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EVALUATION STRATEGY FOR LIQUID CHROMATOGRAPHIC INSTRUMENTATION ALLOWING DIRECT CALCULATION OF PEAK HEIGHTS AND LIMITS OF DETECTION

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SUMMARY

A simple procedure is established for liquid chromatographic (LC) system characterization allowing calculation of peak heights and limit of detections (LODs) for any compound on a column of known plate height in the LC system. Sample, instrumental and chromatographic parameters are all included in the theory, where previous expressions have neglected the system dispersion, flow-cell path-length and sample absorptivity. Experimental evaluation illustrates the power of these new expressions.

INTRODUCTION

Assessing the compatibility of modern liquid chromatographic (LC) instrumentation to the requirements of a particular analysis or laboratory, has necessitated a lengthy empirical approach, as frequently this cannot be ascertained from studying the instrument specifications. For LC instrumentation, these are highly detailed, but often their relevance to real chromatographic operation is unclear. For example noise and drift of a UV absorbance detector with a dry cell at constant temperature is of little significance, if there is increased noise and/or drift for typical LC solvents when flowing and this is not specified. However testing of instrumentation relevant to chromatography necessitates careful planning and execution. Extending our example, use of a flowing solvent can introduce independent solvent effects, from their absorbance, from flow-related phenomena including pressure pulsations or even from incomplete degassing[1]. Specification of system parameters directly relevant to the chromatography, but independent of the test conditions *e.g.* external dispersion, is the ideal. Yet this is highly complex due to the interaction of a large number of parameters.

The two basic questions asked by the chromatographer in assessing the suitability of a particular chromatogram to his analytical problem(s) are: "Is there enough resolution?" and "Is there sufficient sensitivity, and what is the limit of detection?"

The first question is primarily a chromatographic problem and requires a careful selection of a suitable combination of column (dimensions, packing size and func-

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tionality) and solvent(s), necessitating the knowledge of an experienced chromatographer or an effective optimisation system. Instrumental parameters can affect the resolution obtained through the external dispersion, but this can be readily assessed[2]. The second question can subsequently be faced as it combines column, instrumental and sample parameters. It is this question we investigate here.

This paper aims to introduce a practical procedure for characterising an LC system, to allow calculation of chromatographic peak heights and limits of detection on this system.

For a chromatographic peak, eluted from a column under defined conditions, the signal-to-noise ratio (S/N) observed is dependent on four main parameters *viz.*:

(1) the noise observed under these solvent conditions (dependent on incident light on flow cell, solvent absorbance, flow sensitivity, pressure fluctuations and detector electronics);

(2) detection wavelength (affecting signal and noise);

(3) length of flow-cell;

(4) system dispersion (including time constant of detector).

These effects are comprised of static and dynamic components and thus must be evaluated under running conditions. It is the interaction of these effects that determine the observed S/N and the limit of detection (LOD). [To eliminate any confusion, it is the LOD we are considering and not the minimum detectability (MD). The LOD refers to the whole analytical procedure[3] *i.e.* injection, chromatography and detection, whereas the MD refers only to detection.] Relating the observed S/N and LOD to the injected sample, the chromatography must also be included: increasing dilution with increasing retention and increasing column dimensions needs inclusion.

Previous work has involved some, but not all, of these effects in one expression. The effect of column parameters and sample size on peak dilution and LOD was studied[4], leading to expressions in terms of chromatographic parameters, eqns. 1 and 2:

$$\frac{c_{max}}{c_0} = \frac{V_{inj}\sqrt{N}}{V_R\sqrt{2\pi}} \tag{1}$$

$$\mathrm{LOD} = \sqrt{2\pi}\,\frac{[V_0(1 + k')]b \cdot \mathrm{MD}}{\sqrt{N}} \tag{2}$$

where c_{max} = concentration at peak maximum, c_0 = original concentration in sample, V_{inj} = injection volume, V_R = retention volume, V_0 = column void volume, k' = capacity factor, N = column plate count, b = proportionality constant. The factor $\sqrt{2\pi}$ is introduced, as peaks are assumed to be Gaussian. A more recent paper[3], clarifying the role of LODs in LC, showed a practical method for calculating the LOD on any column relative to a reference column, eqn. 3 on the basis of eqn. 2.

$$\mathrm{LOD}(2) = \frac{V_{R(2)}}{V_{R(1)}} \cdot \left(\frac{N_1}{N_2}\right)^{1/2} \cdot \mathrm{LOD}(1) \tag{3}$$

Both papers thus neglected system dispersion and flow-cell characteristics.

In this work expressions are derived to describe the peak heights and LODs observed in terms of (1) chromatographic parameters (allowing for column and system dispersion), (2) instrumental parameters, including flow-cell length and noise levels, and (3) sample parameters, including sample absorptivity and size. These expressions are then evaluated and examples showing direct comparisons with experimental results are included.

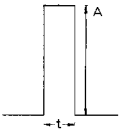
THEORY

In a chromatographic system, when a sample is introduced ideally as a plug, the area can be expressed by

$$\text{peak area (undispersed)} = \frac{At}{b_s} \tag{4}$$

where A = absorbance (AU) of the sample in a cell of length b_s cm (*i.e.* a static measurement) and t = time of injection (s). Let $A' = A/b_s$ (*i.e.* absorbance of sample per cm), now

$$t = \frac{V_{inj}}{v} \tag{5}$$



where V_{inj} = volume of injection (μl) and v = volume flow-rate (μl s^{-1}) then eqn. 4 becomes

$$\text{peak area} = \frac{A'V_{inj}}{v}$$

This plug is dispersed in the chromatographic system, with the area being expressed by eqn. 6:

$$\text{area of peak in chromatogram} = \frac{rp}{b_c}\sigma_{TOT} \tag{6}$$

where p = peak height (AU), b_c = length of chromatographic flow-cell (cm), σ_{TOT} = peak standard deviation, and r is a shape parameter, which for Gaussian peaks would be equal to $\sqrt{2\pi}$. Now, as the area of sample plug and the area of the chromatographic peak are equal (assuming rapid peak height measurements in the chromatographic detector, *i.e.* flow cell volume $\ll$ volume of peak), then

$$A'\frac{V_{inj}}{v} = \frac{rp}{b_c}\sigma_{TOT} \tag{7}$$

Now from additivity of variances

$$\sigma^2_{TOT} = \sigma^2_{COL} + \sigma^2_{EXT} \tag{8}$$

where σ^2_{COL} = column variance and σ^2_{EXT} = external variance, and from definition for N (the column plate count)

$$N = \frac{t_R^2}{\sigma^2_{COL}} \tag{9}$$

or

$$N = \frac{L}{H} \tag{10}$$

where L = column length (mm) and H = plate height (mm). Combining eqns. 8, 9 and 10, and substituting into 7, we obtain

$$\frac{A'^2}{p^2} = \frac{v^2 r^2}{V^2_{inj}\, b_c^2} \cdot \left(\frac{H t_R^2}{L} + \sigma^2_{EXT} \right) \tag{11}$$

or

$$\frac{A'^2}{p^2} = \alpha \cdot \frac{t_R^2}{L} + \beta \tag{12}$$

where

$$\alpha = \frac{v^2 r^2}{V^2_{inj}\, b_c^2} \cdot H$$

and

$$\beta = \frac{v^2 r^2}{V^2_{inj}\, b_c^2} \cdot \sigma^2_{EXT}$$

Treating the chromatographic system as a black-box (p/A') neglects the path-length of the flow-cell, and compares directly the chromatographic peak height (in AU), with the absorbance of the sample solution in a standard 1-cm cell, thus p/A' is the apparent dilution factor [true dilution factor is $p/(A'b_c)$ equivalent to c_{max}/c_0] and p/A' at $t_R^2/L = 0$, *i.e.* $1/\sqrt{\beta}$ is the apparent system dilution factor.

It should be noted that if the β term is ignored (*i.e.* $\sigma_{EXT} \approx 0$), then eqn. 12 reduces to the previous expressions (eqn. 1).

Calculation of LOD

The definition of the LOD of a LC analysis is the minimum component amount injected that produces a defined multiple of the S/N. In this case we shall take this figure as 3.

Thus $p = 3n$ where n = noise, for LOD conditions. When we substitute this value in eqn. 12 we get

$$\frac{A'^2}{9n^2} = \frac{v^2 r^2}{V^2_{inj} b_c^2} \left(\frac{t_R^2}{L} H + \sigma^2_{EXT} \right)$$

Now combining this equation with $\mathrm{LOD} = c \cdot V_{\mathrm{inj}}$ and $A'_{\mathrm{min}} = a \cdot c = a \cdot \mathrm{LOD}/V_{\mathrm{inj}}$, where a = absorptivity, we get

$$\frac{a^2\mathrm{LOD}^2}{9n^2V_{\mathrm{inj}}^2} = \frac{v^2r^2}{V_{\mathrm{inj}}^2\, b_c^2}\left(\frac{t_R^2}{L}H + \sigma_{\mathrm{EXT}}^2\right)$$

$$\mathrm{LOD}^2 = \frac{9n^2v^2r^2}{b_c^2a^2}\left(\frac{t_R^2}{L}H + \sigma_{\mathrm{EXT}}^2\right)$$

i.e.

$$\mathrm{LOD}^2 = \gamma\frac{t_R^2}{L} + \varphi \tag{13}$$

where

$$\gamma = \frac{9n^2v^2r^2}{b_c^2a^2}H = \frac{9n^2}{a^2} \cdot V_{\mathrm{inj}}^2\,\alpha$$

and

$$\varphi = \frac{9n^2v^2r^2}{b_c^2a^2}\sigma_{\mathrm{EXT}}^2 = \frac{9n^2}{a^2} \cdot V_{\mathrm{inj}}^2\,\beta$$

This can be expressed in an alternative form, in terms of L and k', values which are more useful for predictive purposes. Since $t_R = t_0\,(1 + k')$ and

$$t_0 = \frac{\pi d^2}{4}\frac{Le}{v}$$

where e = porosity, d = column I.D., then

$$\mathrm{LOD}^2 = \gamma' L(1 + k')^2 + \varphi \tag{14}$$

where

$$\gamma' = \frac{9n^2}{b_c^2a^2}r^2 \cdot \frac{\pi^2d^4e^2H}{16\,v^2} = \frac{9n^2V_{\mathrm{inj}}^2}{a^2} \cdot \frac{\pi^2d^4e^2}{16}\alpha$$

Again, our expression (eqn. 13) reduces to the simpler expression eqn. 3, if the external dispersion is neglected ($\beta \to 0$), and the LOD of two columns are compared.

The validity of the derived expression for dilution (eqn. 12) is explored, and two LC systems are characterized in terms of α and β. Comparisons of experimental peak heights with values calculated by this theory (eqn. 12) and previous theory (eqn. 1), are made. Experimental and calculated LOD values (*via* eqn. 14) are subsequently compared.

EXPERIMENTAL

Method

The sample selected for characterization of the LC systems contained a homologous series of six alkylphenones, to reduce the possibility of differential peak-shapes from chemical effects. Elution of these components was adjusted to give a typical working range of capacity factors (k' 0.5–10).

Equipment

The two LC systems were composed of the following modules, linked with 0.25 mm I.D. stainless-steel tubing. System A was comprised of PU4003 chromatograph with PU4025 U.V. detector fitted with an 8-μl flow-cell, with the column mounted in an oven (PU4031). System B was comprised of the PU4100 chromatograph utilizing the 8-μl analytical flow-cell. The 2.5-μl flow-cell was used with a fast LC column where noted. On both systems a 7125 Rheodyne valve was employed using a selection of precalibrated loops. System characterization used 19.5- and 21.5-μl loops (loops X and Z) on systems A and B, respectively. The detector output, in both cases, was handled by a PU4810 computing integrator. The data quoted is an average of at least three runs. All instruments are from Philips Analytical (Cambridge, U.K.).

Chromatography

Characterization of the LC systems was carried out on a series of 4.6 mm I.D. cartridge columns of 10 and 22 cm. These were all packed from the same batch of 5 μm RP-18 packing. One fast LC cartridge (10 cm × 3.2 mm I.D., 3 μm Velosep RP-18) was used. Also another 10 cm × 4.6 mm Spheri-5 RP-18 cartridge from a different batch, and a 10-μm RP-18 (of same dimensions) were employed. All cartridges were supplied by Brownlee Labs. (Santa Clara, CA, U.S.A.).

The sample (working mixture) contained acetophenone (51.3 μg/ml), propiophenone (51.1 μg/ml), butyrophenone (49.7 μg/ml), valerophenone (50.1 μg/ml), hexanophenone (81.9 μg/ml), octanophenone (67.7 μg/ml) in acetonitrile–water (60:40). The eluent was acetonitrile–water (60:40) and the ovens were operated at 45°C. Detection was at 274 nm. The octanophenone peak (k' *ca.* 11) was used for estimation of N (to allow estimation of H), from full-width half height (FWHH) measurements, where $N = 5.545\ t_R^2/\mathrm{FWHH}^2$. Measurements of N were carried out simultaneously with the corresponding α and β determinations to ensure relevant comparisons. Approximate values of σ_{EXT} were determined in some cases by the extrapolation method using FWHH measurements[2].

Characterization of sample

Individual solutions of the six alkylphenones were prepared from the same stock solutions as the working mixture, at the same concentrations. The absorbances of these solutions were measured at 274 nm (on a Philips PU8800 UV spectrophotometer) in a 1-cm cell, giving the A' values.

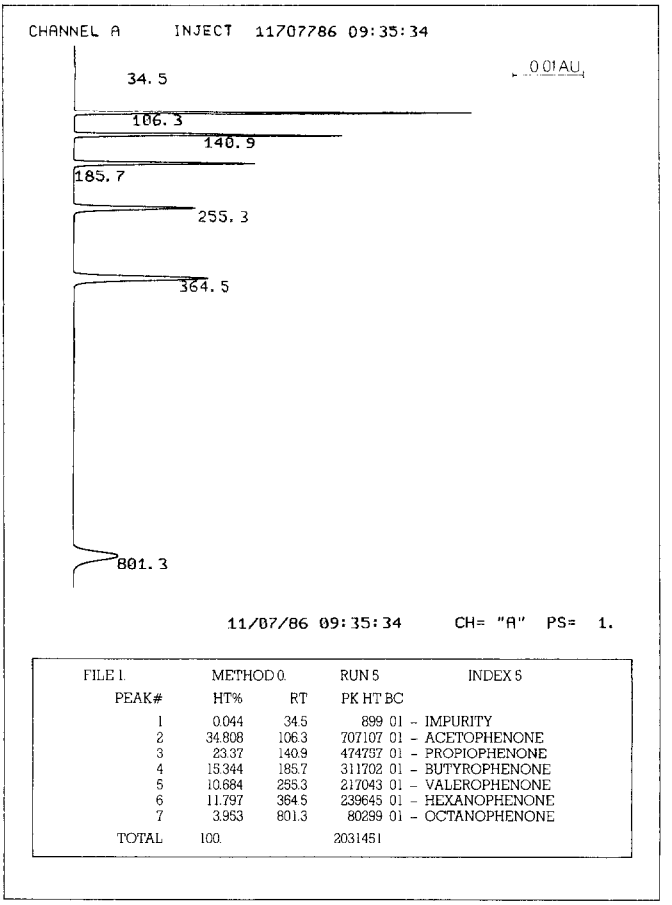
Calibration of sample-loops

With each of the four sample loops (W, X, Y, Z), injections of the sample

solution were made onto one of the 10-cm columns. The six peaks areas from each run were compared with a calibration curve produced by injecting 5, 10 and 20 μl of sample solution into a 50-μl loop. (Volumes were determined as 14.4 μl, 19.5 μl, 24.6 μl and 21.5 μl for W, X, Y and Z respectively).

Characterization of LC systems

Using the 10-cm and 22-cm cartridges, the peak heights for the six alkylphenones were recorded, and converted to p/A' ratios. α and β values were calculated for each column. On system A, various lengths of connecting tubing were used (tubing I, II and III). The volume of injection (V_{inj}) was varied with loops W, X and Y on system A. System B was characterized similarly on 10- and 22-cm cartridges. Variations in flow-rates were studied with the 3-μm fast LC column with the 2.5-μl fast LC cell.



FILE 1	METHOD 0.		RUN 5	INDEX 5
PEAK#	HT%	RT	PK HT BC	
1	0.044	34.5	899 01	- IMPURITY
2	34.808	106.3	707107 01	- ACETOPHENONE
3	23.37	140.9	474797 01	- PROPIOPHENONE
4	15.344	185.7	311702 01	- BUTYROPHENONE
5	10.684	255.3	217043 01	- VALEROPHENONE
6	11.797	364.5	239645 01	- HEXANOPHENONE
7	3.953	801.3	80299 01	- OCTANOPHENONE
TOTAL	100.		2031451	

Fig. 1. Typical chromatogram on a 22-cm cartridge on system B. Eluent, acetonitrile–water (60:40); flow, 2 ml min^{-1}; sample 21.5 μl; detection at 274 nm; 0.5 s response time.

Verification of LOD

A sample solution of propiophenone with concentration 0.160 μg/ml was prepared. With loop Z, injection of 21.5 μl, gave a sample of 3.4 ng (The noise was measured as peak to peak.)

RESULTS AND DISCUSSIONS

A typical chromatogram is shown in Fig. 1, with a listing of the peak retention times and heights below. These heights were converted into absorbance units. These were used to calculate the apparent dilution factors, p/A', which as expected decreased for higher k' peaks and for longer columns (Fig. 2).

Plots of t_R^2/L *vs.* $(A'/p)^2$ produce straight lines with good linear correlations ($r \geqslant 0.9997$). (All correlations are on six data points.) Values of α, β and correlation coefficients are listed for each determination, along with the apparent system dilution factor $1/\sqrt{\beta}$. Results for systems A and B are shown (Fig. 3) and are listed in Table I. Although a lot of data is summarised in these figures and tables, the main points to note here are:

(1) good linear correlations on all lines, (2) low variations in α and β values on both systems. (Variations in α on system B are discussed later.)

As the cartridges are all packed with the same batch of packing, ideally H

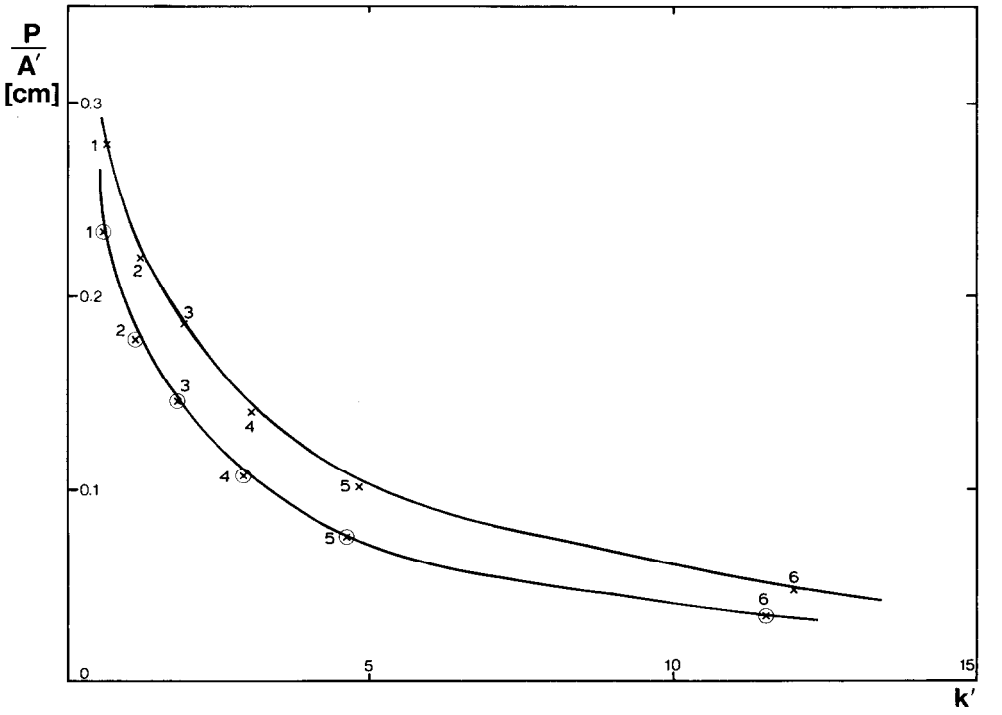


Fig. 2. Plot of apparent dilution p/A' against capacity factor (k'), for system A on a 10-cm column (II) (×) and 22-cm column (⊗), for the six alkylphenone peaks.

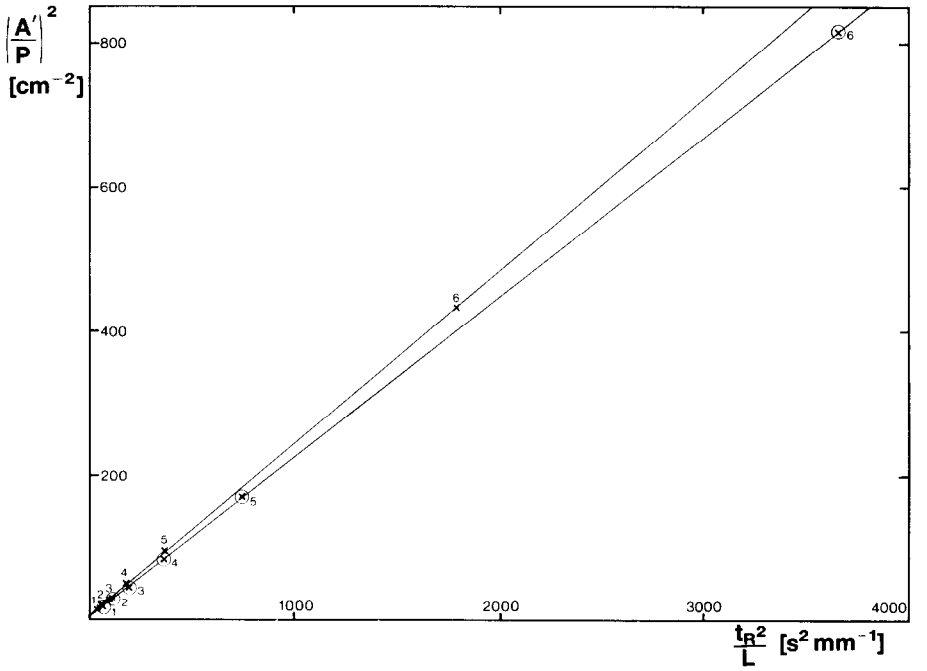


Fig. 3. Plot of $(A'/p)^2$ against t_R^2/L on system A on 10- (II) (×) and 22-cm (⊗) columns (see also Table I), for the six alkylphenone peaks.

should be identical on all cartridges. In each system, v, V_{inj} and b_c are constant, and with a as a constant, any variation in α is due to variations in H.

As α/H is approximately constant for the 10- and 22-cm columns, see Table II, variations in α can be largely accounted for by variations in the estimated H

TABLE I

LISTING OF α AND β VALUES FROM LINEAR CORRELATIONS (SIX DATA POINTS) ON 10- AND 22-cm COLUMNS ON SYSTEMS A AND B

Column length (mm)	*Column*	α	β	*Correlation coefficient*	*Apparent system dilution factor.* $1/\sqrt{\beta}$
System A					
100	I	0.237	6.87	0.99998	0.382
100	II	0.239	8.38	0.99994	0.345
100	III	0.239	8.10	0.99994	0.351
220		0.221	7.26	0.99999	0.371
System B					
100	IV	0.908	13.91	0.99999	0.268
100	V	1.002	15.97	0.99999	0.250
100	VI	0.837	14.10	0.99998	0.266
220		0.864	14.59	0.99999	0.262

TABLE II

LISTING OF α, H AND α/H VALUES FOR 10- AND 22-cm COLUMNS ON SYSTEM B

Column length (mm)	*Column*	α	*H (mm)*	α/H	*Correlation coefficient*
100	IV	0.908	0.0129	70.4	0.99999
100	V	1.002	0.0137	73.1	0.99999
100	VI	0.837	0.0121	69.2	0.99998
220		0.864	0.0116	74.5	0.99999

TABLE III

LISTING OF α AND β VALUES FOR VARIATIONS IN CONNECTION TUBING ON SYSTEM A

Tubing	α	β	*Correlation coefficient*
10-cm column (II)			
I	0.239	8.38	0.99994
II	0.238	17.97	0.9998
III	0.246	11.95	0.9997
22-cm column			
I	0.221	7.26	0.99999
II	0.211	18.25	0.9998
III	0.226	13.49	0.9999

TABLE IV

VARIATION OF α AND β WITH V_{inj} ON SYSTEM A (TUBING III), ON A 10-cm COLUMN (II)

V_{inj} (μl)	Rel. $1/V_{inj}^2$	α	Rel. α	β	Rel. β	*Correlation coefficient*
14.4	1.00	0.454	1.00	21.3	1.00	0.99990
19.5	0.55	0.240	0.53	13.6	0.640	0.9996
24.6	0.34	0.149	0.33	8.72	0.409	0.9997

values. β values for the 10- and 22-cm cartridges are expected to be constant for each system with identical columns, so any variation here indicates the typical error in this determination. It is larger than for α, as small variations in slope cause large variations in the intercept.

Although these approximate values for H can be used for comparisons of relative effects, accurate determination of H and σ_{EXT} values are needed for accurate comparison of theory and experiment. For example determination of the value(s) of r (shape factor) is not possible without such data.

At this point it is pertinent to observe that α is not constant between columns, due to variation in r and/or H. We believe that H varies with column length, probably due to column-end effects, but this requires further proof with accurate column and external dispersion measurements.

TABLE V

LISTING OF α AND β VALUES FOR VARIATION IN FLOW (v), USING FAST LC COLUMN (10 cm × 3.2 mm WITH 3-μm PACKING), WITH A 2.5-μl FLOW-CELL, 5-μl INJECTION ON SYSTEM B

Flow (ml min^{-1})	*H (mm)*	α	*Rel.* α	*Rel.* v^2H	β	*Correlation coefficient*
1	0.0114	0.573	1.00	1.00	56.7	0.9996
2	0.0126	2.85	4.97	4.42	85.6	0.9992

Addition of extra external dispersion affected the values of β, but left α unaffected (Table III). Tubing I was optimum connections, tubing II increased the external dispersion (σ_{EXT} approx. 25 μl) and tubing III increased σ_{EXT} less (σ_{EXT} approx. 15 μl). These affected values by up to 100%, but α values varied less than 7%. This shows the independence of the two parameters.

Variations in α and β were studied with variations in injected volume (V_{inj}) on system A. Precalibrated loops were used and results are shown in Table IV. The variation in α is mirrored by the variation in $1/V_{inj}^2$ as predicted by eqn. 12. Variation in V_{inj} also affects σ_{EXT} and thus β should vary with σ_{EXT}^2/V_{inj}^2, but this could not be checked without accurate σ_{EXT}^2 measurements.

Variations in α and β with variations in flow-rate (v) were investigated on system B (Table V). Correlation of α with v^2H (from eqn. 12) is good.

Variations in α and β with other 10-cm cartridge columns than those in the matched sets were investigated, and results compared to column II (on system A, with tubing III). Agreement in the α/H values for the 5-μm and 10-μm material is good, (Table VI), according to eqn. 12. For the 3-μm material the agreement of α/H values is also good, when allowance for the reduced flow-rate is included. The β values can be expected to be constant for constant flow-rate, which is observed for column II and the 5-μm column. For the 10-μm column, β is observed to drop, which we believe may be due to different end-fittings. Independent external dispersion measurements are needed to investigate this further.

The results of these systematic investigations into the expression (eqn. 12), show that α is dependent on v^2, H and V_{inj}^2, and independent of σ_{EXT}. Now v, H and V_{inj} are closely related to the chromatography rather than the system and are fre-

TABLE VI

LISTING OF α AND β VALUES ON VARIOUS COLUMNS ON SYSTEM A (TUBING III)

Column	α	*H (mm)*	β	*Flow (ml min^{-1})*	*Rel.* α	*Rel.* v^2H	*Correlation coefficient*
II	0.246	0.0156	11.9	2.00	1.00	1.00	0.9997
5 μm	0.239	0.0156	13.2	2.00	0.97	1.00	0.9995
10 μm	0.705	0.0435	2.04	2.00	2.87	2.79	0.9992
3 μm*	0.043	0.0108	11.2	1.00	0.175	0.173	0.990

* Column dimensions 10 cm × 3.2 mm I.D., all others being 10 cm × 4.6 mm I.D.

quently variable, whereas b_c and σ_{EXT} are system parameters. Thus redefining α and β in terms of system parameters only, *i.e.*

$$\alpha' = \frac{r^2}{b_c^2} \quad \text{and} \quad \beta' = \frac{r^2}{b_c^2}\sigma_{EXT}^2 \tag{15}$$

Thus from the definition of α and β as in eqn. 12,

$$\alpha' = \frac{\alpha V_{inj}^2}{v^2 H} \quad \text{and} \quad \beta' = \frac{\beta V_{inj}^2}{v^2} \tag{16}$$

and we can rewrite eqn. 12 as

$$\left(\frac{A'}{p}\right)^2 = \frac{v^2 H}{V_{inj}^2}\alpha' \frac{t_R^2}{L} + \frac{v^2}{V_{inj}^2}\beta' \tag{17}$$

(It should be noted however, that β' will show some dependence on v and V_{inj}, as σ_{EXT} is dependent on these parameters.)

Using these new system parameters for the terms in eqns. 13 and 14 we obtain new expressions for γ, γ' and φ: For eqn. 13 we get

$$\text{LOD}^2 = \gamma \frac{t_R^2}{L} + \varphi \tag{13'}$$

where

$$\gamma = \frac{9n^2}{a^2} \cdot Hv^2 \cdot \alpha' \tag{18}$$

and

$$\varphi = \frac{9n^2}{a^2} \cdot v^2 \cdot \beta' \tag{19}$$

From eqn. 14 we obtain

$$\text{LOD}^2 = \gamma' \cdot L(1 + k')^2 + \varphi \tag{14'}$$

where

$$\gamma' = \frac{9n^2}{a^2} \cdot \frac{\pi^2 d^4}{16} \cdot e^2 \cdot H \cdot \alpha' \tag{20}$$

Several points should be noted though in relation to system parameters α' and β' replacing α and β. α and β included v, H and V_{inj}, parameters which chromatographers often are ignorant of their accurate values; it is the high precision of v and V_{inj} that is generally essential. However for accurate application of these expressions, accurate values will be needed. For H, either accurate values or accurate H ratios

will be needed *i.e.* In the determination of α' and β' for the system, a determination of H on the column(s) used, will be needed under these conditions (*e.g.* FWHH measurement on representative peak at $k' \approx 10$). A similar determination should then be used on the analytical column for the LOD work, giving a consistent ratio of H values, even if the H values are not accurate. (However, if H, V_{inj} or v are not changed between calibration and LOD runs, then their dependence can be eliminated and their accuracy is irrelevant.)

Comparison of predicted and experimental peak heights

An LC system characterized in terms of α' and β', allows calculation of the peak dilutions on given columns. To compare theory and practice, characterization data on system B, produced with the 22-cm column, was used to calculate the apparent dilution factors p/A', produced on two 10-cm columns (V and VI) with different H values.

Characterization of system B on 22-cm cartridges gives $\alpha' = 30.99\ cm^{-2}$, $\beta' = 6.07\ cm^{-2}\ s$ (from eqn. 16 $\alpha = 0.864$, $H = 0.0116$, $V_{inj} = 21.5\ \mu l$, $v = 33.33\ \mu l\ s^{-1}$, $\beta = 14.59$). Calculated, p/A' ratios, for observed retention times (see Table VII), via eqn. 17, are compared to those predicted by previous theory (eqn. 1), and with experimental data. All three data sets are plotted for both 10-cm columns (Fig. 4).

Clearly our predicted dilutions are very close to the experimental results. The discrepancy of previous theory from experimental results is worse at low retention as expected due to neglecting system dispersion. Our calculated data is highly dependent on the accuracy of the H values (or ratios). Yet even with the 7.5% variation in the α/H values observed between the calibration column (22 cm) and the 10-cm column VI (see Table II), where α/H may be expected to be constant, the fit is still good.

TABLE VII

LISTING OF EXPERIMENTAL AND PREDICTED APPARENT* PEAK DILUTIONS (p/A') ON 10-cm COLUMNS (V AND VI), FOR RANGE OF PEAKS (k' 0.5–10), ON SYSTEM B

t_R (s)	p/A' (from eqn. 17)	p/A' (previous theory, eqn. 1)*	Experimental p/A'
Column V			
48.6	0.161	0.226	0.164
63.6	0.134	0.173	0.134
83.3	0.108	0.132	0.109
113.5	0.083	0.097	0.083
160.9	0.060	0.068	0.060
350.8	0.028	0.031	0.028
Column VI			
48.7	0.167	0.241	0.179
63.8	0.140	0.184	0.145
83.6	0.114	0.140	0.119
113.9	0.087	0.103	0.090
161.5	0.063	0.073	0.065
352.1	0.030	0.033	0.031

* As Eqn. 1 predicts true peak dilutions c_{max}/c_0, this must be adjusted by the factor b_c, (0.5 cm) to give p/A'.

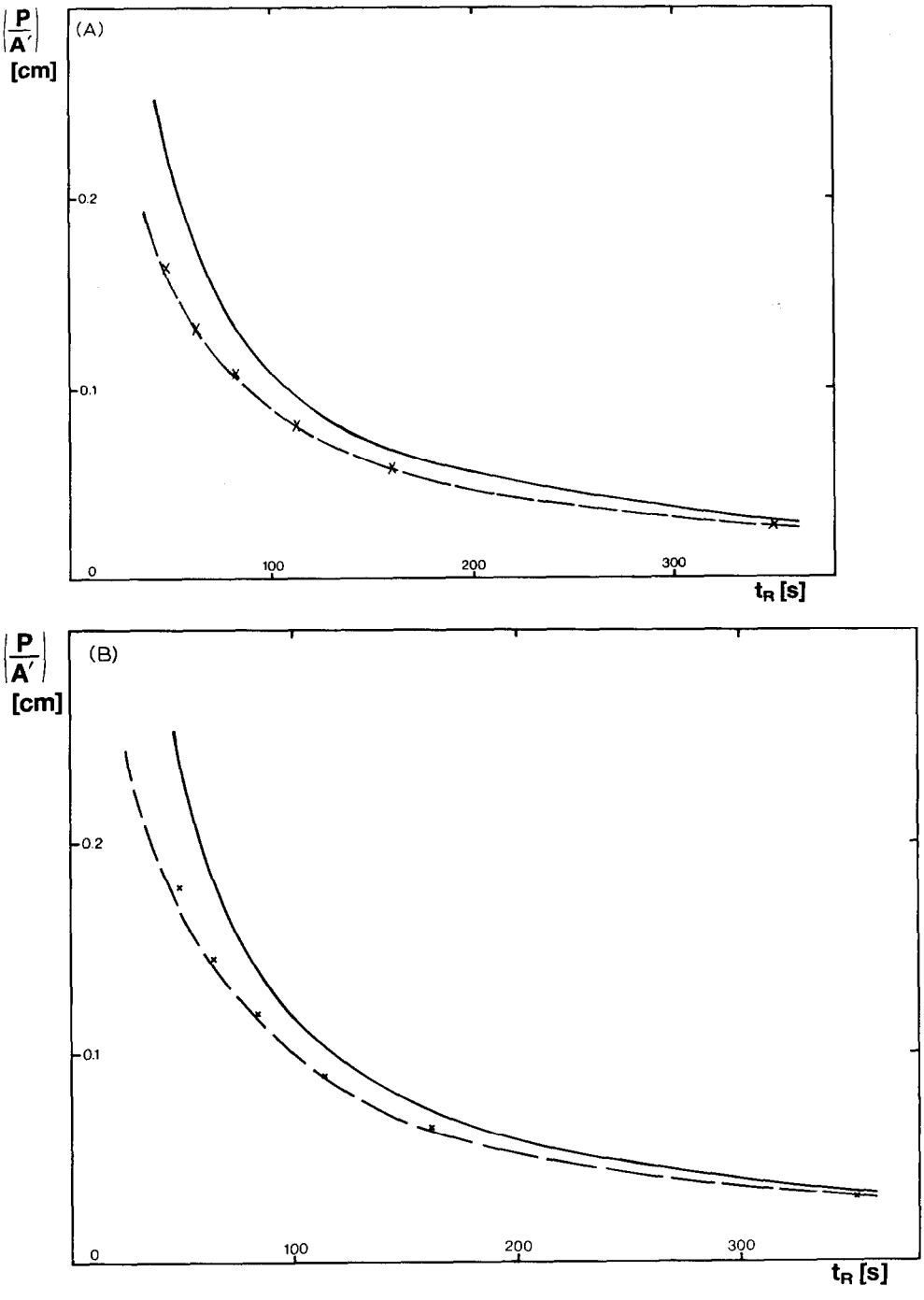


Fig. 4. Comparison of theoretical and experimental results. Plot of p/A' against t_R, on 10-cm columns on (A) column V, (predicted) $\alpha = 1.02$, (experimental) $\alpha = 1.00$; and (B) column VI (predicted) $\alpha = 0.90$ (experimental) $\alpha = 0.84$. (– – –) This theory, (———) previous theory (see also Table VII).

TABLE VIII

LOD VALUES OF PROPIOPHENONE ON SYSTEM B

k'	*LOD (ng)*	
	10-cm column	*22-cm column*
0	2.71	3.20
0.5	3.38	4.25
1.0	4.14	5.37
2	5.79	7.73
3	7.51	10.15
5	11.03	15.05
10	19.99	27.42
1.06*	4.24	–

* For direct comparison with experimental result.

Limit of detection

For an LC system, characterized in terms of α' and β', the LOD of any compound can be calculated on a column of known plate height, using eqn. 14 with the expressions for α' and φ, as defined in eqns. 19 and 20.

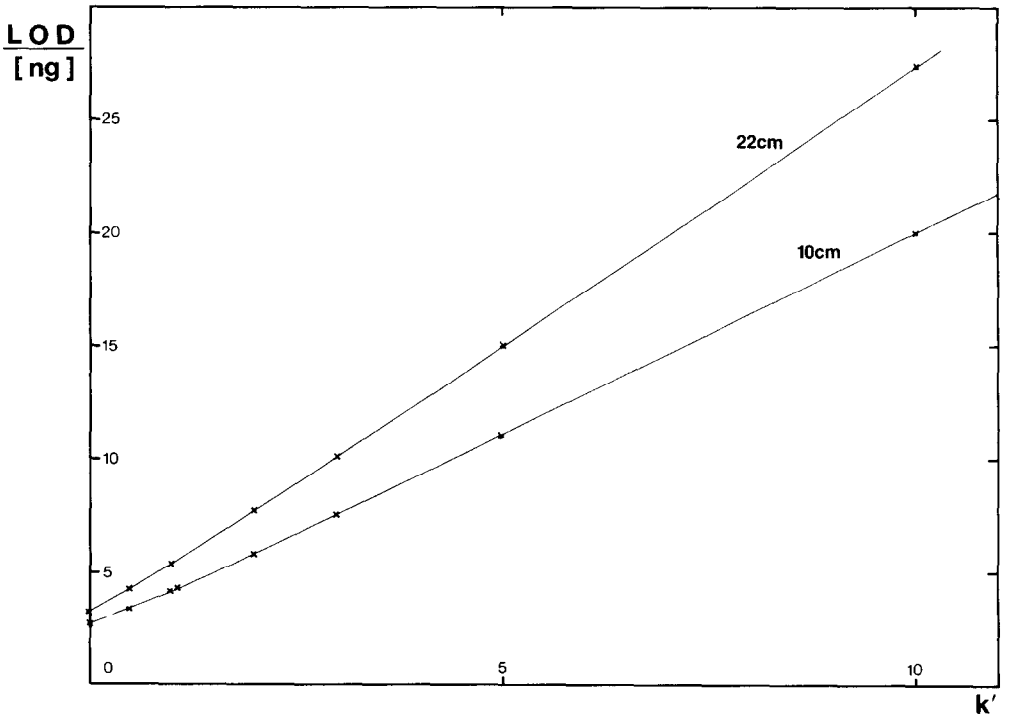


Fig. 5. Variation of LOD (ng), with k' on system B, for propiophenone, on 10- and 22-cm columns (see also Table VIII).

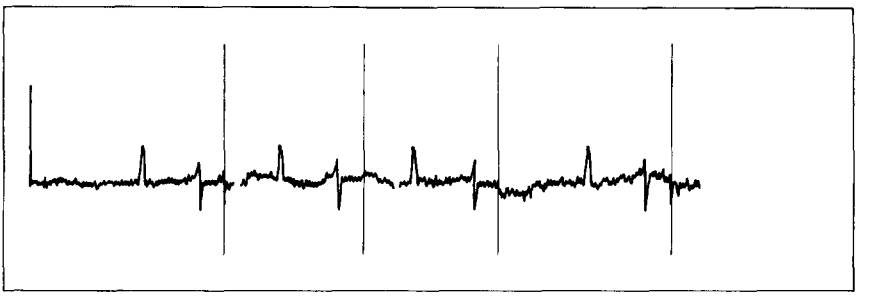


Fig. 6. Chromatogram of propiophenone (k' = 1.06) on a 10-cm column with loading of 3.43 ng producing an S/N value of 2.8 (signal = $1.69 \cdot 10^{-4}$ a.u., noise = $6 \cdot 10^{-5}$ a.u.).

As an example, consider the LOD of propiophenone on 10- and 22-cm cartridges (4.6 mm I.D., 5 μm packing), under the previous chromatographic conditions, on system B. The column plate count for these columns, under conditions close to those of the analysis, needs to be known. For the 10-cm column H = 13.5 μm and the 22-cm column H = 11.6 μm, determined from one run each under the chromatographic conditions on a peak at high k' (10, on octanophenone).

For system B, α/H = 71.8 and β = 14.6 (average values from Tables I and II), where $V_{inj.}$ = 21.5 μl, v = 33.3 μl s^{-1} and thus α' = 29.9 cm^{-2} and β' = 5.27 $cm^{-2}s^2$ (from eqn. 16).

The LOD values were calculated for a range of k' values (from eqns. 14 and 20), on the 10- and 22-cm cartridges. These are listed in Table VIII and plotted in Fig. 5.

This data predicts that for k' = 1.06, propiophenone would have a LOD of 4.2 ng on the 10-cm column. A chromatogram run with a loading of 3.4 ng (Fig. 6) shows an S/N value of 2.8 (determined over nine runs). Thus empirically a LOD of 3.7 ng is found.

CONCLUSIONS

This new procedure for LC system characterization, in terms of two system parameters α' and β', can be effected from one chromatographic separation on one column for several (*ca.* 6) components with a range of k' values 0.5–10. Calculation of peak heights and LODs on other columns, with known H, in the LC system can then be performed for any compound of known absorptivity, and given retention.

Incorporation of instrumental, chromatographic and sample parameters into the one theory allows simple calculation of the practical parameters of peak heights and LODs in real separations. Despite employing a simple model, the experimental evaluation here illustrates the power of such expressions, allowing calculation of peak heights within 7%, and LODs within 12%.

Future investigations will include accurate σ_{COL} and σ_{EXT} measurements by an independent calculation method, (via second statistical moments), as the present measurements of σ_{COL} (or N) are very closely related to our determination of α. These accurate measurements will also allow determination of r, (shape parameter). The contributions from the column end-fittings to the system dispersion (σ_{EXT}), if sig-

nificant, will limit the usefulness of this approach. Thus different column end-fittings need investigation along with a wider range of column dimensions, particularly very short columns and joined cartridges.

These measurements have all been performed with one eluent. Although N is dependent on solvent viscosity, α' should be independent of eluent. This needs verifying.

It can be predicted that such expressions will become increasingly employed as the complete optimization of LC separations, is automated. Here the compatibility of the aim of the analysis, in terms of resolution and S/N, is matched to the characteristics of column, system and sample.

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