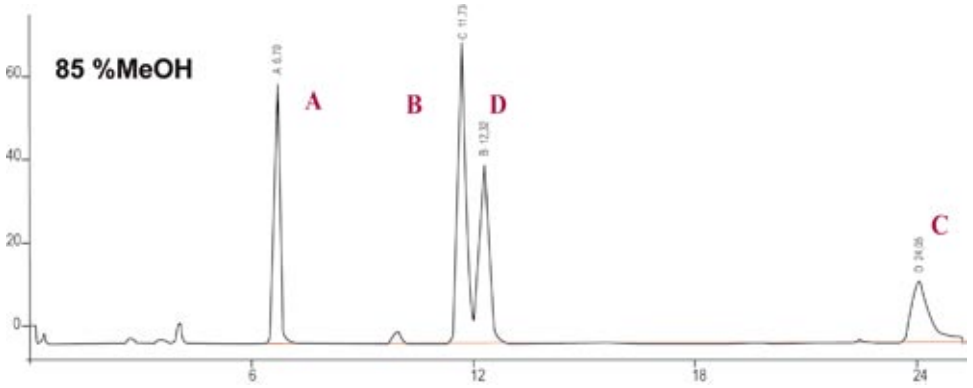
Further considerations

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].

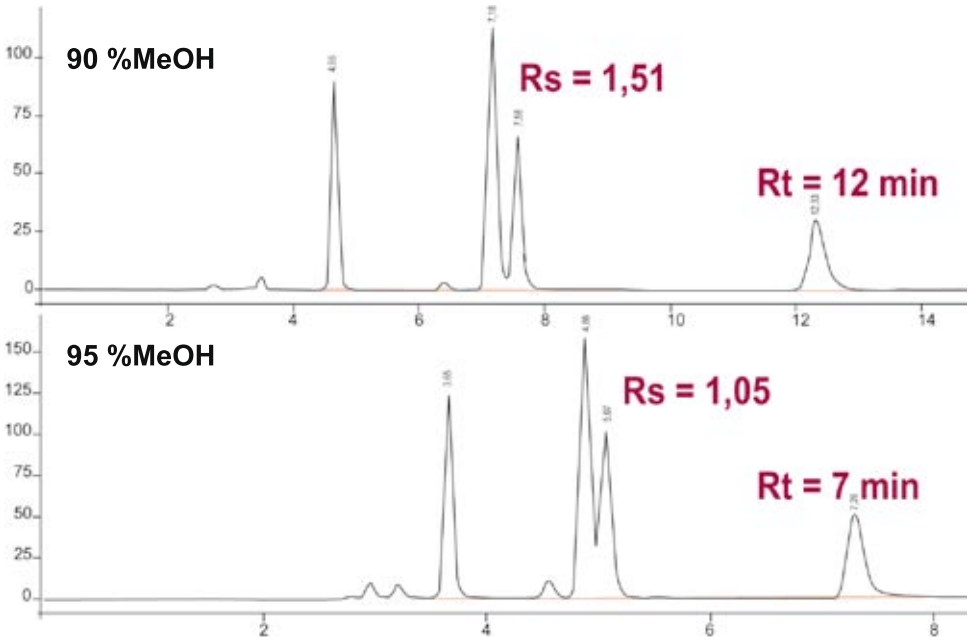
A modification of the organic concentration and temperature, finally achieves a run time of 6.88 min with a resolution of 3.02 for the critical pair (fig1).

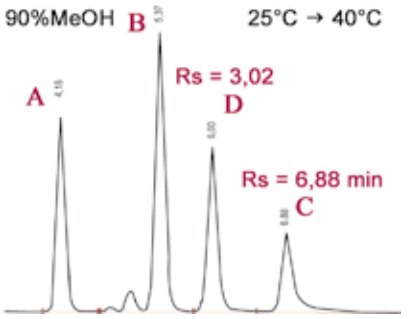
Original Separation of anti-UV agent by Uptisphere® TF



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

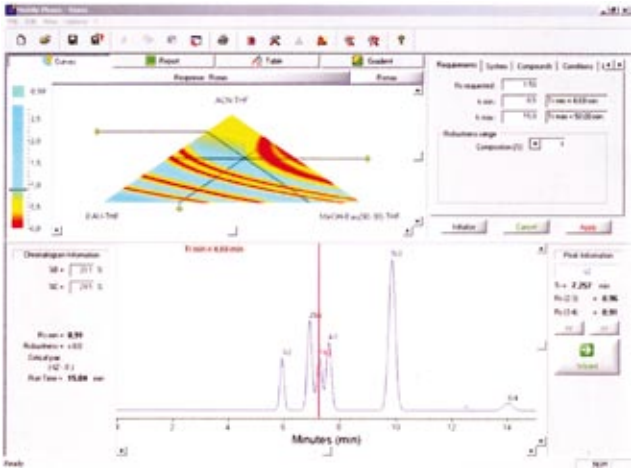
Optimize

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