Experimental Combination of Method Development Strategies in a Working Environment of Different Instrumental Set-ups

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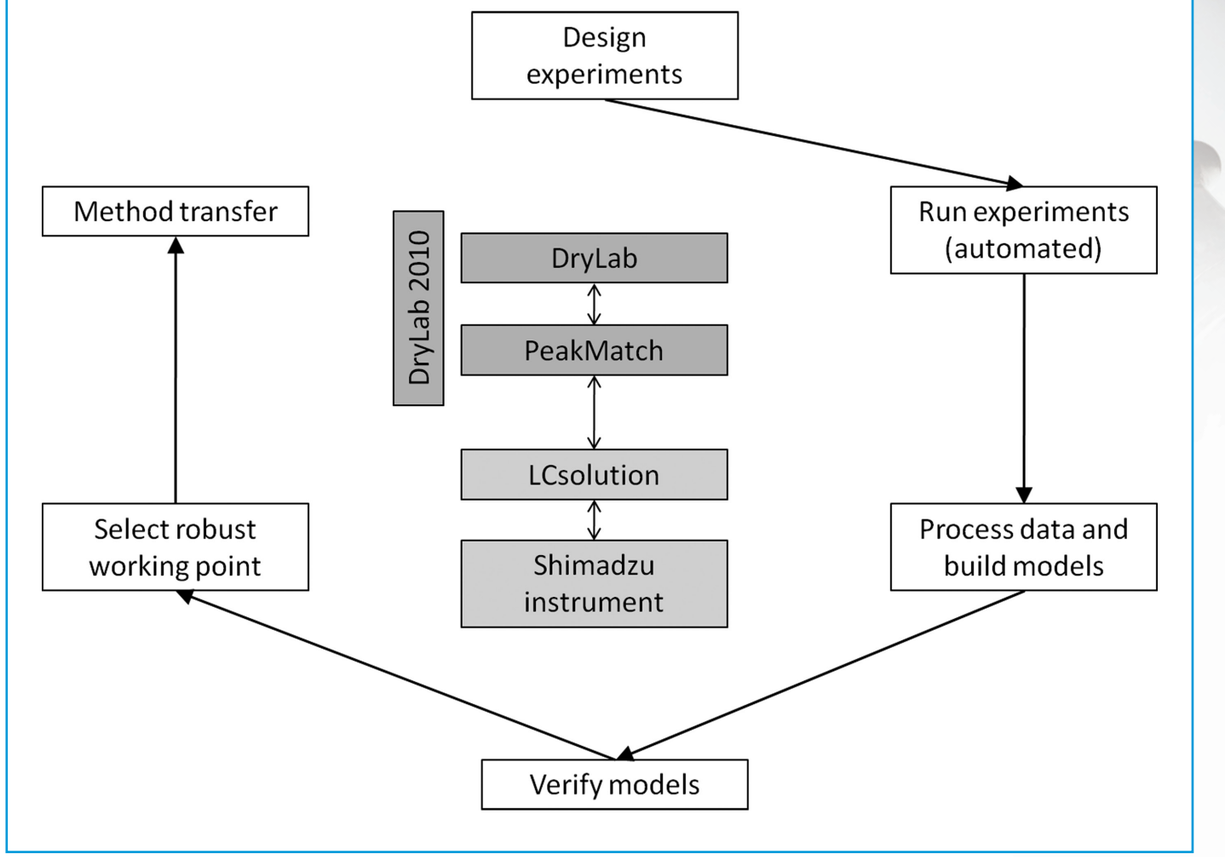
Modelling of retention behaviour of pharmaceutical compounds has a long history.[1] In recent times chromatography modelling software has been shown to considerably aid the application of Quality by Design (QbD) principles in the method development process, with the construction and evaluation of column and eluent design spaces.[2–5]

As described in the ICH Q8 (R2),[6] a QbD approach to reversed-phase high pressure liquid chromatography (RP-HPLC) method development should be systematic, beginning with predefined objectives, emphasizing understanding and control and based on sound science.

One of the advantages of applying QbD principles to the development process of RP-HPLC methods is that robustness and, therefore, easier method transfer can be built in from the outset. This translates into more reliable methods and allows more flexibility to improve a method once established, without the need for revalidation. A commonly occurring problem in the life cycle of a method is that as it is often run on a different instrumental set-up to that on which it was developed, changes in selectivity that compromise the quality of the original separation can occur.

The combination of ultra-fast technologies with the latest modelling visualization software has recently been employed in a Shimadzu presentation.[7] This article presents a specific strategy for applying QbD principles to the development of an HPLC method for a sample of toxicological interest with the evaluation of robustness and method transfer.

Figure 1: Workflow followed.



Experimental

Eluents: Methanol, acetonitrile and HPLC water were purchased from Promochem. Eluent A was prepared by combining varying volumes of aqueous buffers of differing pH (A1 and A2): A1 was a solution of 25 mM phosphoric acid and A2 was a solution of 25 mM monobasic sodium monophosphate. For pH 2.6 25% A1 and 75% A2 (V:V) were mixed, and for pH 1.7 100% A1 was used. Eluent B was methanol, acetonitrile and mixtures of the two. Further details can be found in Table 1.

Sample: Twelve model substances of toxicological interest provided by Charité – Universitätsmedizin Berlin, Institute of Legal Medicine were used: acetaminophene, caffeine, sulphathiazole, sulphadimidine, sulphamerazine, sulphamethodoxypyridazine, sulphamethoxazaole, sulphafurazole, sulphaquinoxaline and propyhenazone. MPPH was purchased from Sigma-Aldrich and N-ethyl oxazepam from Recipe.

Equipment: HPLC separations were performed on three different Shimadzu instruments: a Prominence TOX.I.S, a Nexera and a Prominence UFLC (Shimadzu Corporation). UV detection was performed at 280 nm. SHIM-PACK XR ODS II C18 columns (75 mm × 3 mm, 2.2 µm) and (75 mm × 2 mm, 1.6 µm) were provided by Shimadzu Europe. Further details can be found in Table 1.

Software: HPLC separations were generated using the automation option of DryLab 2010, which includes PeakMatch V. 3.6.3 and DryLab V. 3.95 (Molnár-Institute, Berlin, Germany) coupled with Shimadzu's LCsolution integration software.

Experiments for modelling: Conditions under which input experiments were run for the generation of retention models are detailed in Table 1.

Table 1: Summary of all experimental conditions for input runs used in the generation of DryLab models.

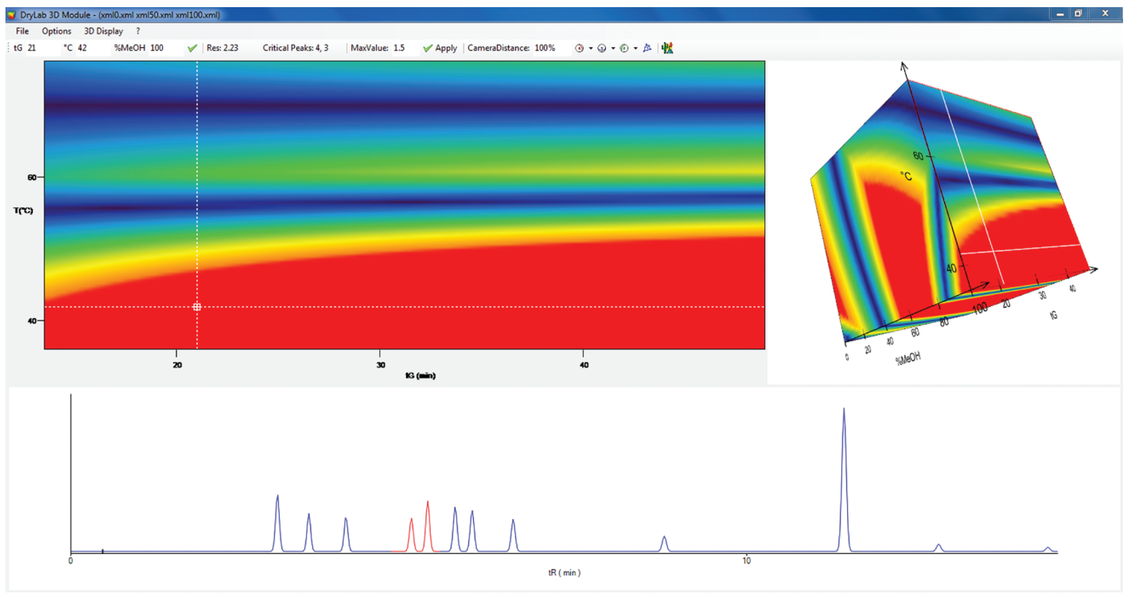
Instrument data		Modelling data		Eluent data						Column data	
Name	Dwell volume	Optimized parameters	Input conditions	Flow-rate	Eluent A		Eluent B	Gradient		Type	Dimensions
					pH	Additive		Start %B	End %B		
Shimadzu Prominence TOX.I.S	1.10 mL	Gradient time (t_G)	t_{G1}: 15 min t_{G2}: 45 min	0.7 mL/min	pH 2.3	25 mM phosphate buffer	Different (AN:MeOH) ratios	5	95	C18, SHIM-PACK XR ODS II	(75 mm × 3 mm, 2.2 µm)
		Temperature (T)	T_1: 40 °C T_2: 70 °C								
		Ternary eluent composition (tC) (AN:MeOH)	tC_1: (100:0) tC_2: (50:50) tC_3: (0:100)								
Shimadzu Nexera	0.14 mL	Gradient time (t_G)	t_{G1}: 4 min t_{G2}: 12 min	0.8 mL/min	pH 1.7	25 mM phosphate buffer	AN	5	70	C18, SHIM-PACK XR ODS II	(75 mm × 2 mm, 1.6 µm)
		Temperature (T)	T_1: 40 °C T_2: 70 °C								
Shimadzu Prominence UFLC	0.35 mL	Gradient time (t_G)	t_{G1}: 4 min t_{G2}: 12 min	1.0 mL/min	pH 1.7	25 mM phosphate buffer	AN	5	70	C18, SHIM-PACK XR ODS II	(75 mm × 3 mm, 2.2 µm)
		Temperature (T)	T_1: 40 °C T_2: 70 °C								

Results and Discussion

A workflow (see Figure 1) was designed combining DryLab with different Shimadzu instruments, generating basic experiments overnight, carrying out peak tracking the next morning, creating the corresponding retention models, and running the confirmation experiments. The DryLab models were then used to evaluate robustness and method transfer without any further experimentation.

Design of experiments (DoE): The experimental design differed from instrument to instrument. On the Shimadzu Prominence TOX.I.S a three parameter optimization was performed by modelling gradient time, temperature and ternary composition of eluent B. The corresponding DoE consisted of 12 experiments resulting from the combination of two different gradient times, two temperatures and three ternary eluent ratios. On the Shimadzu Nexera and Shimadzu Prominence UFLC, gradient time and temperature were modelled and optimized; four experiments were peformed on each instrument.

Figure 2: 3-D resolution space modelling gradient time (x-axis), temperature (y-axis) and ternary eluent composition (z-axis) vs critical resolution ($R_{s,crit}$). Red areas indicated above baseline separation ($R_{s,crit}$ > 1.5) whereas blue regions indicate a coelution.



Automated experimental data generation: Once the experiments were designed, chromatograms were acquired by means of automated data generation. This was executed within the software PeakMatch coupled with Shimadzu's LCsolution software. Chromatographic conditions, instrument, column and eluent data were first keyed into the input data interface. Then, upon "starting runs" the relevant experimental data were loaded into a batch file and read by the chromatographic system. Once the batch had been completed, chromatograms were automatically integrated and imported ready for peak tracking.

Peak tracking and generation of retention models: Upon changing eluent properties and the temperature, chromatographic selectivity (the separation) also changes. Therefore, prior to importing data into DryLab for modelling, peak tracking had to be performed. Peak tracking refers to the matching of bands for the same compound between experimental runs where conditions have been changed. Once peaks were matched data was transferred to DryLab where retention models are generated.

Resolution models map the critical resolution (resolution between the least separated peak pair) for each combination of the study parameters (i.e., t_G, T, ternary). The value of the critical resolution ($R_{s,crit}$) is represented as a colour so that warm colours show large $R_{s,crit}$ values and cold colours show low values. Specifically, red regions are above baseline resolution ($R_{s,crit}$ > 1.5) and blue lines signalize peak overlaps ($R_{s,crit}$ = 0). The retention space generated on the Shimadzu Prominence is shown in Figure 2. Each point within this cube corresponds

Table 2: Potential working points for each instrumental set-up with corresponding tolerances.

	Gradient time (t_G)	Temperature (T)	Ternary eluent composition (tC)
Shimadzu Prominence TOX.I.S	17 ± 2 min	48 ± 3 °C	40 ± 5% AN in MeOH
Shimadzu Nexera	6 ± 2 min	53 ± 3 °C	100% AN
Shimadzu Prominence UFLC	11 ± 2 min	44 ± 2°C	100% AN

to a precise modelled chromatogram and each cube represents over a million virtual experiments.

Confirmation of models: The three retention models were experimentally verified by running confirmation experiments. Correlation between predicted and experimental data, shown in Figure 3, was found to be excellent, with deviations not exceeding 0.03 min (2 s).

Selection of working point and robustness: Once the retention models had been experimentally verified, working points could be selected from within the robust regions (represented by the colour red indicating $R_{s,crit} > 1.5$) of the resolution maps. The choice of working point was done taking into consideration critical resolution, robustness tolerances and run time, maximizing the first two and minimizing the last. The selected working points with corresponding robustness tolerances can be found in Table 2.

Method transfer: To evaluate the practical use of DryLab as a method transfer enabler, the retention model generated on the Shimadzu Prominence UFLC was used to predict results on the Shimadzu Nexera. Instrument data as well as column dimensions and flow-rate were varied so as to fully test the software's predictive capacity. Figure 4(i) compares the DryLab t_G-T model for the Nexera, and the derived DryLab model, originally generated for the Prominence UFLC with modified dwell volume, extra column volume, flow-rate, column diameter and particle size. Both resolution maps correlate well and were experimentally verified [Figure 4(ii)], indicating the software's ability to successfully model method transfer.

Summary

A workflow incorporating QbD principles was applied to the development of a reversed-phase liquid chromatography method for the separation of twelve compounds of toxicological interest. The influence on selectivity of critical parameters was studied and highly accurate retention models were created. Input data for modelling was generated by means of the harmonic combination of various Shimadzu instruments and DryLab software. The results obtained were used to define robust working points with known tolerances without the need for further experimentation. It was also shown how DryLab resolution models can be employed as a knowledgebase to store and transfer chromatographic data from one set-up to another without compromising quality, which is expected to have wide application in the field.

Acknowledgement

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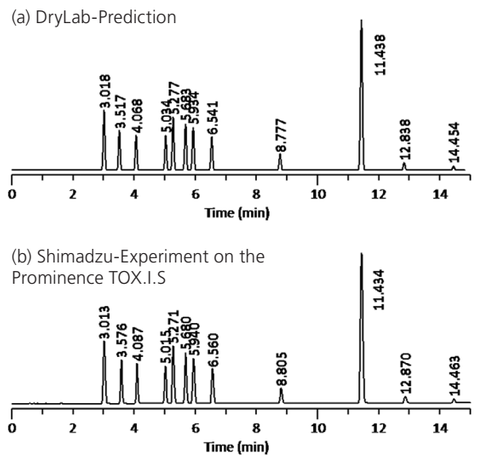
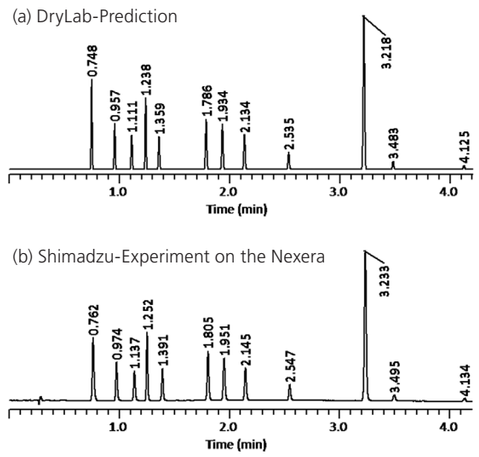
Figure 3: Verification experiments run on each of the three Shimadzu instruments: (i) Prominence TOX.I.S, (ii) Nexera and (iii) Prominence UFLC.

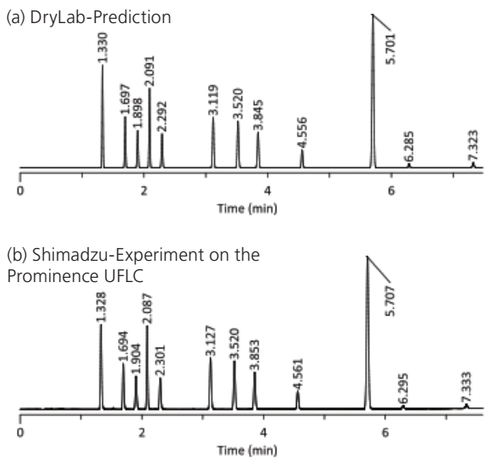
(i) Shimadzu Prominence TOX.I.S: gradient time 21 min, temperature 42 °C with 100% methanol as organic eluent (all remaining conditions identical to those of the input experiments).



(ii) Shimadzu Nexera: gradient time 6.2 min and temperature 53 °C (all remaining conditions identical to those of the input experiments).



(iii) Shimadzu Prominence UFLC: gradient time 11 min and temperature 44 °C (all remaining conditions identical to those of the input experiments)



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Imre Molnár received his PhD from the University of Saarbrücken, Germany, with special training in analytical chemistry. He then worked for two years as a postdoctoral fellow with Csaba Horváth at Yale University, USA, on fundamentals of reversed-phase chromatography. He founded the Molnár Institute 30 years ago and developed the DryLab software with L.R. Snyder, J. Dolan, T. Jupille and their team. He has considerable experience in the development of HPLC instruments, column production and HPLC applications and is specialized in pharmaceutical and biopolymer research and analysis.

Kate E. Monks received her degree in chemistry in Valencia, Spain. She worked at the Faculty of Pharmacy at the Charles University in Prague in the area of synthesizing drug candidates for tuberculosis. Since 2008 she has been working at the Molnár Institute in Berlin, specializing in DryLab 2010 3D method development.

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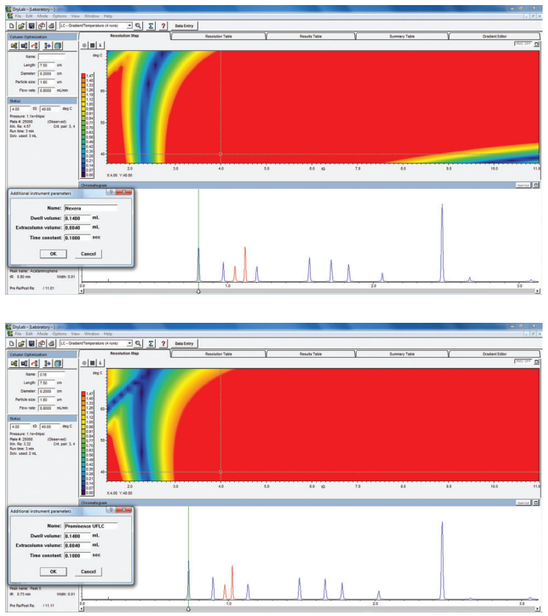
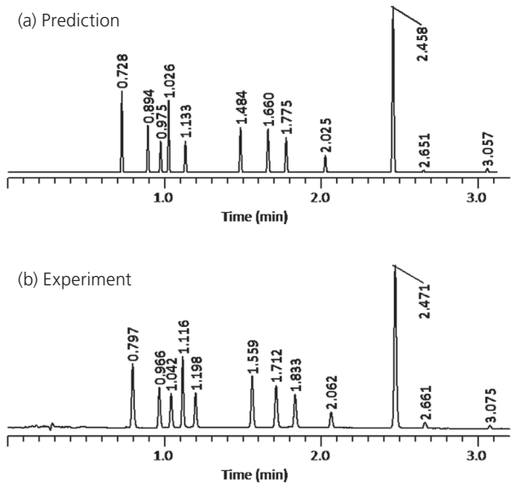
Figure 4: (i) Comparison between DryLab t_G-T robust resolution map ($R_{s,crit}$ > 1.5 shown in red colour) for the Nexera (above) and the predicted map for the Nexera configuration derived from the Prominence UFLC data (below). (ii) Comparison between (a) predicted chromatogram from Prominence UFLC data and (b) experimental chromatogram run on Nexera instrument at t_G 4 min and T 40 °C

(i)



(ii)



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