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Use of overlapping resolution mapping scheme for optimization of the high-performance liquid chromatographic separation of pharmaceuticals

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Abstract

The use of overlapping resolution mapping (ORM) for the optimization of HPLC separations was examined. The ORM scheme was employed to predict the optimum conditions required for the isocratic HPLC separation of a group of basic pharmaceuticals. The ORM scheme involved first performing seven pre-planned experiments located on a triangle representing the mobile phase compositions. From these experiments, and through the use of a Basic program, the optimum conditions required for separation were established. The scheme was initially applied to establish the optimum mobile phase compositions consisting of quaternary mixtures of buffered acetonitrile, methanol and 2-propanol with a C_{18} column. This approach was found to offer a very rapid and versatile means of determining the optimum HPLC conditions required for such a complex mobile phase system. Complete separation of all the peaks in the mixture was achieved using a mobile phase composition of acetonitrile–methanol–2-propanol–buffer (50:15:5:30, v/v) derived from the ORM scheme. The usefulness and versatility of the ORM scheme was further demonstrated through the use of the optimum mobile phase compositions with different C_{18} columns. It was found that satisfactory separation was achieved by adopting the established optimum mobile phase composition without the need to perform any further re-optimization. In addition, the precision, selectivity and linearity of the method developed were found to be highly reproducible and reliable.

1. Introduction

High-performance liquid chromatography (HPLC) is widely used for the analysis of pharmaceuticals [1]. The many advantages of HPLC include its simplicity, accuracy, precision, versatility, reliability, reproducibility and most importantly its selectivity. With increasing complexity of pharmaceutical samples to be analysed, the demands on the use of HPLC techniques have intensified in recent years. The development of HPLC columns with a wide variety of coatings and polarities [2] and the use of multi-modifier systems are some of the popular approaches now employed. Many systems are being developed to meet the current needs, particularly in the area of improving the separation selectivity for the determination of various components in complicated mixtures or matrices.

The use of multi-modifier mobile phase systems in HPLC has been demonstrated in many applications [3,4] and the success of such an

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approach is found to be greatly dependent on the actual determination of the optimum mobile phase compositions required for separation. The usual trial-and-error approach, although simple and easy to apply, suffers from serious drawbacks as it is laborious and contains many uncertainties. This problem is further exacerbated if the number of modifiers used increases. A way to circumvent this is to use systematic experimental designs which require few experiments to be performed and are capable of locating optimum conditions required for the separation. Palasota et al. [5] have successfully employed a sequential simplex algorithm for the separation of amino acids in a constraint simplex mixture space and Heinisch et al. [6] described the use of several prioritized criteria to search for the optimum eluent strength for HPLC separations using an interpretative approach.

In this work, the use of overlapping resolution mapping (ORM), a systematic experimental design for the optimization of HPLC separations, was examined. The scheme is an experimentally based optimization procedure. Compared with the commonly used simplex optimization approach, which tends to locate only the most favourable local optimum, the ORM is more capable of locating the global optimum. More important, in contrast to simplex procedures, which usually require a relatively large number of experiments to locate optimum separation conditions, the ORM scheme requires only a small set of preplanned experiments which are carried out systematically. The number of experiments for the ORM scheme is usually governed by the experimental design and the order of polynomial chosen to relate the resolution to the experimental parameters.

The application of the ORM scheme to predict the optimum conditions required for the isocratic HPLC separation of a group of basic pharmaceuticals was studied in this work. The scheme was initially applied to establish the optimum conditions for reversed-phase HPLC separations using a C_{18} column and a mobile phase consisting of quaternary mixtures of buffered acetonitrile, methanol and 2-propanol. This approach was found to offer a very rapid and versatile means of determining the optimum HPLC conditions involving complex mobile phase systems such as quaternary mixtures. The usefulness and versatility of the ORM scheme is further demonstrated through the use of the optimum mobile phase compositions derived on different C_{18} columns. The aim is to demonstrate the ruggedness of the ORM scheme in which satisfactory separation can be achieved for another column by simply adopting the established optimum mobile phase compositions without the need to perform any further optimization. The precision, linearity and selectivity of the method were also evaluated.

2. Experimental

2.1. Instrumental

All chromatographic separations were performed using either a Shimadzu (Kyoto, Japan) LC-6A isocratic instrument equipped with an SPD-6A variable-wavelength UV detector or a Hewlett-Packard (HP) Model 1050 instrument equipped with a variable-wavelength UV detector. Both detectors were set at 216 nm. With the Shimadzu system, chromatographic data were collected and analysed on a Shimadzu CR6A Chromatopac integrator. The chromatographic data from the HP 1050 LC system were collected and analysed using an HP ChemStation.

The reversed-phased HPLC columns used were Phase-Sep ODS-2, 5 μm (20 cm $\times$ 4.6 mm I.D.) (column I) and YMC-Pack ODS-A, 5 μm (25 cm $\times$ 4.6 mm I.D.) (column II). The Phase-Sep column (column I) was first employed to determine the optimum mobile phase composition using the ORM scheme. Once the optimum mobile phase had been established, the YMC column (column II) was then used.

2.2. Reagent and materials

A basic drug substance, ondansetron (abbreviated as DS1), and its related impurities GDL1, GDL2 and GDL3 were investigated (structures are given elsewhere [7]). Standards of

DS1 and the impurities were obtained from Glaxo Development. All other chemicals used, unless stated otherwise, were of the purest grade available.

3. Results and discussion

In a previous paper [8], we reported the use of the ORM scheme for the optimization the mobile phase compositions for the separation of environmental pollutants. In this work, the ORM scheme was employed to establish the mobile phase compositions required to separate a group of basic pharmaceuticals. The need for the use of a multi-modifier system in this study derives from the fact that binary mobile phases are unable to separate this group of compounds satisfactorily.

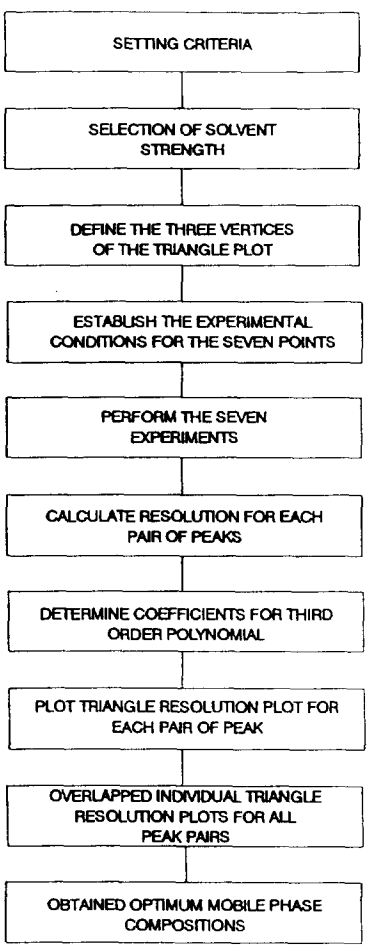

Fig. 1. Schematic diagram of the ORM scheme.

The ORM scheme consisted of a number of steps, shown schematically in Fig. 1. For this work the criteria for the separation were first the resolution (at least 1.5 between adjacent peaks) and second the analysis time of the separation (capacity factor not more than 20). These values were defined to ensure that separation could be achieved within a reasonable time range. As the ORM scheme is based on the Snyder selectivity triangular experimental design [9], binary mixtures of buffered methanol, buffered acetonitrile and buffered 2-propanol were chosen to represent the three vertices of the triangle. At the same time, four other experimental points were strategically chosen on the triangular resolution plot. The locations of the seven experiments in this triangular plot are shown in Fig. 2. It should be noted that the choice of the three modifiers for this work was largely based on the consideration that a wide range of selectivity can be achieved using the combination of these three modifiers [9].

In order to determine the appropriate solvent strength [10] required for the system, the proportion of methanol in the binary mixture representing the first vertex was adjusted until the retention time of the last-elution peak was within the pre-set limit (capacity factor not more than 20). It was found that a mobile phase composition of 74% (v/v) of methanol in the buffer gave

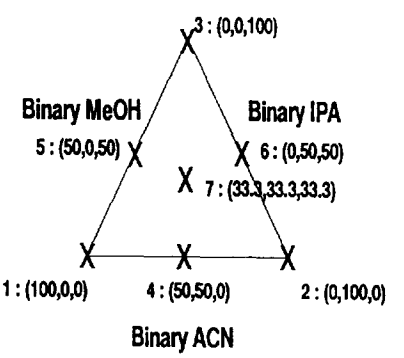

Fig. 2. Triangle plot showing composition portion of mobile phase consisting of mixtures of binary solvents (buffered methanol, buffered acetonitrile, buffered 2-propanol).

Table 1
Eluent mixtures used in the seven preliminary experiments

Experimental point No.	Methanol	Acetonitrile	2-Propanol	Buffer[a]
1	74.0	0.0	0.0	26.0
2	0.0	71.6	0.0	28.4
3	0.0	0.0	52.9	47.1
4	37.0	35.8	0.0	27.2
5	37.0	0.0	26.5	36.6
6	0.0	35.8	26.5	37.7
7	24.7	23.9	17.6	33.8

[a] 0.02 *M* sodium dihydrogenphosphate adjusted to pH 5.4 with 50% (v/v) sodium hydroxide.

retention times which satisfied this criterion (capacity factor within the limit) and was thus chosen to represent one of the vertices. It should be emphasized that at this stage of the ORM scheme there is no requirement to ensure that solvent strength selected must meet the resolution criterion. Based on this composition, the solvent strength of the system was known. The solvent strength found was then utilized to calculate the compositions of the other two binary mixtures at the other two vertices. Similarly, the modifier compositions at the remaining four experimental points of the triangle in Fig. 2 were calculated. Table 1 shows the mobile phase compositions of the seven experimental points.

Experiments based on Table 1 were conducted and the results of these seven preliminary experiments are given in Table 2. From these results, the resolution between adjacent peaks, R, could be calculated and they are given in Table 3.

Subsequently, the R values of each pair of peaks over the seven sets of experiments were fitted into a third-order polynomial:

$$R = a_1x_1 + a_2x_2 + a_3x_3 + a_{12}x_1x_2 + a_{23}x_2x_3 + a_{13}x_1x_3 + a_{123}x_1x_2x_3$$

where a_i are constants and x_i are the proportion of binary mixture (modifier and buffer) at the respective positions on the triangular plot shown in Fig. 2.

With the aid of a modified version of the Basic program given in Ref. [10], the constants for each pair of peaks were determined. Subsequently, the R values at other mobile phase compositions besides the seven experiment points were calculated using the above equation. These R values were then used to construct the triangular resolution plots. As there were three pairs of adjacent peaks, three resolution plots were obtained. A typical resolution plot for one of these pairs is shown in Fig. 3. From Fig. 3, it

Table 2
Retention times (min) using the seven eluents listed in Table 1

Experimental point No.	GDL1	GDL2	DS1	GDL3
1	3.792	8.903	4.642	26.175
2	2.918	12.628	4.308	28.557
3	3.145	5.812	5.812	43.082
4	3.357	5.873	10.573	13.408
5	3.343	6.253	4.565	32.378
6	2.892	4.142	3.267	15.475
7	3.123	4.512	3.365	14.958

Table 3
Resolution, R, between adjacent peaks

Experimental point No.	Peak pair 1–2	Peak pair 2–3	Peak pair 3–4
1	0.906	1.433	1.540
2	1.805	1.809	1.459
3	1.053	0.065	2.242
4	0.753	2.172	1.854
5	1.016	0.495	1.963
6	0.889	0.852	1.913
7	0.645	1.229	2.123

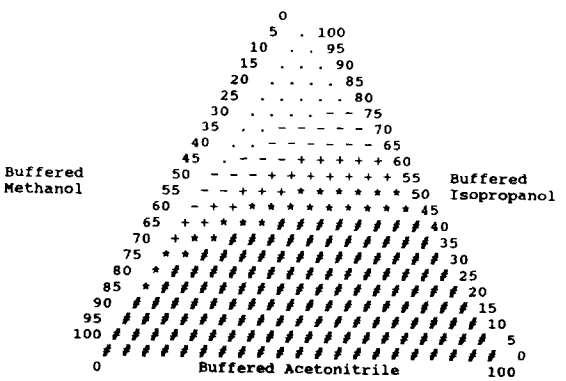

Fig. 3. Typical triangle resolution plot for a particular peak pair, where · = $R \leq 0.4$; – = $0.4 < R \leq 0.6$; + = $0.6 < R \leq 0.8$; * = $0.8 < R \leq 1.0$; and # = $R > 1.0$.

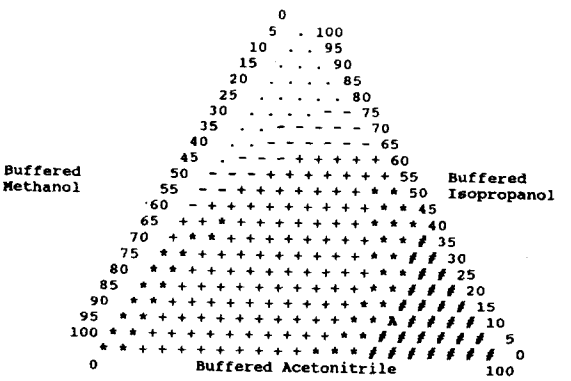

Fig. 4. Overlapped triangle resolution plot for all peak pairs, where · = $R \leq 0.4$; – = $0.4 < R \leq 0.6$; + = $0.6 < R \leq 0.8$; * = $0.8 < R \leq 1.0$; and # = $R > 1.0$.

can be seen that the resolution plot is divided into regions. Each of these regions is characterized by a symbol which gives the expected resolution range/value for that particular pair of peaks at the mobile phase composition concerned. In other words, for a mobile phase composition found in the region marked #, the separation is expected to have a resolution equal to or greater than unity for that particular pair of peaks. Therefore, by overlapping all the three triangular resolution plots and retaining the minimum resolution values, the mobile phase compositions which could provide resolution equal to or greater than unity for all four peaks in the mixture could also be determined. The overlapped triangular plot is shown in Fig. 4.

To confirm the success of the ORM optimization procedure, an additional experiment using one of the mobile phase compositions in the optimum region (marked A in Fig. 4) was performed to verify that a satisfactory separation of all the peaks could be achieved. This corresponds to a mobile phase composition of acetonitrile–methanol–2-propanol–buffer (50:15:5:30, v/v). The chromatogram obtained using this mobile phase is shown in Fig. 5. A satisfactory separation was achieved and the resolution of all the peaks exceeded 1.5.

To demonstrate further the usefulness of the ORM scheme and to validate the methodology developed, a series of experiments were performed. First, to demonstrate the ruggedness of the method, the same mobile phase was employed with another C_{18} column (column II). The results obtained are shown in Fig. 6. It can be seen that a satisfactory separation of all the peaks was achieved with a resolution greater than 1.5 for all the peaks. In addition, the precision, selectivity and linearity of the method were determined. The results obtained in the precision and linearity study are summarized in Table 4. It can be seen that highly reproducible results (R.S.D. not greater than 0.25%) are achievable using this method. The linearity study also yielded satisfactory results, R^2 values greater than 0.99 being obtained for all four compounds.

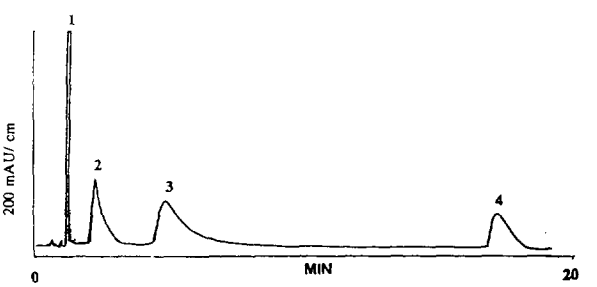

Fig. 5. Chromatogram of DS1 and its related impurities Conditions: mobile phase, acetonitrile–methanol–2-propanol–0.02 *M* NaH_2PO_4 (50:15:5:30, v/v); flow-rate, 1.0 ml/min; column, I. Peak identification: 1 = GDL1; 2 = DS1; 3 = GDL2; 4 = GDL3.

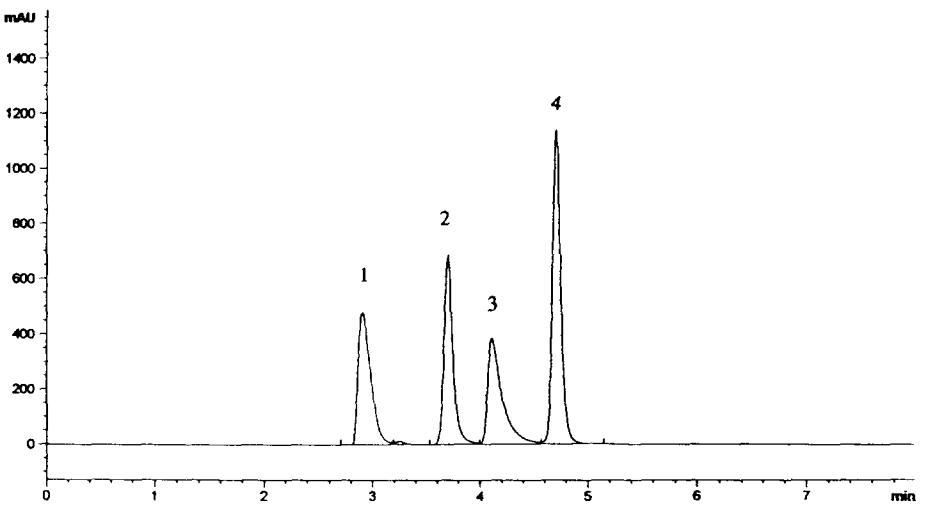

Fig. 6. Chromatogram of DS1 and its related impurities Conditions: mobile phase, acetonitrile–methanol–2-propanol–0.02 *M* NaH_2PO_4 (50:15:5:30, v/v); flow-rate, 1.0 ml/min; column, II. Peak identification: 1 = GDL1; 2 = DS1; 3 = GDL2; 4 = GDL3.

4. Conclusions

The use of a systematic scheme for the optimization of the HPLC separation of a group of pharmaceuticals was demonstrated. The scheme required seven pre-planned experiments located on a triangular resolution plot. The optimization procedure was systematic, simple to use and capable of locating global optimum conditions for separations involving complex mobile phase systems. In addition, once the optimum conditions had been established, satisfactory separation was also obtained even when another column with the same type of stationary phase was used.

Table 4
Precision and R^2 values for the four compounds

Compound	R.S.D. (%)[a]		R^2[b]
	Retention time	Area	
GDL1	0.10	0.11	0.997
DS1	0.11	0.11	0.999
GDL2	0.15	0.14	0.999
GDL3	0.12	0.21	0.999

[a] $n = 5$.
[b] From 1.0 to 0.2% (w/w).

References

[1] A. Aszalos (Editor), *Modern Analysis of Antibiotics*, Marcel Dekker, New York, 1986, Ch. 7, p. 239.
[2] W.T. Wahyuni and K. Jinno, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 10 (1987) 687.
[3] G. Baier, G. Wollensak, E. Mur, B. Redl, G. Stoffler and W. Gottinger, *J. Chromatogr.*, 525 (1990) 319.
[4] G. Werner, V. Schneider and J. Emmert, *J. Chromatogr.*, 525 (1990) 265.
[5] J.A. Palasota, J.M. Palasota, H.L. Chang and S.N. Deming, *Anal. Chim. Acta*, 270 (1992) 101.
[6] S. Heinisch, P. Riviere and J.L. Rocca, *Chromatographia*, 36 (1993) 157.
[7] K.D. Altria, *Chromatographia*, 35 (1993) 177.
[8] S.F.Y. Li, H.K. Lee and C.P. Ong, *J. Chromatogr.*, 506 (1990) 245.
[9] L.R. Snyder, *J. Chromatogr Sci.*, 16 (1978) 223.
[10] J.C. Berridge, *Techniques for the Automated Optimization of HPLC Separations*, Wiley, Chichester, 1985.