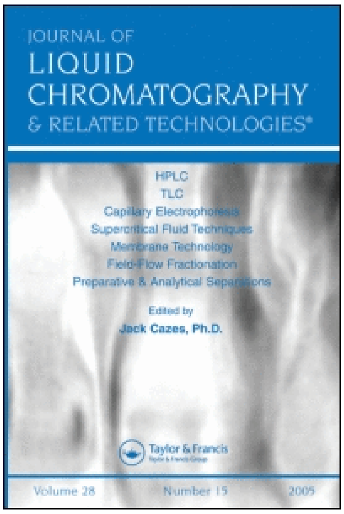

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COMPUTATION OF HPLC PARAMETERS & COMPARISON OF NOISE LEVELS OF A DUAL PISTON RECIPROCATING AND A TRIPLE PISTON PUMP

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ABSTRACT

Amongst HPLC parameters such as k', α, N, R, and tailing factor (T), the first two are computed from Eq. 1 and 2 which are universally used. For computation of N, R & T there are a variety of equations. N and R are best computed from Eq. 4 and 7, and T from Eq. 12. By 't' and 'F' tests it is shown that there is no significant difference in the noise levels of a dual piston reciprocating and a triple piston pump, at the commonly used flow rates (1-3 ml/min), and at a reasonably high sensitivity (0.01 AUFS).

INTRODUCTION

Separation of a mixture of compounds by HPLC is influenced by the nature and particle size of column material, dimensions of column, number of theoretical plates per column length or efficiency of column (N), column temperature, mobile phase composition, and mode of separation (adsorption/partition/ion exchange/ion pair). The compounds are characterized by the retention time (t_n). The degree of separation is characterized by the capacity factor (k'), relative

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* Correspondence

retention or selectivity (α), resolution (R), and tailing factor (T). The expressions for k', α, N, R and T as given in various texts are given below. While the expressions for k' and α do not differ in various texts, those for N, R and T do differ.

$k'_n = (t_n/t_o) - 1$, (Eq.1)(1,2) where t_n and t_o are the respective retention times for the nth component and the non-retained component.

$\alpha = (t_{n+1} - t_o) / (t_n - t_o)$, (Eq.2)(1,2)

$N_n = 16\ t_n^2 / W_{y,n}^2$, (Eq.3)(1), where $W_{y,n}$ = the peak width of the nth component along the base line (i.e. at y = 0% of the peak height); it is the intercept on the baseline made by the tangents to the Gaussian curve (tangent method).

$N_n = 5.54\ t_n^2 / W_{y,n}^2$ (Eq.4)(2), where $W_{y,n}$ is the width at y = 50% of the peak height of the nth component (half-width method).

$N_n = 4\ t_n^2 / W_{y,n}^2$ (Eq.5)(3), where y = 60.7% of peak height of the nth component.

$R_{n,n+1} = 2(t_{n+1} - t_n)/(W_{y,n} + W_{y,n+1})$, (Eq.6)(1,2)

$R_{n,n+1} = 1.198(t_{n+1} - t_n)/(W_{y,n} + W_{y,n+1})$ (Eq.7)(4)

where y = 50% of peak height.

$$R_{n,n+1} = \frac{1}{4}\ \frac{\alpha - 1}{\alpha}\ \frac{k'_{n+1}}{1 + k'_{n+1}} \sqrt{N_{n+1}}\ \text{(Eq.8)}^{(5)}$$

$$R_{n,n+1} = \frac{1}{4}\ \frac{\alpha - 1}{\alpha}\ \frac{k'}{1 + k'} \sqrt{N},\ \text{(Eq.9)}^{(6)}$$ where

$k' = (k'_n + k'_{n+1})/2$, and $N = (N_n + N_{n+1})/2$.

$R'_{n,n+1} = AB/AC$, (Eq.10)(7) where C is the point of intersection of the base line and the perpendicular at B (the valley point) to the baseline, and A is the point of intersection of the perpendicular and the line joining the adjacent peak maxima.

$T = W_{y,n}/2\ f_{y,n}$ (Eq.11)(1), where $W_{y,n}$ is the peak width of nth component at y = 5% peak height and $f_{y,n}$ is the distance between the perpendicular dropped from the peak maximum and the leading edge of the nth peak at y = 5% peak height.

$T = (W_{y,n} - f_{y,n})/f_{y,n}$ (Eq.12) where y = 10%(3,8,9) 15%(10), or 60.65%(11) of peak height of the nth component.

Modern micro-computers and data acquisition techniques permit on-line computation of column efficiency (by statistical moments)(12) and peak symmetry(13).

In this article, we compare the various equations for a manual calculation of N, R and T, and the noise levels encountered with a dual reciprocating pump and triple piston pump.

APPARATUS

(A) Dual reciprocating HPLC pump, Constammetric II (Laboratory Data Control, N.J., USA)

(B) Triple piston HPLC pump, Model 870 (DuPont, DE, USA).

(C) DuPont 830 HPLC's thermostated column compartment (DuPont).

(D) Septumless injection valve 70-10 with 10 µl loop (Rheodyne, Berkley, USA).

(E) Fluorescence/Absorbence detector, Model 836 with 254nm filter (DuPont).

(F) Dual pen recorder Model 7130A (HP, Calif, USA)

(Note: All were connected to mains supply through a voltage stabilizer).

MATERIALS

Tyrosine(1) (Fluka, puriss); benzoic acid GR(2); o-phthalic acid GR(3) and salicylic acid(4), (E.Merck); (4) was recrystallised from water.

Mobile phase(V): citrate-phosphate buffer, 0.0225 M(pH 3.8).

Solution for injection contained 0.7, 2.3, 3.6 and 29.9 microgram of (1), (2), (3) and (4) respectively in 10 microlitre of (V). The concentrations were so chosen as to give peak heights of about 80 divisions on the recorder chart.

Column: Zipax-SAX (2.1mm x 1m) (DuPont)

PROCEDURE

(i) The compounds (1), (2),(3) and (4) were separated at 50^{o}C by injecting 10 microlitres of their solution on the column connected to pump (A). Elution was carried out with mobile phase (V) at a flow rate of 1ml/min. Detector attenuator was set at 0.04 AUFS. The chromatograms were recorded at a chart speed of 1 inch/min.

The values of retention times (t_n), peak heights (h) and widths (W_y) were measured from the chromatogram (Fig.1) for calculation of k',α, N, R and T from the equations given earlier. The peak heights were measured from the corrected base lines.

(ii) The noise levels were recorded for about 30 min at flow rates of 1, 2, and 3 ml/min with pumps (A) and (B) coupled alternately to the column maintained at 50^{o}C and using water as mobile phase and detector

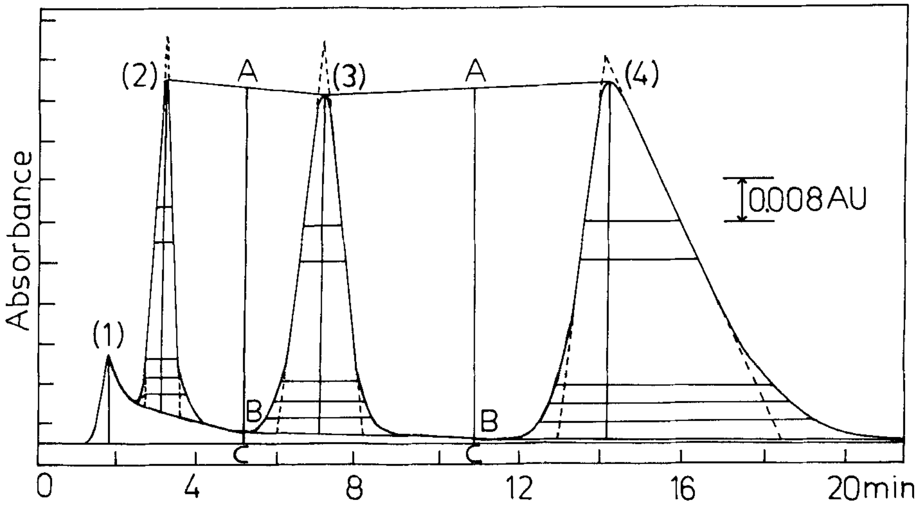

FIGURE 1
HPLC profile of a mixture of (1) tyrosine, (2) benzoic acid, (3)o-phthalic acid and (4) salicylic acid on Zipax-SAX Column

attenuator set at 0.01 AUFS. The chart speed was 1 inch/min. The amplitudes of the signals were measured in 10 portions of the 30 min recording.

RESULTS AND DISCUSSIONS

(i) Parameters

The values of t_n, h, k',α, N and T are given in Table 1. The elution time of (1) was used as t_o.

Column Efficiency

The values of N-3, as determined by using Eq.3 are less than N-4 and N-5 obtained from Eq.4 and 5 respectively. Eq.3 lacks precision as the drawing of tangents is subject to considerable error. Eqs.4 & 5 are more precise as $W_{0.5h}$ and $W_{0.6065h}$ can be

Table - 1 : Retention time, Capacity Factor, Selectivity, Column Efficiency and Tailing Factor for compounds (1) (2) (3) and (4)

Compound	t_n (min)	k'	α	h (mm)	y (% h)	$W_y \times 10^2$ (min)	$f_y \times 10^2$ (min)	N	T	
									Eq.11	Eq.12
(1)	1.8	-	-	-	-	-	-	-	-	-
(2)	3.1	0.72	-	209.5	0	95	-	170^a	-	-
					5	110	49	-	1.1	1.2
					10	96	43	-	(1.1)	1.2
					15	88	39	-	(1.1)	1.3
					50	54	29	182^b	(1.0)	(0.9)
					60.65	45	24	190^c	(0.9)	0.9

(3)	6.9	2.83	3.93	213	0	209	-	174^a	-	-
					5	250	120	-	1.0	(1.1)
					10	224	106	-	(1.1)	1.1
					15	199	97	-	(1.0)	1.0
					50	119	59	186^b	(1.0)	(1.0)
					60.65	100	49	190^c	(1.0)	1.0
(4)	14.1	6.83	2.41	216.5	0	549	-	106^a	-	-
					5	693	168	-	2.1	(3.1)
					10	608	147	-	(2.1)	3.1
					15	547	131	-	(2.1)	3.2
					50	299	74	123^b	(2.0)	(3.0)
					60.65	230	60	150^c	(1.9)	2.8

The values in parentheses are for values of y other than those specified by the equations in the text : a: N-3, b: N-4, c: N-5 more reliably measured and give nearly concordant values. For Gaussian profiles cf. (2) and (3), the difference between N-3, N-4 and N-5 are not significant. For the asymmetric profile of (4), N-3 differs significantly from N-4 and N-5.

Tailing factor

'T' should be ideally 1(1) but it is often in the range of 1.2 to 3.0(11,14). Some HPLC column manufacturers guarantee their columns with $T = 0.9 - 1.4$(9) or $T_{max} = 1.8$(10). If the T values (calculated by using Eq.11 and 12 at various values of y) are compared, it is seen that the differences are not significant for the nearly Gaussian profiles of (2) and (3), but significant for the asymmetric profile of (4), as can be seen from Table 1 and the following additional data:

% h of peak (4)	(20)	(30)	(40)
T(Eq.11)	(2.1)	(2.1)	(2.0)
T(Eq.12)	(3.2)	(3.2)	(3.0)

The above data indicates that Eq.12 containing the term $(W_y - f_y)$ is able to express the degree of asymmetry better than Eq.11 which gives deceptively low values, and that measurement made at 10 or 15% may be preferred.

Resolution

The resolution $R_{2,3}$ and $R_{3,4}$ as calculated from Eqs. 6, 7, 8, 9 and 10 are given below:

Eqs.	6	7	8			9			10	
			a	b	c	a	b	c		
$R_{2,3}$	2.4	2.6	1.8	1.9	1.9	1.6	1.6	1.6	$R'_{2,3}$	0.97
$R_{3,4}$	1.8	2.1	1.2	1.4	1.6	1.4	1.5	1.6	$R'_{3,4}$	0.99

(Note: a,b,c: For N-3, N-4 and N-5 respectively)

If R = 1, the separation is 98% and if R = 1.5, it is almost complete separation(15,16). Eq.6 is subject to considerable error owing to the uncertainty due to tangent drawn and this error increases with peak asymmetry. Eqs.8 and 9 give generally lower values than Eqs. 6 & 7 whether N-3, N-4 or N-5 is used. Though Eqs.8 & 9 could be less accurate than Eq.6, they are useful for analysis of effects of N, k' and α on R(6). According to Eq.10, which is not commonly used by chromatographers, the ideal value of R' is 1, and as overlapping increases, the value of R' becomes

Table 2 : Noise level (microabsorbance unit) at various Flow Rates for Pumps A and B

	Flow Rate (ml/min)					
	1		2		3	
	A	B	A	B	A	B
	Microabsorbance units					
$\bar{x}$	392	402	365	405	398	400
s.d.	28	32	22	26	35	22
R.s.d.,%	7	8	6	7	9	6
d.f.	9	9	8	9	9	8
t	0.72		1.21		0.07	
	$t_{18,0.05} = 2.10$		$t_{17,0.05} = 2.11$		$t_{17,0.05} = 2.11$	
F	1.30		1.92		2.47	
	$F_{9,9,0.05} = 4.03$		$F_{9,8,0.05} = 4.36$		$F_{9,8,0.05} = 4.36$	
't' & 'F'	Not significant		Not significant		Not significant	

progressively less than 1. However, for calculation of R, Eq.7 appears to be more satisfactory than Eq.6 as the peak width at half height would be more precise than on the baseline.

ii) The Noise levels

The mean ($\bar{x}$)of the amplitudes of the signals (acceptable by Chauvenet's criterion(17)), s.d., and R.s.d.(%), the 't' values of the means, and the 'F' values of the variances are given in Table 2. The 't' and 'F' tests(18) show that the differences in the means of noise levels and their variances for the dual reciprocating and triple piston pumps are not significant even at a reasonably high sensitivity of 0.01 AUFS.

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