

Efficient HPLC Validation

Background

HPLC is one of the most widely used analytical techniques because it offers a powerful means of separating complex mixtures of pharmaceuticals, agrochemicals, chemical intermediates and pollutants.

HPLC methods are developed by varying many factors such as column type, solvent type & proportions, buffer type and pH, flow rate and mobile phase additives. Once a method is developed it often forms the basis of a Standard Operating Procedure (SOP) for analysis.

Before such a method can be used routinely it must be validated which requires that small changes in the operating conditions do not result in a failure of the method. This ensures that operators in other laboratories are able to repeat the method successfully.



Picture © Waters Corporation

DoE: A Strategy for Method Validation

Testing the robustness of an HPLC method involving many factors can lead to an impractical number of experiments, adding to the time and cost of the assay.

Design of Experiments (DoE) is a statistical approach which can dramatically reduce the number of experiments required whilst retaining essential information about the factors.

Experiments are conducted so that all factors are simultaneously varied according to a mathematical design. Popular choices for robustness testing are *fractional factorial* and *Plackett-Burman* designs (FFD).

Example Validation

A HPLC separation required a resolution of > 1.5 to account for anticipated inter laboratory variation.

The first step is to define the relevant factors and the ranges over which they are expected to vary during normal operating conditions. For example the worst expected error in pH measurement leads to the range 3.8 to 4.2.

The second step is to define the responses, here the capacity factors of two of the peaks and the resolution.

Next, using the *Design Wizard* functionality in MODDE, an 8 run 2^{5-2} fractional factorial design, supplemented with 4 centre points was chosen.

Factors						
	Name	Abbr.	Units	Type	Use	Settings
1	AcN	Ac	%	Quantitative	Controlled	25 to 27
2	pH	pH		Quantitative	Controlled	3.8 to 4.2
3	Temp	Te	°C	Quantitative	Controlled	18 to 25
4	OSA	OS	mM	Quantitative	Controlled	0.09 to 0.11
5	Column	Co		Qualitative	Controlled	CoA,CoB

Responses			
	Name	Abbr.	Units
1	k1	k1	-
2	k2	k2	-
3	Res1	Re1	-

Modelling the Responses

DoE is normally used to investigate which factors affect the responses and in what way. In robustness testing, however, the aim is to demonstrate that small changes in key factors **do not** significantly affect the responses.

In this case significant models were found for both capacity factors but not for resolution. The results show that resolution is both **within specification** and **robust** which is the ideal situation.

Setting Specifications

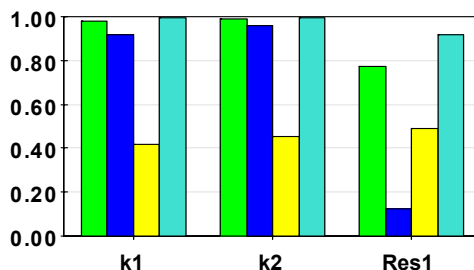
If significant models are found then predictions of responses may be made at the worst possible combination of factor settings to check that the responses remain within specification even in the worst case.

If responses are found to lie outside specification then the model may be used to predict the 'tightening' of the factor ranges required to maintain robustness.

MODDE is fully validated software and contains an audit trail and is 21 CFR 11 compliant (electronic records).

Investigation: itdoe_rob01a (MLR)
Summary of Fit

Green	R2
Blue	Q2
Yellow	Model Validity
Cyan	Reproducibility



MODDE software summarises the quality of the models using easily interpretable bar charts

Conclusions

- Design of Experiments is an efficient way to conduct validation experiments.
- MODDE is easy to use and guides the user through the process of choosing the most appropriate design.
- Modelling of the results highlights any significant response variation.
- DoE models may be used to set factor limits for robustness.

MODDE 7 is our user friendly software for Design of Experiments. The design wizard and analysis advisor guide the user to a successful result.



In all countries:
Umetrics AB
Box 7960
SE-90719 Umea
Sweden
Tel +46 (0)90 184800
Fax: +46 (0)90 184899
Email: info@umetrics.com
info.uk@umetrics.com

www.umetrics.com

In USA and Canada:
Umetrics, Inc.
17 Kiel Ave.
Kinnelon, NJ 07405
USA
Tel: +1 973 492 8355
Fax: +1 973 492 8359
E-mail: info.us@umetrics.com

In UK:
Umetrics UK Ltd.
Woodside House, Winkfield
Windsor, SL4 2DX
UK
Tel: +44 (0)1344 885605
Fax: +44 (0) 1344 885410
E-mail