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## EVALUATION OF METHODS OF MEASURING GAS LIQUID CHROMATOGRAPHIC RETENTION ON SKEWED PEAKS

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### SUMMARY

Of the various circumstances in which peaks are skewed, one of the commonest is that caused by a partition isotherm which is too strongly curved for the sample size used. In this case five different methods of estimating the infinite dilution retention from measurements on the peak have been described in the literature. They are evaluated both *a priori* and by experimental comparison with the true infinite dilution retention for four gas-liquid partitioning systems free from adsorption effects. Peaks were studied for both directions of skew with skew ratios between about 0.2 and 5. Only the method of Littlewood, Phillips and Price is satisfactory for both directions of skew though it is about equalled in accuracy (a few percent) by one of the other methods for each direction of skew. Methods based on the peak maximum can give very large errors.

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### INTRODUCTION

In a recent review<sup>1</sup> we have categorised the different causes of peak distortion and discussed methods of measuring retention in each case. Of these cases perhaps the most difficult to deal with, yet also one of the most common, is that of non-linear, non-ideal chromatography. Here the solute concentration is in a region where the distribution coefficient and other factors controlling retention depend on concentration (non-linearity) yet the resultant peak skewing is insufficient to overwhelm the influence on the profile of normal band-spreading processes such as slow mass transfer between phases (non-ideality). The peaks are therefore only moderately asymmetrical, *i.e.* neither side of the peak is vertical. Nevertheless, the time to the peak maximum varies with sample size and fails to provide a measure of the retention at infinite dilution.

The possibility of moving to a linear regime, *e.g.*, by raising or lowering the solute concentration or by changing the temperature, has been discussed previously<sup>1</sup>. These approaches are not always feasible or appropriate. The problem, therefore, is how to find a good estimate of the true retention volume at infinite dilution of the solute, given that the peak is moderately asymmetrical due to non-linear, non-ideal

chromatographic behaviour. We also restrict consideration to the case where the main cause of non-linear behaviour, apart from the sorption, thermal and viscosity effects<sup>2</sup>, is the isotherm for partitioning of the solute between gas and liquid; significant retention contributions from adsorption on the solid support or at the gas-liquid interface are excluded.

Five methods of measuring retention have been proposed at various times, four empirical and one arising from a mathematical model. We first describe the methods and their limitations and then their experimental evaluation for a variety of solute-solvent systems and conditions.

#### *Methods of measuring retention*

*Peak maximum method (PM).* In this method no correction is made for distortion. Retention is simply measured to the maximum of the asymmetrical peak.

*Method of Littlewood, Phillips and Price (LPP).* The second method is based on the finding<sup>3-5</sup> that, if profiles for different sample sizes are superimposed (Fig. 1), tangents to the leading sides intersect the baseline more or less at a common point  $t_I$ . Tangents to the trailing sides cut the baseline at points  $t_F$  which are nearly at constant distance ( $\Delta t = t_F - t_{\max}$ ) from the peak maximum. Littlewood, Phillips and Price<sup>3</sup> therefore proposed that the time  $\Delta t$  be added to  $t_I$  to give a corrected infinite dilution value for the retention  $t_R$ . Their formula is accordingly

$$t_R = t_I + (t_F - t_{\max}) \quad (1)$$

This formula was applied only to peaks skewed in the direction  $\eta > 1$ , as shown in Fig. 1. (The skew ratio,  $\eta$ , is defined as the ratio of the slope of the trailing edge of the peak to that of the leading edge, both slopes being obtained at the points of inflection.) If the formula could be extended to peaks of opposite skew,  $\eta < 1$ , it would be expected to take the form

$$t_R = t_F - (t_{\max} - t_I) \quad (2)$$

which turns out to be identical to eqn. 1. An alternative form of these equations, more directly comparable with eqn. 5 below, is

$$t_R = t_{IF} + (t_{IF} - t_{\max}) \quad (3)$$

where  $t_{IF}$  is the arithmetic mean of the initial and final retention times:

$$t_{IF} = \frac{1}{2}(t_I + t_F) \quad (4)$$

*Method of Pollard and Hardy (PH).* Instead of applying a formula to a single peak, Pollard and Hardy<sup>4</sup> superimpose peaks for different sample sizes. A line is then drawn through the peak maxima and its intersection with the baseline is taken as the retention of an infinitely small sample. This procedure, in contrast to the second, was originally applied only to peaks skewed in the direction  $\eta < 1$ .

*Method of Conder and Young (CY).* Conder and Young<sup>2</sup> have returned to the

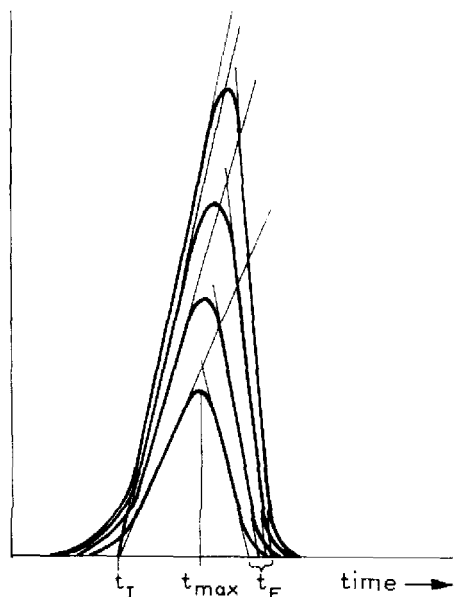


Fig. 1. Definitions of  $t_I$ ,  $t_F$  and  $t_{max}$ .

use of a single peak but propose replacing eqn. 3 by the simpler one

$$t_R = t_{IF} \quad (5)$$

The basis for this suggestion is that, in linear, non-ideal chromatography, it provides an excellent approximation to the retention of the mass centre of the peak<sup>6</sup>, which is the proper, thermodynamic measure of retention in the linear, non-ideal case. In the non-linear case the mass centre loses its significance, but  $t_{IF}$  still appears, from the general geometry of Fig. 1, to be a not unreasonable estimate of  $t_R$ .

*Method of Haarhoff and Van der Linde (HL).* Several authors have developed mathematical models for non-linear, non-ideal peak profiles<sup>-13</sup>. In all these treatments simplifying assumptions have been made to make the mathematics manageable. For long columns, Buys and De Clerk<sup>12</sup> have derived a semi-empirical equation which can be cast in a form<sup>14</sup> relating the first statistical moment of the peak to the retention  $t_R$  at infinite dilution. The equation is not suitable for predicting  $t_R$  because it involves a non-linearity parameter which is not measurable unless  $t_R$  is known. Potentially a more useful approach is that of Haarhoff and Van der Linde<sup>13</sup> who show that under certain conditions  $t_R$  can be found by the following procedure. If  $c_{max}$  is the concentration at the peak maximum and  $t_{1/2}^-$  and  $t_{1/2}^+$  are the times measured from injection to the points at  $1/2c_{max}$  on the leading and trailing edges of the peak, an asymmetry ratio  $s$  is calculated from equation

$$s = 2(t_{max} - t_{1/2}^-)/(t_{1/2}^+ - t_{1/2}^-) \quad (6)$$

A second ratio  $r$  is defined by

$$r = \frac{1}{2}(t_R - t_{\max}) / (t_{1/2}^+ - t_{1/2}^-) \quad (7)$$

Since  $r$  is related to  $s$  by

$$\begin{aligned} r &= 1.32(1 - s) && \text{for } 0.75 \leq s \leq 1 \\ r &= 1.08 - s && \text{for } 0.25 \leq s \leq 0.75 \end{aligned} \quad (8)$$

$t_R$  can be calculated from these equations after finding  $t_{\max}$ ,  $t_{1/2}^-$  and  $t_{1/2}^+$  from the chromatogram. This procedure was defined only for peaks skewed in the direction  $\eta < 1$  (tailing peaks,  $s < 1$ ) though the model on which it is based is valid for both directions of skew. We have investigated the effect of extending the method empirically to peaks of opposite skew  $\eta > 1$  by replacing  $t_{1/2}^-$  in the numerator of the right hand side of eqn. 6 by  $t_{1/2}^+$  and interchanging  $t_R$  and  $t_{\max}$  in eqn. 7.

Haarhoff and Van der Linde's model incorporates several simplifying assumptions. Non-ideal behaviour is adequately accounted for by means of a single, lumped dispersion term in the differential equation but only two sources of non-linear behaviour, *viz.* curvature of the distribution isotherm and the sorption effect (variation in velocity of the moving phase where the solute is being sorbed or desorbed<sup>15</sup>), are taken into account. The effects<sup>15</sup> of gas compressibility, temperature changes and viscosity variations are excluded but it was argued that the peak profile should still be described satisfactorily by the model provided one theoretical parameter in the model was allowed to take on empirical values and provided also that the inlet band width is negligible and the column contains a large number of theoretical plates ( $N > 2000$ ). These last two conditions, on inlet width and plate number, are also prerequisites for the description of the basic non-linear and non-ideal behaviour to be valid and so underlie the procedure of eqns. 6-8 under all circumstances. They are both rather restrictive conditions in practice. When they are satisfied, however, the model predicts that both the procedure of eqns. 6-8 and Pollard and Hardy's method should yield the true infinite dilution retention  $t_R$ .

A further restriction on the model is that it is not accurate in any temperature range where the contributions to non-linearity from isotherm curvature and the sorption effect are close to cancelling each other (see below). If cancellation occurs at a temperature  $T^*$ , the excluded range is from *ca.* 20°C below  $T^*$  to *ca.* 40°C above<sup>13</sup>. However, many measurements of retention are conducted in this region.

## EXPERIMENTAL

To evaluate the five methods of estimating  $t_R$  we have made an experimental comparison of the estimates derived from asymmetrical peaks with the measured retention at infinite dilution. The comparison was conducted on four gas-liquid chromatographic (GLC) systems to study the effect of skew direction and magnitude.

In GLC the sorption effect and isotherm curvature are opposed in skewing direction, tending to cause, respectively,  $\eta < 1$  and  $\eta > 1$ . At a temperature  $T^*$ , which is usually about, or a little above, the boiling point of the solute, the two effects cancel and  $\eta = 1$  in an ideal column; at lower temperatures  $\eta > 1$  whereas, at higher

temperatures,  $\eta < 1$  (refs. 1 and 16). In a column containing only a few hundred plates, non-ideal behaviour provides a further skewing influence towards  $\eta < 1$ , and the temperature at which  $\eta = 1$  is lower. To obtain the two skew directions, therefore, two solute-solvent systems (1 and 2 in Table I) were run *ca.* 40°C below the solute boiling point and two (3 and 4) *ca.* 20°C above. (System 4 is an example of a case where the effect of a low temperature in causing  $\eta > 1$  is reinforced by an unusually low activity coefficient of about 0.38<sup>17</sup>.) These particular temperatures were chosen to allow convenient study of peaks of varying asymmetry, while still permitting infinite dilution to be achieved, for comparison purposes, with an acceptable signal-to-noise ratio on a katharometer detector. For each of these solute-liquid-support systems chosen, retention is expected to arise solely from partitioning in the bulk and not from any adsorption effects.

Two chromatographs were used and one was run by several operators to provide a check on the reproducibility of the results. The chromatographs were based, respectively, on a Pye Unicam Series 204 and a Phase-Sep LC2 equipped with twin-channel thermal conductivity detectors. With both instruments the flow-paths were rearranged to place the column between the two detector channels so that the shape and width of the peak could be monitored at the column inlet as well as the outlet. Claimed recorder response times were less than 0.6 sec. The columns and operating conditions are listed in Table I. All standard deviations quoted in this paper refer to the quantity  $[\Sigma(x - \bar{x})^2/(n - 1)]^{1/2}$  and not the (smaller) standard error of the mean. Solutes and stationary phases were supplied by B.D.H. (Poole, Great Britain) and were of AnalaR grade or "for gas chromatography".

A simple, highly reproducible, origin of measurements was obtained by measuring all retentions from the beginning of the peak produced by the first detector channel situated between the injector and column. The fact that this procedure overestimated the retention by a constant 1 and 2% was of no account in comparison with the need for accurate, reproducible measurement of relative retentions. Retentions were usually expressed in their primitive form of distance measured on the chart. Where necessary, corrections were applied for small fluctuations in temperature<sup>18</sup> and flow-rate.

## RESULTS

The ratio of peak widths at outlet and inlet of the column is shown in Table I. The adequately large values of this ratio, coupled with the rapid response of the detection system and care taken to avoid excessive dead spaces in tubing and connectors, indicate that the shape of the eluted peak was determined almost entirely by column processes (non-ideality and non-linearity) rather than by apparatus effects<sup>1</sup>.

Chromatograms were obtained over a range of sample size from well within the infinite dilution range up to skew ratios of approximately 1/5 or 5/1. Illustrative chromatograms are shown in Fig. 2.

The maximum sample size for infinite dilution was determined as that below which no significant concentration dependence was observed in retention or skew ratio. The true retention at infinite dilution,  $t_R$ , required high precision of measurement as the reference value against which predictions were judged. It was determined as the mean of replicate infinite dilution runs carried out at regular intervals during the course of the higher concentration runs. This procedure also provided a check on the stability of the operating conditions. The Conder and Young (CY) method was used for the infinite dilution retention since it is theoretically justified in this case<sup>1,2,6</sup>.

TABLE I  
SOLUTE-SOLVENT SYSTEMS AND OPERATING CONDITIONS

	System: 1			4
	$\eta > 1$	$\eta > 1$	$\eta < 1$	
Direction of non-linear skewing	$\eta > 1$	$\eta > 1$	$\eta < 1$	$\eta < 1$
Solute (b.p.)	Benzene (80.1°C)	Ethyl acetate (77.6°C)	Benzene (80.1°C)	Chloroform (61.7°C)
Stationary liquid	Polyethylene glycol 20M	Di- <i>n</i> -nonyl phthalate	Di- <i>n</i> -nonyl phthalate	Di- <i>n</i> -nonyl phthalate
Support	Phase-Sep P	Silanised Chromosorb G*	Silanised Chromosorb G	Silanised Chromosorb G
Mesh size	60-85	60-85	60-85	60-85
Liquid loading	21.0%	19.5%	19.5%	19.5%
Mass of stationary liquid, g	5	8.85	8.85	8.85
Column dimensions	1.8 m $\times$ 4 mm I.D.	1.5 m $\times$ 4 mm I.D.	1.5 m $\times$ 4 mm I.D.	1.5 m $\times$ 4 mm I.D.
Column temperature (°C)	37.6 $\pm$ 0.03	38.05 $\pm$ 0.01	102.0 $\pm$ 0.3	79.0 $\pm$ 0.05
Carrier flow-rate (cm <sup>3</sup> /min)	71.0 $\pm$ 0.3	36.3 $\pm$ 0.2	47.3 $\pm$ 0.05	54.6 $\pm$ 0.2
Number of theoretical plates	422	582	681	481
Sample size range ( $\mu$ l)	0.06-35 (liq.)	0.1-18 (liq.)	0.01-18 (liq.)	0.01-8 (liq.)
Ratio of peak base widths at column outlet and inlet at infinite dilution, ( $w_o/w_i$ )	11.5	7.3	5.0	8.9
Carrier gas	Nitrogen	Hydrogen	Hydrogen	Nitrogen
Chromatograph	Pye 204	Pye 204	Phase-Sep LC2	Pye 204

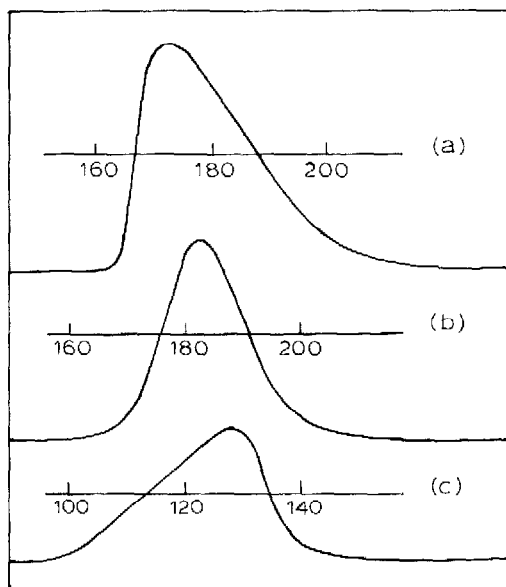


Fig. 2. Illustrative chromatograms. (a) System 3,  $\eta = 0.20$ . (b) System 3,  $\eta = 0.73$ . (c) System 1,  $\eta = 3.4$ . Scales show time (chart cm) since injected peak passed column inlet.

The true infinite dilution data are given in Table II. The limiting skew ratios of 0.7–0.8 are typical of columns containing a few hundred theoretical plates. As expected, this limiting skew ratio does not depend on the direction of skewing at higher solute concentrations.

The estimates of  $t_R$  derived from each of the five predictive methods were plotted against peak height. Examples of the plots are shown in Fig. 3. Scales of approximate skew ratio  $\eta$  and sample size are also shown. Over the range of concentrations used the peaks become progressively more asymmetrical with increasing solute concentration. For peaks where  $\eta$  is initially less than unity and then greater than unity, the skewing first decreases and then increases.

The predictive methods under test are intended for use when infinite dilution cannot be attained. To minimise the extent of prediction required the samples used should nevertheless be as small as practicable. Implicitly, therefore, the PM, LPP, CY and HL methods use a single peak of the minimum feasible sample size. Pollard and Hardy's method involves a series of samples from this size upwards. To compare the methods retentions have been read off the smoothed plots for two arbitrarily chosen sample sizes simulating two different values of the minimum feasible sample size for each system. These retentions are the predicted values of the infinite dilution retention, and are compared with the measured true values in Table III. No prediction was

TABLE II  
INFINITE DILUTION DATA

	System: 1	2	3	4
Standard deviation $t_R$ (%)	$\pm 2.2$	$\pm 0.6$	$\pm 0.4$	$\pm 0.5$
$\eta$	$0.69 \pm 0.08$	$0.80 \pm 0.11$	$0.78 \pm 0.04$	$0.73 \pm 0.12$
No. or runs measured	13	13	9	6
Sample size ( $\mu$ l)	$0.06 \pm 0.7$ (liq.)	0.1–1.0 (liq.)	0.01–0.2 (liq.)	0.01–0.06 (liq.)

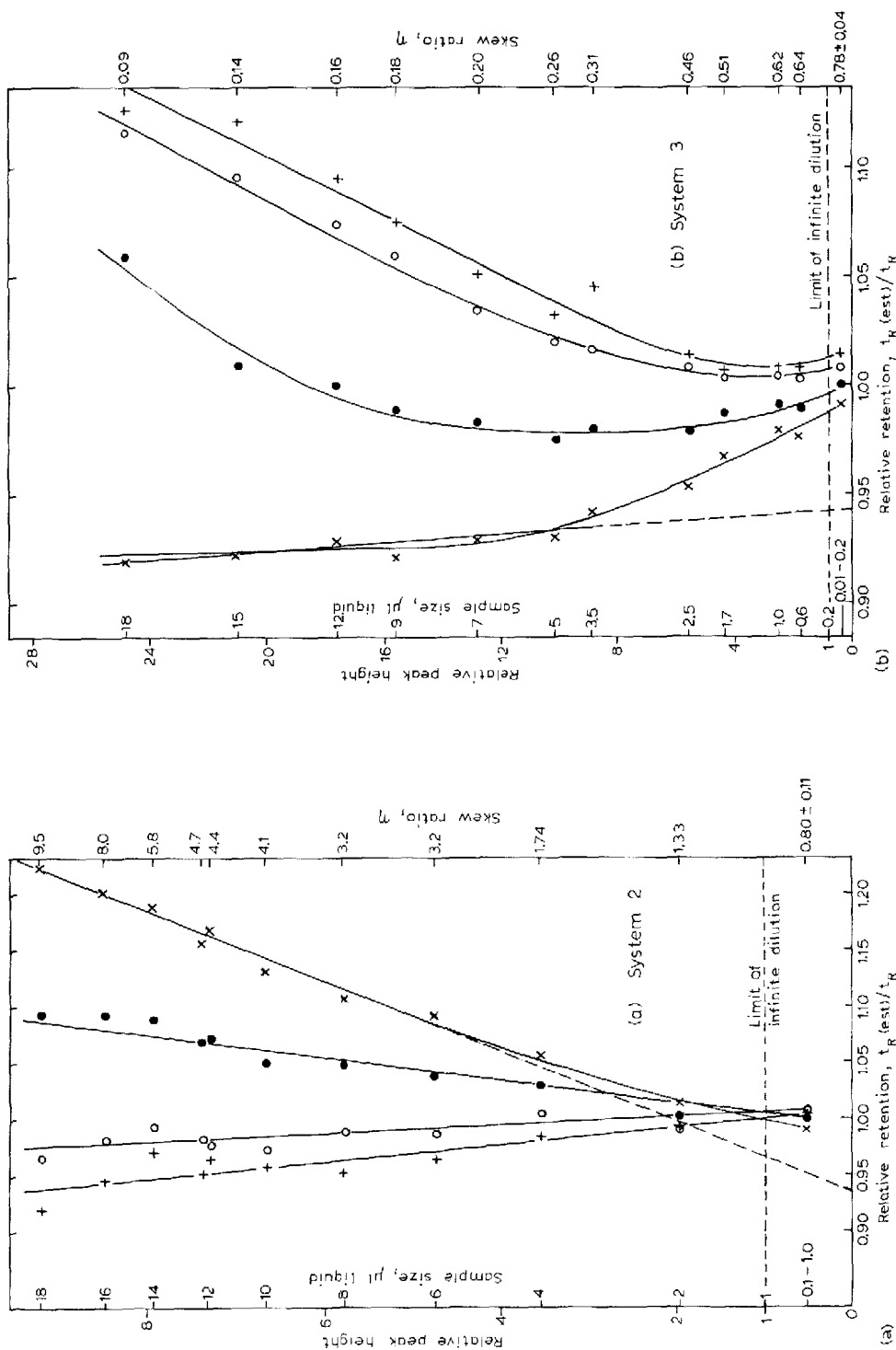


Fig. 3. Plots of ratio of retention estimate,  $t_R(\text{est})$ , to true infinite dilution retention,  $t_R$ , against relative peak height, for each method of estimating infinite dilution retention: x = PM; O = LPP; ● = CY; + = HL. Scales of sample size and skew ratio are also shown. (a) System 2; ethyl acetate + DNP. (b) System 3; Benzene + DNP.



available for Pollard and Hardy's (PH) method at the higher of the two sample sizes because this was always chosen close to the highest size used. For this method a straight line was drawn through the maxima of peaks from the lower assumed minimum sample size upwards. Extrapolation to zero peak height (Fig. 3) yielded the PH prediction of  $t_R$  corresponding to this minimum sample size.

## DISCUSSION

The data in Table III for the peak maximum method (PM) show that measured retentions can be grossly in error, even for peaks of relatively small asymmetry, if no attempt is made to correct for asymmetry. This is particularly so for peaks exhibiting fronting ( $\eta > 1$ ).

The data further show that, generally speaking, whichever method is used, the error is much greater for fronting peaks of a given skew ratio  $\eta (> 1)$  than for tailing peaks of the reciprocal skew ratio  $1/\eta$ . The reason is probably that the linear, non-ideal skew ratio,  $\eta = 0.7-0.8$ , is less than unity. A greater degree of non-linearity is therefore required to raise the ratio to, say, 4/1 than to reduce it to 1/4.

The differences reported here between the various measures of retention in non-linear chromatography are far larger, by some one or two orders of magnitude, than those encountered<sup>19</sup> with a linear isotherm, *i.e.* at infinite dilution.

Of the various methods, the peak maximum (PM) and Pollard and Hardy's (PH) method are the least satisfactory for all three systems studied. This is as expected. The PM method makes no allowance for asymmetry. The PH method entails some loss of accuracy and questionable assumptions about isotherm behaviour in going to higher concentrations in order to be able to extrapolate back down to zero concentration. The failure of the linear extrapolation assumption is very obvious in Fig. 3. The extrapolation procedure also magnifies any imprecision in retention measurement, particularly when the retention of the peak maximum varies strongly with sample size. This was confirmed by the poor reproducibility of the PH retention values observed for system 1 when the equipment and operator were changed.

Choice between the other three methods is less clear cut. For the two systems with  $\eta < 1$ , the CY method gives the most stable estimate, *i.e.* that least dependent on skew ratio; the accuracy of the HL and LPP estimates worsens with increasing asymmetry. The position is reversed for the two systems of  $\eta > 1$ . Here the CY method is the least stable of the three, and there is little to choose between HL and LPP. Change of equipment and operator for system 1 gave similar results (not shown), but with LPP marginally better than HL.

We deduce that the LPP and CY methods are the best for systems of  $\eta < 1$ , the CY method being the better of the two when the skew is strong ( $< 0.3$ ). For  $\eta > 1$ , the LPP method is the best overall.

Although the LPP method was originally devised for systems of  $\eta > 1$ , and HL for systems of  $\eta < 1$ , the performance of both methods (in the extended forms defined here) is little affected by whether the skew ratio is greater or less than unity. The HL method is based on a model strictly valid only for columns containing a large number of plates ( $N > 2000$ ). It remains a possibility, though we have not tested it, that the HL method would perform better under this condition.

## CONCLUSIONS

When peak asymmetry is due to a non-linear partition isotherm, gross errors can arise if retention is measured to the peak maximum, particularly when the peaks

TABLE III

## COMPARISON OF METHODS FOR ARBITRARILY CHOSEN DEGREES OF PEAK ASYMMETRY

Tabulated values are percentage deviation of retention estimate  $t_R$  (est) from true infinite dilution retention,  $t_R$ , i.e.  $100 [t_R(\text{est}) - t_R]/t_R$ . PEG = polyethylene glycol; DNP = di-*n*-nonyl phthalate.

Method of measuring retention	1: Benzene + PEG		2: Ethyl acetate + DNP		3: Benzene + DNP		4: Chloroform + DNP	
	$\eta = 2.3$	$\eta = 4.4$	$\eta = 3.2$	$\eta = 9.5$	$\eta = 0.31$	$\eta = 0.14$	$\eta = 0.48$	$\eta = 0.29$
Peak maximum (PM)	+13	+32	+9	+22	-6	-8	-4	-6
PH	-15		-6		-6		-3	
HL	-1	-2	-3	-6	+3	+11	+1	+5
LPP	+3	+6	-1	-3	+2	+9	+1	+3
CY	+8	+19	+4	+9	-2	+2	-2	-2

have a fronting skew ( $\eta > 1$ ). The error has been found to be in the range 4–40%, depending on the skew ratio. Four other methods have been investigated for predicting the true infinite dilution retention. Of these the LPP method is the best overall for skew ratios in the approximate range 0.2–5, with an accuracy in the region of 0–5%. The CY method is at least as good for  $\eta < 1$  and the HL method is nearly as good for  $\eta > 1$ . The PH method, hitherto probably the most often used, is not recommended. These findings apply to typical circumstances where the columns contain a few hundred plates (Table I).

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