

CHROM. 13,258

## Note

---

### Optimisation of reversed-phase high-performance liquid chromatographic separations with the aid of a programmable calculator

J. C. BERRIDGE

*Analytical Chemistry Department, Pfizer Central Research, Pfizer Ltd., Sandwich, Kent (Great Britain)*

(First received June 20th, 1980; revised manuscript received August 18th, 1980)

The usual procedure for the optimisation of separations in liquid chromatography is by trial and error, based on the chromatographer's experience and intuition. A paper<sup>1</sup> was published recently which discussed some of the more systematic procedures available and described an alternative method. The authors presented a semi-empirical approach which combines a number of simple mathematical relationships to produce a scheme which allows the chromatographer to investigate the effects of varying experimental parameters such as column length, mobile phase composition or temperature. Data were presented which demonstrated the applicability of the approach to methanol-water reversed-phase systems.

A particular feature of the paper was the inclusion of a program, designed for a small programmable calculator, which was intended to allow optimisation of a separation following the acquisition of a minimum amount of experimental data. In attempting to apply the presented calculator program to systems similar to those described it was found that there were several errors in the adaptation of the mathematical concepts into a computer acceptable format. There are also many errors in the program as written for the recommended calculator (Texas Instruments TI59): these errors prevent the program presented from running correctly<sup>2</sup>.

In this paper it is shown that the semi-empirical mathematical approach is also applicable to acetonitrile-water reversed-phase systems. An expression has been derived which describes the dependence of viscosity upon the composition and temperature of acetonitrile-water mobile phases. The calculator program has been rewritten to remove the errors present in the original and to allow its use for either methanol-water or acetonitrile-water mobile phases\*.

## EXPERIMENTAL

### *Chromatography*

The liquid chromatograph comprised an M6000A pump (Waters Assoc.), a variable-wavelength UV detector (Pye Unicam), a six-port sampling valve (Rheodyne Model 70-10), a constant temperature water bath (Gallenkamp) and a Servoscribe

---

\* A listing of the equations used and the calculator program is available from the author.

Model IS recorder. The column was a  $\mu$ Bondapak C<sub>18</sub> (300 × 3.9 mm I.D., 10  $\mu$ m, Waters Assoc.).

All solutes were pharmaceutical grade and used as received: solutions were prepared in acetonitrile-water (50:50).

Values of  $k'$  were determined after 30 min of system equilibration.

### Calculations

A TI59 programmable calculator (Texas Instruments) was used throughout the study. The mathematical principles are those published<sup>1</sup> but with some minor changes in accordance with other workers<sup>3,4</sup>. Calculated values of the reduced plate height ( $h$ ) were scaled by multiplying by a constant factor  $C$ . The value of  $C$  was chosen such that predicted  $h$  values coincided with real values for the experimental system. A fixed value (500) (ref. 1) of the column resistance factor  $\varphi$  was not used: experimentally derived values were incorporated.

For the calculation of the viscosity of acetonitrile-water systems it was found that published data<sup>5</sup> could be fitted ( $R^2 = 0.986$ ) (ref. 6) to the empirical equation:

$$\log \eta_t = \log \eta_{40} + 10^3 (0.7 + 0.2 \eta_{40}) \left( \frac{1}{T} - \frac{1}{313} \right),$$

where

$$\eta_{40}^* = 0.26 + 0.78 (1-F) - 0.374 (1-F)^3$$

where  $\eta_t$  is the viscosity at temperature  $T$  (Kelvin) from 15 to 60°C;  $\eta_{40}$  is the viscosity at 40°C and  $F$  is the fractional composition of the mobile phase (*i.e.*  $F = 0.6$  corresponds to 60% acetonitrile).

The other data needed prior to operation of the optimisation program are exactly those described previously<sup>1</sup>.

## RESULTS

The three solutes used to verify the applicability of the concept to acetonitrile-water systems were the antimicrobial agents methyl, propyl and butyl *p*-hydroxybenzoate (parabens). Table I shows the derived data ( $k'$ ,  $a$ ,  $b$ ,  $c$ ,  $d$  and  $e$ ) for these solutes obtained from the experimental data presented in Tables II–IV. Comparison of the experimental results with the predicted values shows close agreement.

TABLE I  
DERIVED DATA USED FOR OPTIMISATION<sup>a</sup>

	<i>Methyl paraben</i>	<i>Propyl paraben</i>	<i>Butyl paraben</i>
$k'$ (52°C, 50%)	1.15	2.24	3.27
$a$	306	455	523
$b$	0.55	1.22	1.60
$c$	0.97	1.74	2.17
$d$	538	998	846
$e$	385	905	505

<sup>a</sup> Calculated using the TI59 solid state statistic module.

TABLE II  
EXPERIMENTAL AND CALCULATED  $k'$  VALUES  
 $T$ , 300 °K.

Solute	$F$			
	0.5		0.7	
	Exp.	Calc.	Exp.	Calc.
Methyl paraben	1.41	1.42	0.82	0.87
Propyl paraben	3.09	3.09	1.24	1.25
Butyl paraben	4.64	4.65	1.58	1.61

TABLE III  
EXPERIMENTAL AND CALCULATED  $k'$  VALUES  
 $T$ , 309 °K

Solute	$F$			
	0.6		0.85	
	Exp.	Calc.	Exp.	Calc.
Methyl paraben	1.09	1.04	0.73	0.57
Propyl paraben	1.64	1.77	0.85	0.60
Butyl paraben	2.12	2.42	1.03	0.66

TABLE IV  
EXPERIMENTAL AND CALCULATED  $k'$  VALUES  
 $T$ , 325 °K

Solute	$F$							
	0.5		0.6		0.7		0.85	
	Exp.	Calc.	Exp.	Calc.	Exp.	Calc.	Exp.	Calc.
Methyl paraben	1.15	1.16	0.88	0.93	0.70	0.74	0.70	0.53
Propyl paraben	2.24	2.24	1.36	1.50	1.00	1.01	0.82	0.56
Butyl paraben	3.27	3.27	1.76	1.99	1.21	1.21	0.94	0.57

The exceptions are for  $F = 0.85$ . Examination of  $\log k'$  vs.  $F$  plots for the three solutes shows a marked curvature of the plots for acetonitrile contents greater than 80%. All three solutes show minima in their  $\log k'$  vs.  $F$  plots in the region  $F = 0.85$  with the extents of curvature in the order methyl > propyl > butyl.

Table V shows the comparison of other derivable parameters with experimental data for two solutes (methyl and propyl *p*-hydroxybenzoate).

TABLE V  
EXPERIMENTAL AND PREDICTED SEPARATION OF METHYL AND PROPYL PARABENS

$F$ , 0.6;  $T$ , 309 °K.  $t_2$  is retention time (sec).  $k'_M$  and  $k'_P$  are capacity factors (methyl and propyl, respectively);  $N$  is number of theoretical plates and  $P$  is pressure (p.s.i.g.).

	$t_2$	$R$	$k'_M$	$k'_P$	$N$	$P$
Experimental	174	2.86	1.09	1.64	2450	1387
Predicted	167	3.80	1.04	1.77	2506	1237

Again good agreement is evident although the resolution is over-estimated: this is due in part to the problem referred to earlier of the non-linearity of  $\log k'$ .

#### CONCLUSION

The concept of using mathematical expressions, capable of being handled by small programmable calculators, has been extended to acetonitrile-water reversed-phase systems.

The improved software developed can be used for either methanol-water or acetonitrile-water reversed-phase systems. Further uses of the program have been previously discussed<sup>1</sup>.

#### REFERENCES

- 1 J. R. Gant, J. W. Dolan and L. R. Snyder, *J. Chromatogr.*, 185 (1979) 153.
- 2 J. R. Gant, J. W. Dolan and L. R. Snyder, *J. Chromatogr.*, 192 (1980) 504 (erratum to ref. 1).
- 3 L. R. Snyder, *J. Chromatogr. Sci.*, 15 (1977) 441.
- 4 P. A. Bristow and J. H. Knox, *Chromatographia*, 10 (1977) 279.
- 5 H. Colin, J. C. Diez-Masa, G. Guiochon, T. Czajkowska and I. Miedziak, *J. Chromatogr.*, 167 (1978) 41.
- 6 *GLIM 3.12*, Royal Statistical Society, London, 1977.