Improving Lab Sustainability using Retention Modelling for LC Method Development

INTRODUCTION

HPLC method development can be a lengthy process which can consume large amounts of resources in the analytical laboratory. Apart from time and money, this will also include solvents and additives for mobile phases and electricity to power the instrumentation. This also leads to the production of significant quantities of waste.

Often, a trial and error (or one factor at a time) approach is adopted during method development, where parameters are adjusted and decisions made according to the analytical results obtained for each iterative step. By adopting a more structured approach to method development the number of experiments needed can be reduced, useful retention knowledge for analytes can be generated and significant savings in time, money and environmental impact can be achieved. In addition to this, the use of retention modelling software can dramatically reduce the number of practical experiments even further, yielding substantial reductions of environmentally damaging solvent waste and electricity usage, making the whole process much more sustainable.

A popular approach is to begin method development by using screening protocols to systematically explore individual chromatographic parameters (such as column stationary phase, eluent composition, pH etc.) and their effects upon retention/separation.[1,2] Once screening is complete, the most promising combination of conditions can be further optimised, if needed, to produce the final method. This approach is useful, informed and highly recommended.[3]

Taking this process further, analyte retention data from screening experiments can be entered into LC retention modelling software to generate retention models and predict analyte retention behaviour.[4]

By modelling analyte retention times, for one or more experimental parameters, the need for large numbers of practical experiments can be eliminated and the associated chemical waste and other environmental impacts are removed. This can make a significant contribution towards achieving sustainability targets. Once a successful separation has been modelled it can be experimentally verified with just a few injections.

Avantor® ACE®

RETENTION MODELLING USING CHROMSWORD 5.1 LITE

The Avantor® ACE® ChromSword Method Development Kit (MDK) is designed to be the perfect way to develop robust analytical methods and introduce a combined screening/modelling approach to the lab in an extremely sustainable manner.

Often, the optimum result from the column screening approach doesn't provide a full separation of all the sample analytes. In this case, further method development is required through varying other chromatographic parameters such as temperature and gradient time.

The ChromSword 5.1 Lite software included in the Avantor® ACE® ChromSword MDK can be used to model these parameters, avoiding the need for further practical experiments, together with the associated solvent consumption and waste generation.

By entering retention data into the software, from as few as two experimental runs, a retention model can be established. From the model, it is then possible to simulate thousands of potential separations without the need to perform additional experiments.

In the example shown in Figure 1, a simple mixture of six analytes was run at three different pH values (3, 4.5 and 6). In all three experiments there is co-elution of at least two of the analytes. Even though a successful separation has not been achieved, at this point the work can move away from the laboratory. By entering analyte retention data into the ChromSword Lite software, a retention model can be generated. From this model, an optimum separation can be identified and simulated, thereby saving solvent, eliminating chemical waste and reducing energy consumption (A typical HPLC instrument may require over 5 kWh per day when in use).
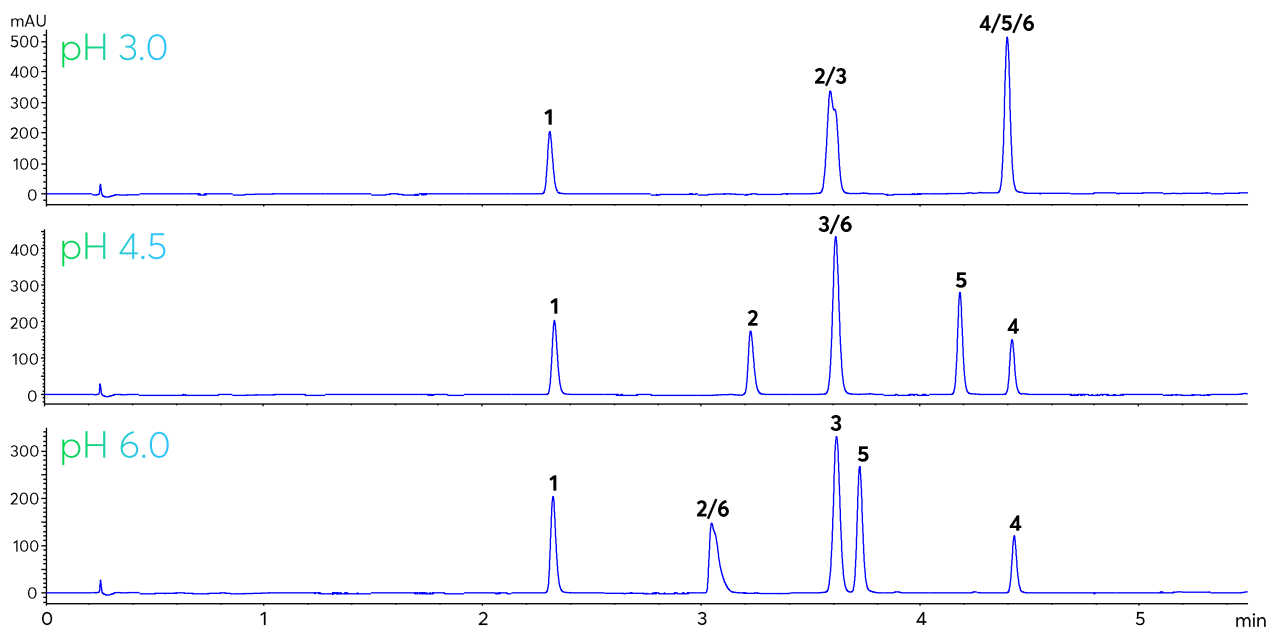

Figure 1: Separation of a six analyte test mix at pH 3, 4.5 and 6. Column: Avantor® ACE® Excel® 3 C18-AR, 50 x 2.1 mm; Mobile phase A: 20 mM ammonium formate (aq) B: 20 mM ammonium formate in MeOH/H_2O 9:1 v/v; Gradient: 3 – 100% B in 5 minutes; Flow rate: 0.6 mL/min; Temperature: 40 °C; Injection volume: 5 µL; Detection: UV, 254 nm. Sample: **1:** Caffeine, **2:** Furosemide, **3:** 1,3,5-Trinitrobenzene, **4:** Butylparaben, **5:** Ketoprofen, **6:** 3,4-Dichlorobenzoic acid.

To generate a retention model, experimental retention times are entered into the ChromSword 5.1 Lite software (Figure 2), along with details of the column and experimental conditions used. In this example, retention data from the three pH's shown in Figure 1 were used to construct a model of the logarithm of analyte retention factor vs pH for all six analytes (Figure 3). Each coloured line corresponds to a single analyte. Points where the lines intersect correspond to analyte co-elution, whilst in regions where no intersections occur, full analyte resolution can be achieved.

The retention model is often best viewed as a resolution map, which plots the resolution of the critical pair as a function of pH (Figure 4). In this case, the minimum
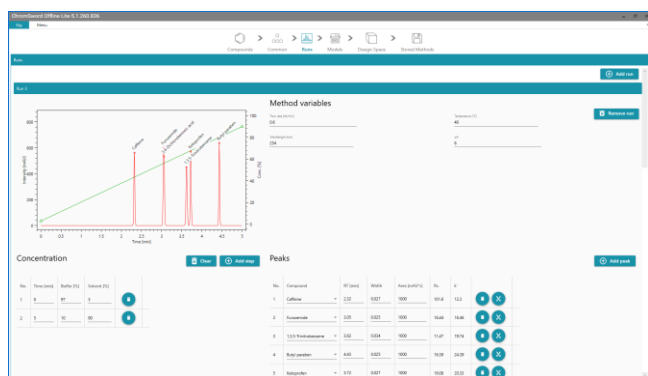
acceptable resolution has been set to 1.5 and is denoted by the red line. At any pH where the resolution map is above 1.5, an acceptable separation can therefore be obtained. This resolution map clearly shows that a mobile phase pH value of 5.1 will provide the maximum resolution.

Another major advantage of the modelling approach is that it can be used to build robustness into the final method. In Figure 4, an acceptable separation can be obtained at pH 4.2, however, any slight variation in the pH would lead to failure of the analytical method. A trial and error approach to method development could lead to the final method being set at this non-robust point. The modelling approach, however, has revealed that a far more robust separation is achieved at a mobile phase pH of 5.1. At this pH, the mobile phase would need to vary by at least ±0.3 pH units to risk method failure, resulting in a much more robust final method.

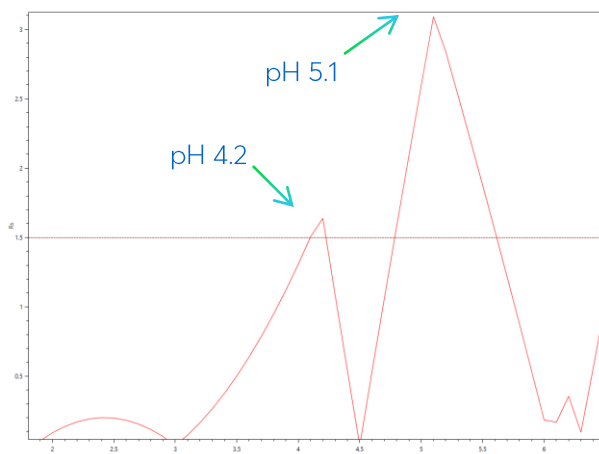**Figure 2:** Data entry screen from ChromSword 5.1 Lite.
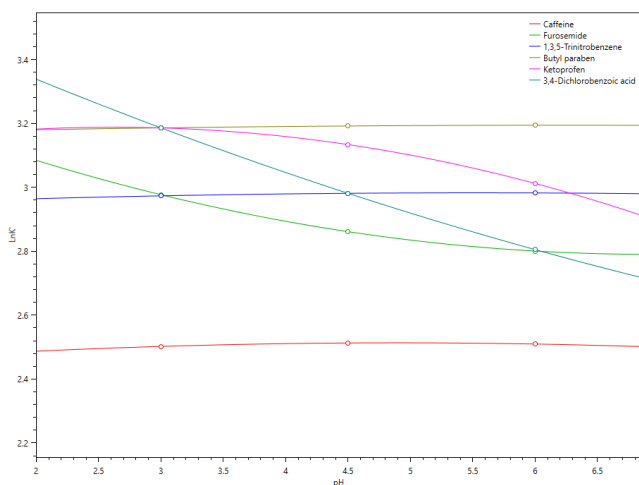
Figure 3: Quadratic lnK vs pH retention model generated from the experimental runs shown in Figure 1.

Figure 4: Resolution map (pH vs critical pair resolution) generated automatically from the retention model in Figure 3.

At any point on the retention model or resolution map, a virtual chromatogram can also be generated (Figure 5), which shows the predicted retention times of all the analytes.

Once the optimal conditions for the final method have been identified from the model, a single experimental run is used to confirm the modelled result (Figure 6). Table 1 compares the retention times predicted by the modelling software and the experimentally obtained results and demonstrates the high degree of accuracy that can be obtained from retention modelling.
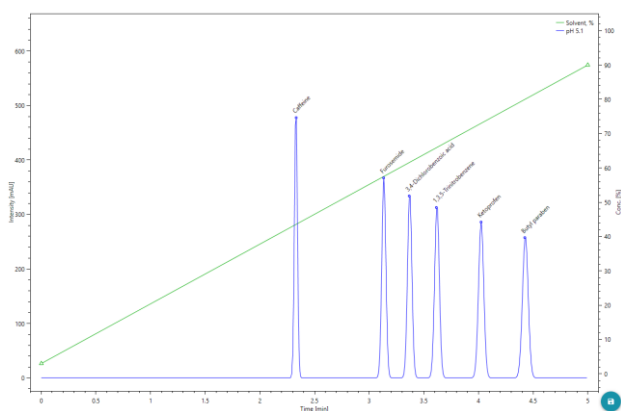
Figure 5: Simulated chromatogram showing all six peaks separated at pH 5.1

Table 1: Comparison of the predicted retention times (pH 5.1) from the simulated separation shown in Figure 5 vs experimentally obtained retention times (Figure 6).

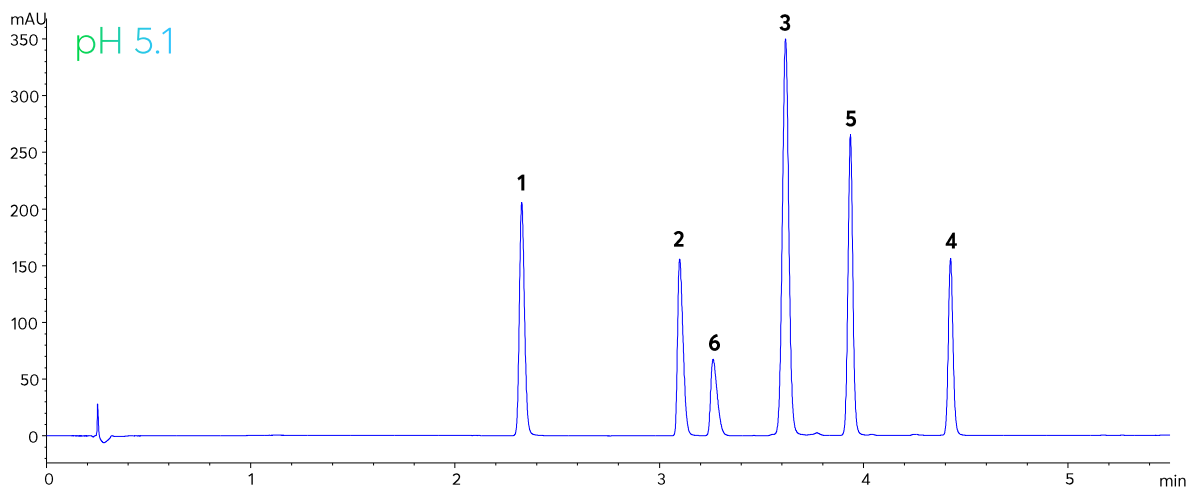| pH 5.1: Retention Times (mins)			
Analyte	**Predicted**	**Actual**	**% Difference**
Caffeine	2.33	2.33	0.0%
Furosemide	3.13	3.10	–1.0%
1,3,5-Trinitrobenzene	3.62	3.62	0.0%
Butylparaben	4.43	4.43	0.0%
Ketoprofen	4.02	3.93	–2.2%
3,4-Dichlorobenzoic acid	3.37	3.26	–3.3%

Figure 6: Experimental results at pH 5.1. Comparison of the experimentally generated data to the simulated data obtained in ChromSword 5.1 Lite is presented in Table 1. Column: Avantor® ACE® Excel® 3 C18-AR, 50 x 2.1 mm; Mobile phase A: 20 mM ammonium formate (aq) B: 20 mM ammonium formate in MeOH/H$_2$O 9:1 v/v; Gradient: 3 – 100% B in 5 minutes; Flow rate: 0.6 mL/min; Temperature: 40 °C; Injection volume: 5 µL; Detection: UV, 254 nm. Sample: **1:** Caffeine, **2:** Furosemide, **3:** 1,3,5-Trinitrobenzene, **4:** Butylparaben, **5:** Ketoprofen, **6:** 3,4-Dichlorobenzoic acid.

All six analytes are now well resolved. To reach this stage using practical experiments would have consumed large quantities of solvents and electricity, generated substantial volumes of chemical waste, and may not even have achieved this fully optimised separation. By using retention modelling, the optimised separation required just three experimental runs to generate the retention model and one run to confirm the final method. This example therefore demonstrates that by incorporating retention modelling software, the environmental impact of LC method development can be substantially reduced.

CONCLUSIONS

The use of retention modelling software during LC method development enables the generation of comprehensive retention models from just a few experimental input runs, and can be used to simulate thousands of potential separations. This means that the amount of experimental work that is required to produce a new method can be streamlined and drastically reduced. From an environmental perspective, this can dramatically reduce the amount of waste solvent generated and electrical power consumed by LC instrumentation during method development. Additionally, the modelling approach is often quicker, thereby improving lab efficiency and provides the added benefit of providing more confidence in the robustness of the final method, which will impact results obtained downstream during the analytical method lifecycle.

The Avantor® ACE® ChromSword Method Development Kit provides a cost effective solution to introduce user-friendly retention modelling software into method development workflows.

REFERENCES

1. Avantor® ACE® Knowledge Note #0018 "Step-by-Step Protocol for Streamlined Reversed-Phase Method Development using Avantor® ACE® MDKs" (https://uk.vwr.com/cms/ace_knowledge_notes)

2. Avantor® ACE® Knowledge Note #0021 "A Simple Step-by-Step Protocol for HILIC Method Development" (https://uk.vwr.com/cms/ace_knowledge_notes)

3. P. Petersson, M. R. Euerby, M. Fever, J. Hulse, M. James and C. Pipe, *LCGC Europe*, **29** (2016), 8-21

4. Avantor® ACE® Technical Note #006 "Streamlined Method Development Using the Avantor® ACE® ChromSword Method Development Kit" (https://av.cmd.vwr.com/rq/ddl/avantorace_technicalnote_atn006)

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