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Short Communication

The use of temperature to empirically determine system suitability validation parameters for liquid chromatography*

GRAHAM S. CLARKE

Bristol–Myers Squibb, Pharmaceutical Research Institute, Moreton, L46 1QW, UK

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Introduction

In routine analysis of pharmaceutical substances and product, it is very important from a GMP perspective that a chromatographic separation achieved during development of a method should be reproduced by the end user laboratory. System suitability parameters for LC methods are, therefore, described to control the analytical chromatographic performance. As well as determining the precision of the LC system, a system suitability test often includes the determination of parameters such as capacity factor (k'), column efficiency (theoretical plates), tailing factor [1], and if there are multiple peaks in the chromatogram, a minimum resolution ratio (as defined in USP) [2] for the separation of critical components within a chromatogram. These parameters are often defined subjectively by the analyst developing the method. Often a minimum resolution ratio is arbitrarily set at 2 [3, 4] when typical resolution ratios achieved during development of the method may be much higher than this. Although such an approach will indicate that the required separation has been achieved, it cannot be used as an indication that the chromatography is comparable to that at the development stage. This paper describes how using column temperature to control the chromatographic separation of three components, an objective definition for minimum system suitability specifications can be given.

Experimental

A substituted pyrimidylpiperazine derivative, currently under development by Bristol–Myers Squibb was assayed by LC. A separation was required to resolve the drug from two process related impurities, and these compounds have been used as a model for this work.

Chromatographic conditions

The chromatography was performed using a modular LC system consisting of a Kontron 420 pump (Kontron Instruments, Watford, UK) a Kontron 460 autosampler, a column oven (Jones Chromatography, Hengoed, Mid-Glamorgan, UK), and a Spectroflow 773 (ABI Analytical Kratos Division). Data was collected and integrated using a PeakPro Chromatography Data System (Beckman Instruments, Inc., Allendale, NJ, USA). UV detection at 287 nm was used with an injection volume of 50 µl. The column stationary phase was 10 µm µBondapak CN, 3.9 mm × 150 mm (Millipore, Waters Chromatography Division, Chester, UK). The mobile phase consisted of acetonitrile–0.1 M sodium acetate

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(1:3, v/v) adjusted to pH 6.5 with 10% (v/v) acetic acid, at a flow rate of 1.5 ml min^{-1}. The column temperature was controlled using the column oven, except for the 6°C temperature which was maintained using an ice bath, and 22.7°C which was the ambient temperature of the laboratory during analysis.

The chromatographic performance parameters of the three components were determined under the LC conditions described above using a column temperature controlled over the range 6–99°C. The capacity factor (k'), tailing factor, column efficiency (mean value for the three peaks) and the resolution ratio between adjacent peaks was calculated using USP procedures. The peak areas of the individual components were also integrated and quantitated by peak normalization. Examples of the chromatographic separation obtained are shown in Fig. 1.

Results and Discussion

Retention

The Van't Hoff plot (ln k' vs $1/T$) for each component was first demonstrated to be linear (Fig. 2) indicating that the interaction of each analyte with the column stationary phase was a simple function of temperature, and that the chromatographic mechanism was not unusual (see Table 1).

Resolution ratio and column efficiency

The important consideration, was how the critical resolution between impurity 2 and the drug, which is monitored using the system suitability parameters, affected quantitation of the components. To determine this, the peak areas of the components in the chromatogram were quantitated by peak normalization, and the ratio of the drug/impurity 2 peak areas

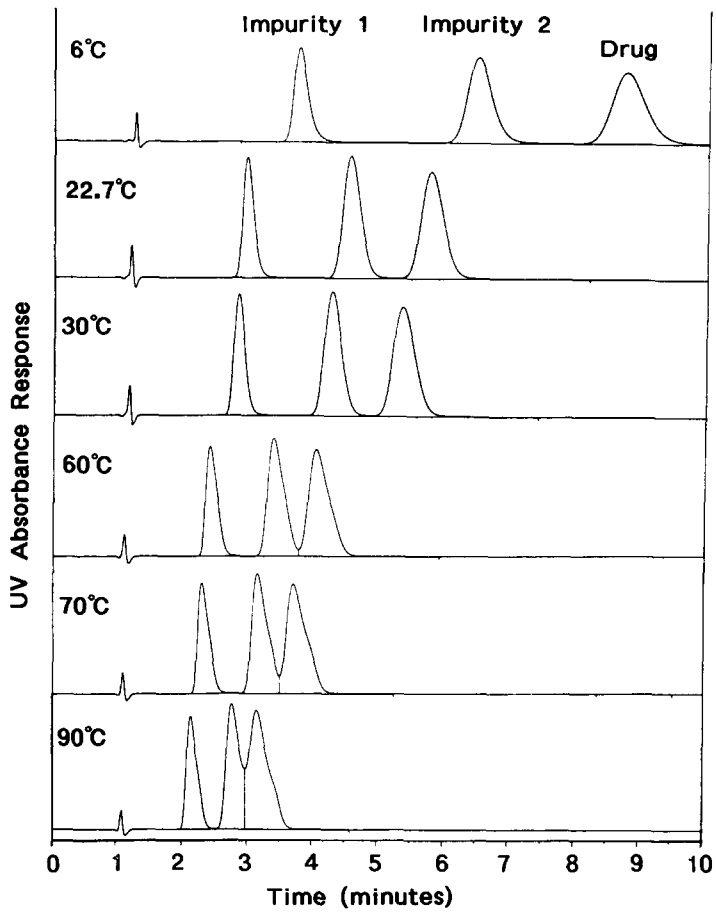

Figure 1
Chromatograms of the drug/impurity separation used to determine system suitability specifications.

Table 1
Capacity factors at each temperature

		k'		
Temp. °K (°C)	1/°K	Impurity 1	Impurity 2	Drug
279.0 (6)	3.58	2.14	4.41	6.30
295.7 (22.7)	3.38	1.48	2.81	3.82
303.0 (30)	3.30	1.37	2.58	3.47
313.0 (40)	3.19	1.26	2.31	3.06
323.0 (50)	3.10	1.14	2.06	2.71
333.0 (60)	3.00	1.03	1.85	2.40
343.0 (70)	2.92	0.93	1.65	2.11
353.0 (80)	2.83	0.85	1.46	1.84
363.0 (90)	2.75	0.78	1.31	1.63
372.0 (99)	2.69	0.73	1.17	1.42

Table 2
Component quantitation

Temperature (°C)	Impurity 1 area (%)	Impurity 2 area (%)	Drug area (%)	Drug/impurity 2 ratio area
6.0	23.2	36.4	40.5	1.11
22.7	22.9	36.3	40.8	1.12
30.0	23.0	36.2	40.7	1.12
40.0	23.2	36.1	40.7	1.13
50.0	23.3	36.1	40.6	1.12
60.0	23.2	36.1	40.7	1.13
70.0	23.2	36.0	40.8	1.13
80.0	23.1	35.3	41.6	1.18
90.0	23.1	32.1	44.9	1.40
99.0	23.0	28.7	48.1	1.68

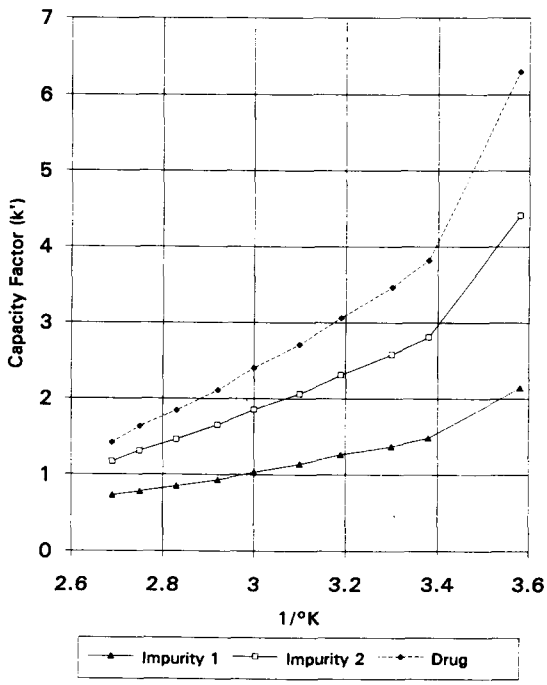

Figure 2
Van't Hoff plot (ln k' vs 1/°K) for each chromatographic component.

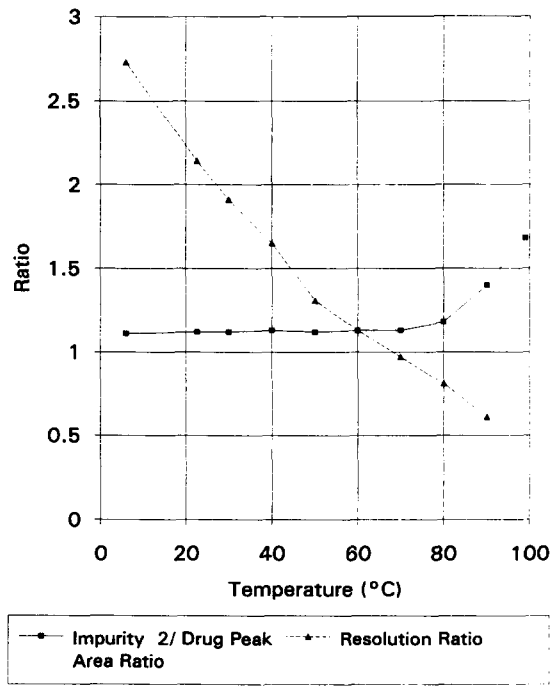

Figure 3
Peak area ratio and resolution ratio for the impurity 2/drug separation.

Table 3
Resolution ratios and column efficiency

Temperature (°C)	Impurity 1/impurity 2 resolution	Impurity 2/drug resolution	Efficiency plates/col.
6.0	4.91	2.73	1369
22.7	3.91	2.14	1353
30.0	3.57	1.91	1217
40.0	3.13	1.65	1083
50.0	2.56	1.31	803
60.0	2.25	1.13	697
70.0	1.98	0.97	623
80.0	1.72	0.81	551
90.0	1.47	0.61	455
99.0	RNA*	RNA	430

* Resolution not achieved.

calculated (Table 2) and plotted on the same axis as the resolution ratio for the two peaks (Fig. 3). The drug/impurity 2 area ratio is constant up to the separation achieved at 70°C, indicating that quantitation under these conditions is acceptable. Having determined this, minimum system suitability specifications for the separation were set based on the 70°C separation. Examples of the chromatography achieved at different temperatures (Fig. 1) show that acceptable quantitation achieved at 70°C does not require baseline resolution between the drug and impurity 2. The resolution ratio for these peaks is approximately 1 and shows that for peaks which do not tail badly (i.e. near-Gaussian), minimum resolution ratio specifications may be set which are less than the value recommended by other investigators [5]. A resolution ratio of 1 approximates to the theoretical value which would be achieved if the peaks were represented as non-overlapping isosceles triangles [6].

The resolution ratio between the two impurities and the drug are given in Table 3, together with the calculated column efficiency (number of theoretical plates/column — an average of all three peaks). The column efficiency under these conditions is approximately 600 plates, which would generally be considered an unacceptably low value for an HPLC separation.

Tailing factor

The tailing factor for all three peaks was determined at each temperature, except where the resolution between peaks is such that a tailing factor could not be calculated, and a plot of these values with respect to temperature is shown in Fig. 4. Although an optimum tailing factor of about 1.2 is achieved for the drug under ambient conditions, a tailing factor of up to 1.7 for the drug will give acceptable conditions for quantitation.

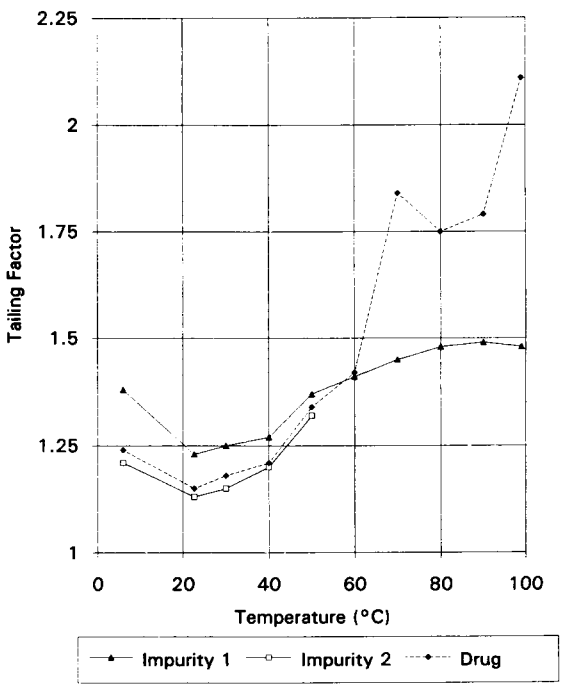

Figure 4
Plot of tailing factor against temperature for each chromatographic component.

Conclusions

Using temperature to control the separation of components in an HPLC chromatogram is both a useful and simple method for objectively setting system suitability specifications. Column efficiency (number of theoretical plates) taken in isolation is not a useful system suitability parameter to control a chromato-

Table 4
System suitability specifications for impurity/drug separation

System suitability parameter	Specifications
Capacity factor	2
Resolution ratio	1
Column efficiency	600 plates
Tailing factor	1.7

graphic separation. The use of temperature to control chromatographic separation is both robust and simple and having determined the resolution which gives acceptable quantitation of the components, minimum system suitability specifications can be objectively specified, and for the separation described would be exemplified as shown in Table 4.

References

[1] G.S. Clarke, *J. Pharm. Biomed. Anal.* **12**, 643–652 (1994).
[2] *United States Pharmacopeia XXII*, pp. 1558–1568. USPC, Rockville, MD (1990).
[3] J.A. Timmons, J.C. Meyer, D.J. Steible and S.P. Assanza, *J. Assoc. Off. Anal. Chem.* **70**, 510–513 (1987).
[4] J.P. Zodda, J.A. Monteferrante and M.N. Eakins, *J. Pharm. Biomed. Anal.* **12**, 573–577 (1994).
[5] D.E. Wiggins, *J. Liq. Chromatog.* **14**, 3045–3060 (1991).
[6] P.J. Karol, *J. Chromatogr. Sci.* **27**, 578–581 (1989).

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