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Short communication

Computer-assisted method development and optimization in high-performance liquid chromatography

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Abstract

This paper reports the use of DryLab, a computer simulation software package, to assist in the development and optimization of a reversed-phase high-performance liquid chromatographic (HPLC) method for the separation of a model drug candidate and its degradation products. Prior to the optimization process, columns with various bonded phases are evaluated for their chromatographic performance using the sample of interest. Simultaneous optimization of two separation variables and the use of resolution maps to predict the optimal conditions are illustrated. Options to optimize column conditions (column length and flow-rate) to further reduce run time are briefly discussed. The accuracy of DryLab-predicted retention times and resolution is compared with experimental values. The DryLab software used in this study provided satisfactory predictions for the selected model, with average errors of less than 3.5 and 11.8% for retention time and resolution, respectively.

Keywords: Computer-assisted optimization; Computer simulation; DryLab

1. Introduction

A major challenge in the development of chromatographic separation methods is the rational selection of optimal experimental conditions that can provide an adequate resolution and a reasonable run time. This task becomes more complicated with an increasing number of operating variables and consequently requires a larger number of experimental runs. To simplify and accelerate the optimization process, several computer simulation software packages have been introduced [1,2]. With a limited number of experimental runs, chromatographers can model changes in experimental conditions, optimize method conditions and predict method robustness [3–5].

In the present study, a computer simulation software (DryLab) was employed in developing a reversed-phase HPLC separation of a drug candidate (Compound I) and its degradation products (Fig. 1). The importance of the evaluation of columns using the sample of interest prior to optimization process is emphasized. Features that allow simultaneous optimization of two variables and optimization of column conditions are discussed. Comparisons of

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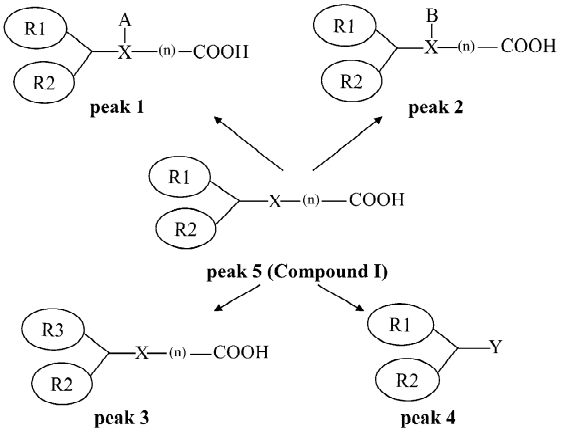

Fig. 1. Functional structural scheme of model drug candidate (Compound I) and its degradation products.

predicted and experimental values for selected separation conditions are also examined.

2. Experimental

2.1. Equipment and software

Chromatographic measurements were made on a HP1100 Series HPLC system (Agilent Technologies, Palo Alto, CA, USA) equipped with a binary pump, a vacuum degasser module, an autosampler with injection volume of 5 μl, a temperature-controlled column compartment and a variable wavelength UV detector set at 220 nm.

Multichrom chromatography data system, version 2.1-1 (Thermo LabSystems, Cheshire, UK) was used to acquire the chromatographic data.

Retention times and peak areas of individual peaks from experimental runs were used as input data for Drylab Software for Chromatography Modeling, version 3.0.09 (LC Resources, Walnut Creek, CA, USA).

HPLC columns evaluated in this study were purchased from YMC (Milford, MA, USA) and Agilent Technologies (Palo Alto, CA, USA).

2.2. Materials

Milli-Q water (Millipore, Bedford, MA, USA), HPLC-grade Omnisolve acetonitrile, methanol (EM Science, Gibbstown, NJ, USA), ammonium formate, ammonium acetate (American Chemicals, Montreal, QC, Canada) and trifluoroacetic acid (TFA), sequanal grade (Pierce, IL, USA) were used.

Standard solutions of Compound I (Merck & Co. Inc., Rahway, NJ, USA) were prepared in water–methanol (25:75, v/v) at a concentration of 0.2 mg/ml, The standard solution was exposed to light (30 min) for generation of peak 3 and then spiked with authentic standards of degradation products (peaks 1, 2 and 4).

3. Results and discussion

3.1. Selection of columns

The model compound and its degradation products used in this study are weak acids, except for one (peak 4) where the acidic functional group is absent (Fig. 1). Due to the ionic character of the model compounds, TFA was added to the mobile phase to provide an acidic medium and to minimize adverse ionic interactions between analytes and residual silanol groups of the silica backbone. Columns with various bonded phases were evaluated for optimum capacity factor (k'), plate number (N) and tailing factor. The organic content of the mobile phase and flow-rate were varied to accommodate the differences in the nature of the bonded phase and column dimension. The results (Table 1) suggested the YMC ODS-AM column as the best choice since it provided excellent peak symmetry, appropriate k' range and greatest plate number. The results also emphasize the fact that the chromatographic information about different phases supplied by many HPLC column manufacturers using their test probes and protocols may not reflect the performance using the sample of interest. Columns must be screened using the actual sample to select the most appropriate for the separation.

3.2. Experimental designs

Since the Drylab software allows for simultaneous optimization of only two variables in a given set of optimization variables, three combinations are possible in this study, based on organic content, pH and column temperature. They include organic content/

Table 1
Initial conditions and evaluation criteria for selection of columns

Columns	%B	Flow-rate (ml/min)	k' range	Tailing[a]	Plate[a] (N)
YMC ODS-AM (3 μm, 150×4.6 mm I.D.)	45	1.5	4.5–22	1.04	17 000
Zorbax SB-CN (3.5 μm, 75×4.6 mm I.D.)	38	1.0	4.0–12	1.71	6000
Zorbax SB-phenyl (5 μm, 150×3.0 mm I.D.)	35	1.5	8.5–27	0.73	4000
Zorbax SB-C_{18} (3.5 μm, 75×4.6 mm I.D.)	42	1.0	5.5–27	1.13	8000
Zorbax XDB-C_{18} (3.5 μm, 75×4.6 mm I.D.)	42	1.0	5.5–31	1.06	8000

[a] Calculated using the main peak.

column temperature, organic content/pH and column temperature/pH. The last combination has been reported to be less satisfactory in controlling selectivity and maximizing resolution [5]. Two combinations of organic content/column temperature and organic content/pH were selected for this study. Fig. 2 shows the experimental designs and the number of experiments required for each combination.

3.3. Simultaneous optimization of organic content (%B) and column temperature

Simultaneous optimization of organic content (%B) and column temperature was first performed using a YMC ODS-AM, 3 μm, 150×4.6 mm I.D. column with aqueous acetonitrile modified with 0.1% TFA as the mobile phase. The flow-rate was set at 1.5 ml/min. The experimental design for this combination is illustrated in Fig. 2A.

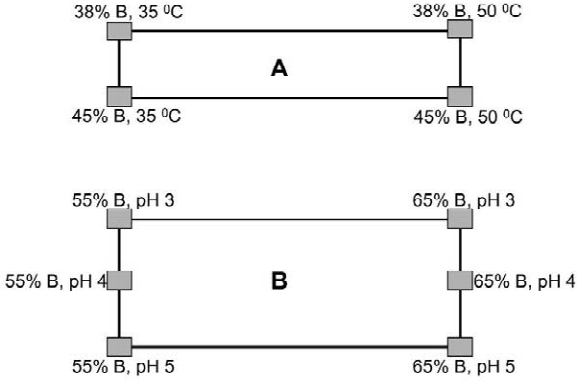

Fig. 2. Experimental designs for simultaneous optimization of (A) organic content/column temperature and (B) organic content/pH.

The result is shown as a resolution map (Fig. 3), where the smallest value of resolution (R_s) of any two critical peaks in the chromatogram is plotted as a function of two simultaneously varied experimental parameters. A maximal R_s of greater than 4 was obtained at higher column temperature and low %B (<30%) at the cost of a long run time (>250 min, not shown on the resolution map). An R_s of less than 1.5 and a significantly shorter run time were obtained when organic content exceeds 48%, regardless of column temperature. An optimal R_s of at least 2.0 and a run time of less than 40 min was obtained at 41–45% B and at 50 °C. Table 2 shows a comparison of predicted and experimental data for the optimized separation at 44% B and column temperature at 50 °C. A good agreement between the predicted and experimental values was obtained, with average errors of 0.8 and 6.6% for retention time and resolution, respectively.

Additional optimization was performed with a column temperature of 50 °C and higher organic content to reduce the run time is described in the following section.

3.4. Simultaneous optimization of organic content (%B) and pH

Experimental design for simultaneous optimization of %B and pH requires six experiments, as illustrated in Fig. 2B. The mobile phase consisted of (a) 25 mM ammonium formate (pH 3.0) or (b) 25 mM ammonium acetate (pH 4.0 and 5.0) and acetonitrile as

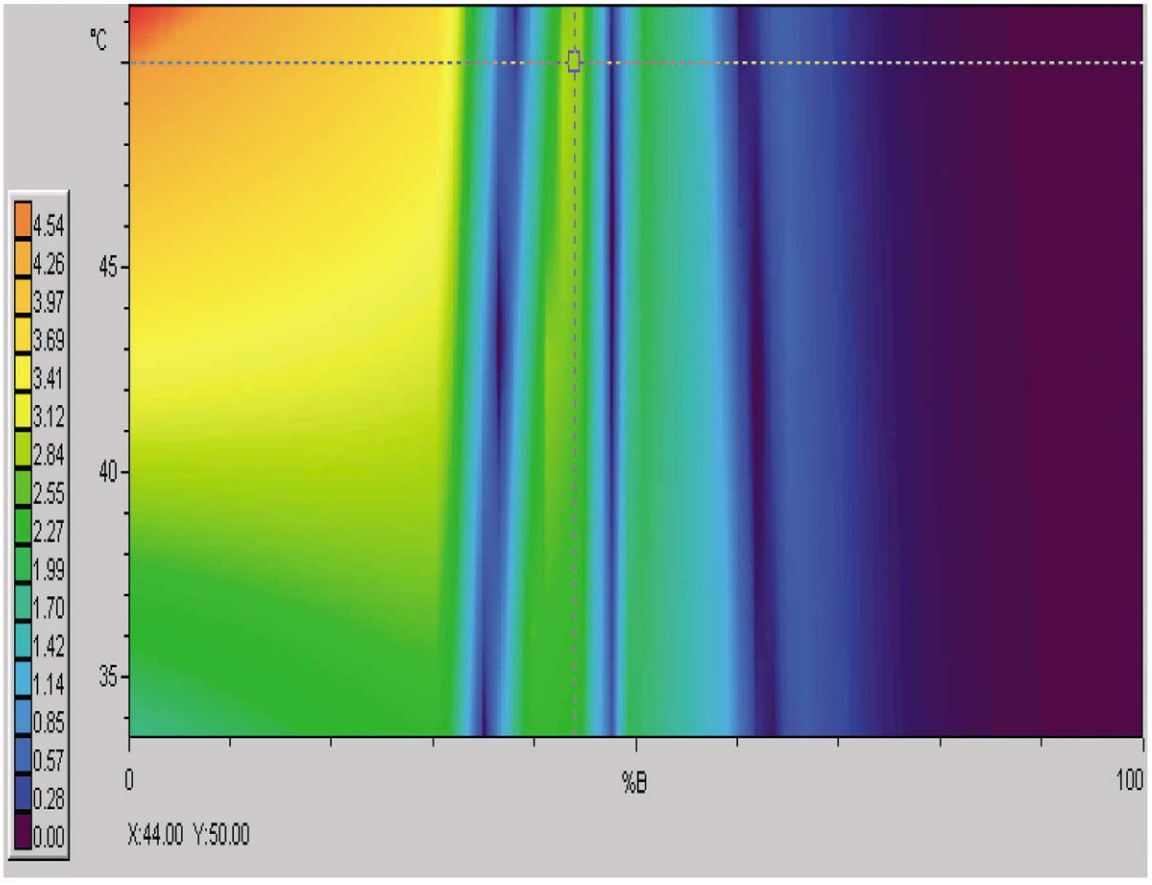

Fig. 3. Resolution map obtained with simultaneous optimization of organic content and column temperature. Cross-hair marks the region with optimal R_s.

the organic modifier. The flow-rate was at 1.5 ml/min and column temperature at 50 °C.

The resolution map (Fig. 4) showed a maximal resolution ($R_s \geq 4$) for conditions of 50–60% B and pH 5.0, at the cost of long run times (25–70 min, not shown on the resolution map). This condition could provide an option for further optimization of column parameters in order to shorten the run time (see section below). At the current column conditions, an optimal R_s of 2.1 and run time of less than 10 min were obtained at 70% B and pH 5.0. Table 3 shows the predicted and experimental data for the optimized separation at 70% B and pH 5.0. A good agreement between the predicted and experimental values was obtained with average errors of 0.9 and 6.3% for retention time and resolution, respectively.

It is interesting to note the change in the elution order for the main peak (peak 5) and one of its

Table 2
A comparison of predicted and experimental data for the optimized separation of model compounds (44% B and 50 °C)

Peak I.D.	Retention time (min)		Resolution	
	DryLab	Exp.	DryLab	Exp.
1	5.37	5.38	2.8	2.5
2	5.84	5.82	32.3	33.3
3	16.66	16.50	3.4	3.6
4	18.22	18.45	3.6	3.8
5	20.53	20.30	NA	NA
Average % error	0.8		6.6	

NA, not applicable.

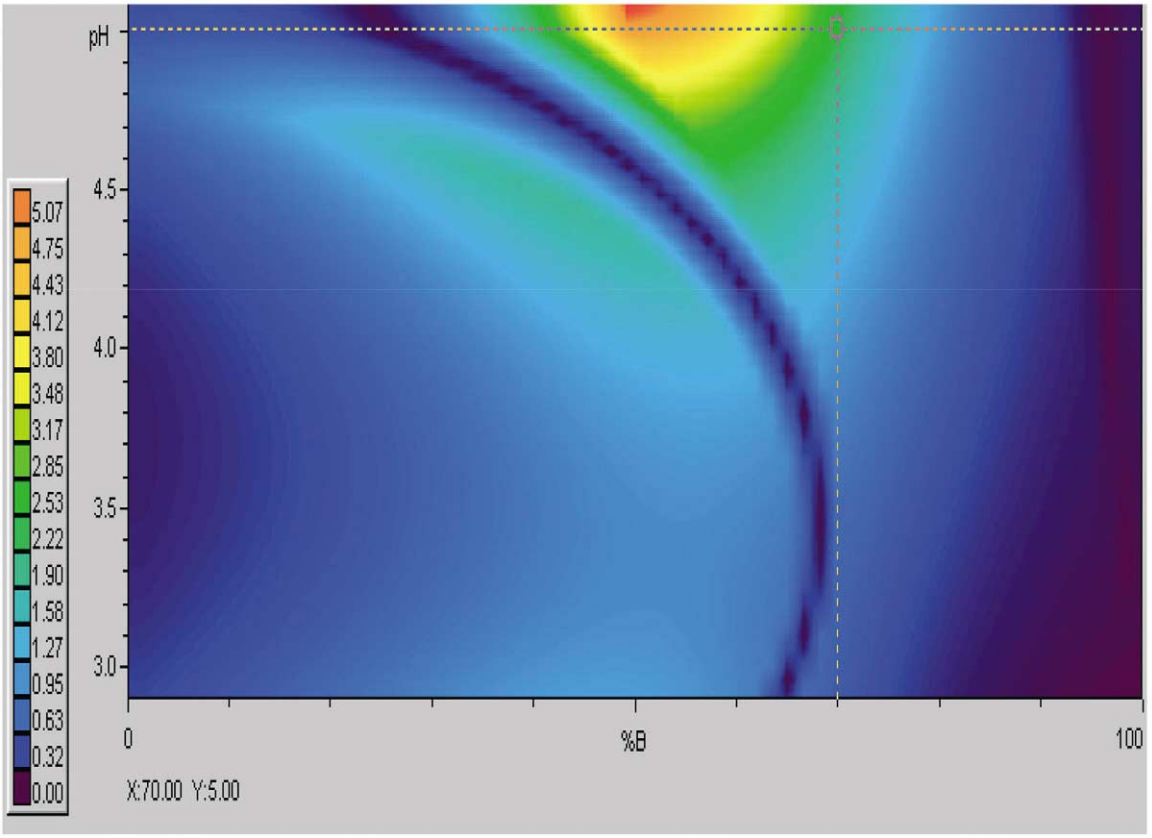

Fig. 4. Resolution map obtained with simultaneous optimization of organic content and pH. Cross-hair marks the region with optimal R_s.

degradation peaks (peak 4) in the pH range of 4–5. It may be due to the impact of pH on the retention of the main peak since it possesses an acidic functional group, while the retention of peak 4 was almost unchanged with pH, probably due to the absence of the acidic group.

Table 3
A comparison of predicted and experimental retention data for the optimized separation of model compounds (70% B and pH 5)

Peak I.D.	Retention time (min)		Resolution	
	DryLab	Exp.	DryLab	Exp.
1	2.11	2.08	2.09	1.90
2	2.28	2.25	18.86	19.11
3	4.51	4.53	6.95	7.88
5	5.72	5.80	10.80	12.26
4	8.37	8.37	NA	NA
Average % error	0.9		6.3	

NA, not applicable.

3.5. Optimization of column conditions

As mentioned in the previous section, the conditions for maximal R_s were 50–60% B and pH 5.0, with run time of 25–70 min. DryLab can be used to model changes in the separation when column parameters, such as column length, particle size and flow-rate, are varied. The goal was to achieve adequate resolution, acceptable column pressure and minimum run time. Fig. 5 shows predicted chromatograms obtained at 60% B and pH 5.0 when (A) flow-rate was increased to 2 ml/min (B) the column length was changed from 15 to 5 cm and (C) simultaneous change in column length and flow-rate. In all cases, the resolution was reported to be of at least 1.6 for the critical pair (peaks 1 and 2) and the

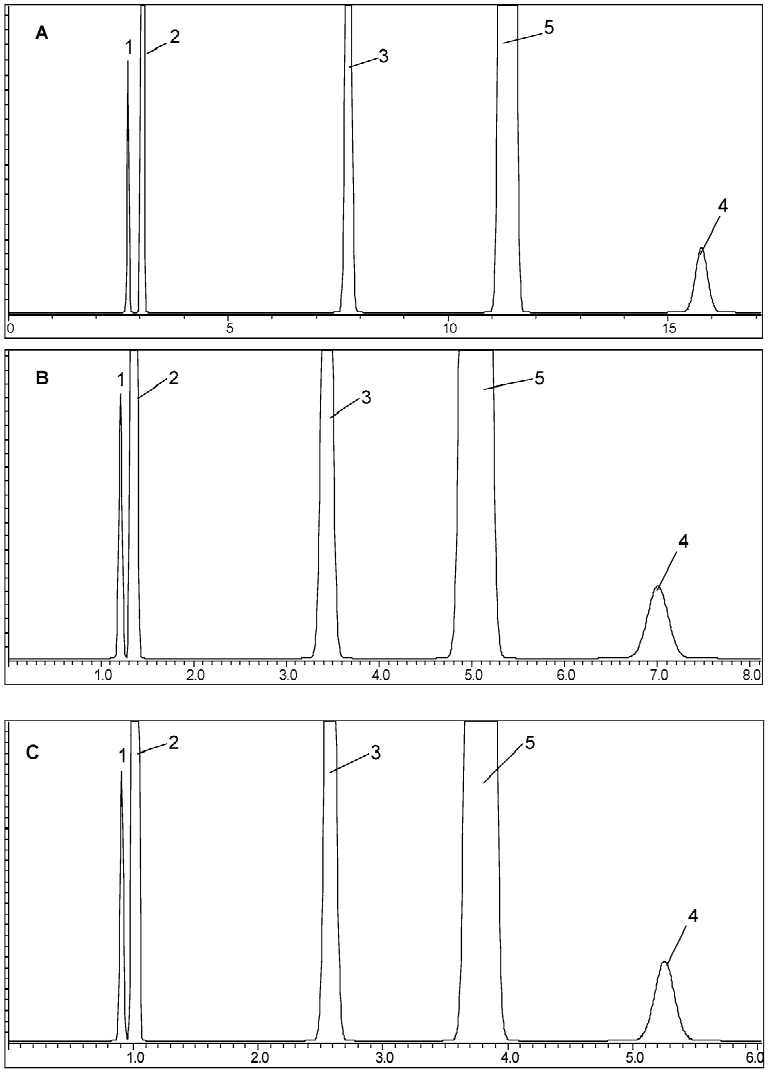

Fig. 5. Predicted chromatograms of the separation when (A) flow-rate was changed from 1.5 to 2 ml/min, (B) column length changed from 15 to 5 cm, (C) simultaneous change in column length from 15 to 5 cm and flow-rate from 1.5 to 2 ml/min.

run time was less than 6 min when both column length and flow-rate were modified (C). To validate the prediction, an experiment was therefore carried out using a 5-cm column (of different lot) and a flow-rate of 2 ml/min. The results showed a satisfactory agreement between the predicted and experimental values, with average errors of 3.5 and 11.8% for retention time and resolution, respectively. The greater percent errors in the predictions were probably due to small k' and column-to-column variation [6–8].

4. Conclusion

The DryLab simulation software was demonstrated to be a useful tool for optimizing the separation of the model drug candidate and its degradation products. After appropriate column selection and with a minimum number of experimental runs, accurate predictions for a broad range of separation conditions could be achieved. This could lead to substantial timesaving and more effective use of staff and resources. In addition, the software is an excel-

lent instructional tool for users who are new to chromatography. DryLab software comes equipped with a number of sample data files that allows the new users to familiarize themselves with the variables affecting a separation. The learning curve can be shortened by replacing long instrument runs with rapid simulation and immediate feedback.

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