

CHROM. 17,397

USE OF RETENTION PLOTS OF *n*-ALKYL BENZENES FOR DETERMINING DEAD TIMES IN LIQUID AND GAS CHROMATOGRAPHY

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(Received October 29th, 1984)

SUMMARY

The retention behaviour of the homologous series of *n*-alkylbenzenes from toluene to hexylbenzene has been examined in reversed-phase liquid chromatography (RPLC) and in gas chromatography (GC) using columns of varying polarity. The series was found to be inappropriate for the mathematical estimation of dead time in GC. The series ethylbenzene to hexylbenzene provided a reasonable estimate of system hold-up volume in RPLC when compared with the retention volume for uracil. However, when toluene was included in the estimation procedure erroneous results were obtained and it is therefore suggested that the use of the alkylbenzene series is unwise in dead time determination in both chromatography systems.

INTRODUCTION

Many studies of practical and theoretical significance¹⁻¹⁴ have employed various means of determining the system hold-up volume, V_0 , which is the value of the eluent volume contained between the injector and the detector cell. Methods reported include: (i) the measurement of the retention volume of radioactively labelled molecules of the eluent itself; (ii) the injection of a volume of eluent, the composition of which is slightly different from that of the actual mobile phase; (iii) the injection of pure water into aqueous mixtures; (iv) the injection of a homologue of lower carbon number than that of the eluent; and (v) the calculation of V_0 from consideration of the column packing density, solvent viscosity and pressure drop.

Many of the methods have been compared in a recent study¹. Most of these procedures have been applied to chromatographic systems equipped with refractive index detectors and are not appropriate if sample components are to be detected with a light absorbance detector. In such circumstances, a non-retained compound capable of detection needs to be injected in order to measure the column hold-up volume, a combination that is not always feasible.

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Several workers have noted the linearity of the semi-logarithmic plots of retention volume *versus* carbon number for members of homologous series in high-performance liquid chromatography (HPLC)^{4,12}. A similar phenomenon of linearity has been used to advantage in gas chromatography (GC); several mathematical procedures for the evaluation of the column dead time have been developed¹⁵⁻²¹ and reviewed²². This procedure has also been applied to the determination of column hold-up volumes in reversed phase (RP) LC^{1,23}.

In this study, we were interested in whether or not the mathematical procedure of Grobler and Balizs¹⁵ as adopted by this laboratory for GC studies^{18,19,24} is applicable to HPLC systems equipped with an ultraviolet absorbance detector. It was also important to determine if the carbon number of the lowest homologue employed affected the estimate of V_0 , as is indeed the case in gas chromatography and as has been implied for RPLC⁶.

CALCULATIONS

Linearity of $\ln(\text{retention time})$ *versus* carbon number plots for members of a homologous series can be represented mathematically by the equation

$$\ln(t_r - t_d) = bZ + c \quad (1)$$

where t_d is the column hold-up time, t_r is the absolute retention time of the homologue of carbon number Z and b and c are the slope and intercept, respectively¹⁸.

The method of Grobler and Balizs¹⁵ requires the use of a series of at least four adjacent homologues (n -alkanes in GC studies^{15,18,19,23}). The slope, intercept and mathematical estimate, t_m , of the column hold-up time, t_d , are determined from the following equations¹⁸:

$$b = \frac{(n-1) \sum_{i=Z_1}^{Z_{n-1}} Z_i \log[t_{r(i+1)} - t_{r(i)}] - \sum_{i=Z_1}^{Z_{n-1}} Z_i \sum_{i=Z_1}^{Z_{n-1}} \log[t_{r(i+1)} - t_{r(i)}]}{(n-1) \sum_{i=Z_1}^{Z_{n-1}} Z_i^2 - \left(\sum_{i=Z_1}^{Z_{n-1}} Z_i \right)^2} \quad (2)$$

$$t_d = \frac{\sum_{i=Z_1}^{Z_n} q^{Z_i} \sum_{i=Z_1}^{Z_n} t_{r(i)} q^{Z_i} - \sum_{i=Z_1}^{Z_n} q^{2Z_i} \sum_{i=Z_1}^{Z_n} t_{r(i)}}{\left(\sum_{i=Z_1}^{Z_n} q^{Z_i} \right)^2 - n \sum_{i=Z_1}^{Z_n} q^{2Z_i}} \quad (3)$$

$$c = \frac{\sum_{i=Z_1}^{Z_n} \log t'_{r(i)} - b \sum_{i=Z_1}^{Z_n} Z_i}{n} \quad (4)$$

where $t_{r(i)}$ is the absolute retention time of the i th homologue, $t'_{r(i)}$ is the net retention time of the i th homologue of carbon number Z_i , q is antilog b and n is the number

of homologues used. The value of the column hold-up volume, V_0 , is calculated from the equation

$$V_0 = t_d F \quad (5)$$

where F is the eluent volumetric flow-rate.

In order to facilitate the calculation of b , c and t_d , a program was written for use on a Texas Instruments TI 59 programmable calculator.

EXPERIMENTAL

The HPLC apparatus was a Waters Assoc. liquid chromatograph equipped with a μ Bondapak C_{18} 30 cm \times 4.6 mm I.D. analytical column, a differential refractometer and an ultraviolet absorbance detector. The absolute retention time of each peak was determined to the nearest 0.001 min by means of a Hewlett-Packard 3390A reporting integrator. In order to verify that the mathematical procedure is applicable to specific detectors, we injected 1.6 mm³ volumes of a solution containing 0.4% (v/v) each of benzene, toluene, ethylbenzene, *n*-propylbenzene and *n*butylbenzene dissolved in a mobile phase consisting of methanol-water (80:20). The effluent was monitored by both a Waters Assoc. Model 440 ultraviolet absorbance detector ($\lambda = 254$ nm) and an R401 differential refractometer. Each detector was coupled to a Hewlett-Packard Model 3390A reporting integrator. These integrators were modified to permit simultaneous starting on the injection of a sample. The eluent flow-rate was measured by the time taken to fill a 25.0-cm³ volumetric flask.

Gas chromatography was carried out using a Hewlett-Packard 5750 research chromatograph interfaced to a 16K PDP 11/40 digital computer. Interfacing was achieved by the use of an LPS 11 Laboratory Peripheral System comprising a 12-bit analog-to-digital converter, a programmable real-time clock with two Schmitt triggers and a display controller with two 12-bit digital-to-analog converters. All on-line programming was written in CAPS II Basic with LPS options. The columns were each 12 ft. \times 1/4 in. O.D. packed with 10% of the four stationary phases used, *i.e.*, SE-30, OV-7, SILAR 5CP and SILAR 9C, on Chromosorb W AW DMCS (80-100 mesh) operated isothermally at 120°C.

RESULTS AND DISCUSSION

It has been reported elsewhere^{1,2,3} that retention data for homologous series of compounds, such as *n*-alkanols in the case of refractive index detectors and *n*-alkylbenzenes in the case of UV detectors, can be treated mathematically to calculate column hold-up in HPLC. In principle, this procedure obviates the classical difficulty in the determination of the column hold-up volume of predicting whether or not a particular compound elutes with the solvent front. Although there is apparently little advantage in the use of this procedure when monitoring the eluent by means of a refractive index detector, it is useful when other so-called specific detectors are employed; one simply injects a solution containing at least four appropriate consecutive homologues. Such data for the retention volumes of *n*-alkylbenzenes measured by ultraviolet and refractive index detectors are presented in Table I.

TABLE I

RETENTION VOLUMES AND CALCULATED COLUMN HOLD-UP VOLUMES FOR *n*-ALKYL-BENZENES

Conditions: methanol-water (80:20) at 2.0 ml/min nominal flow-rate, μ Bondapak C₁₈ column, RP-8 guard column, UV at 254 nm, RI at $\times 4$.

Sample component	Average retention volume (cm ³)		$\Delta V(RI - UV)$
	UV	RI	
Benzene	4.434	4.709	0.275
Toluene	5.033	5.306	0.273
Ethylbenzene	5.709	5.971	0.262
Propylbenzene	6.863	7.126	0.263
Butylbenzene	8.597	8.871	0.274
V_0 (math) (cm ³)	3.23 \pm 0.01	3.5 \pm 0.1	Av. = 0.269 \pm 0.006

The fact that benzene has no alkyl residue ($Z_i = 0$) makes it unique among compounds normally used to estimate dead times. Therefore, it raises the question of whether it can properly be employed as a member of the homologous series of *n*-alkylbenzenes in the calculation of the mathematical estimate of the column hold-up volume. Recalculation of this value after the exclusion of benzene revealed a significant increase in the estimate of V_0 (math), implying that some compound(s) departed from logarithmic retention behaviour. In order to investigate the dependence of V_0 (math) on the homologues used, an extended series of *n*-alkylbenzene hydrocarbons was injected into the liquid chromatograph. A 0.1 wt.-% solution of uracil was also injected, as this compound is believed to be unretained by a chromatographic system consisting of aqueous methanol eluents and a C₁₈ column²⁵. The results of this study are presented in Table II, where the calculated column hold-up volume is shown as a function of the compounds used in its determination.

The results in Table II show that the omission of benzene from the series increases the value of V_0 by 14% above the value obtained for all seven benzenes, thus confirming the non-linearity of the retention data. The results show that the mathematical estimate depends on the carbon number of the lowest homologue employed and also the number of members of the series used. It was found that five *n*-alkyl benzenes (C₂-C₆) yield an estimate that is identical with that obtained from uracil. However, the discrepancy between the C₃-C₆ estimate and uracil suggests that the *n*-alkylbenzene homologues may not be suitable for use in this mathematical procedure.

Also presented in Table II are the values of \hat{b} and b . These correspond to the slopes of the logarithmic retention plot using eqns. 1 and 2, respectively. The values of \hat{b} were calculated using the retention volumes corrected with the retention volume for uracil, whereas the b value only employs the raw retention data. The values of b show significant non-linearity of the logarithmic retention plot whereas correction of the data using uracil considerably improves the linearity. The nearly constant value of 0.41 for the values of the slope of the line corrected for uracil retention shows that, with the exception of benzene and toluene, the *n*-alkylbenzenes follow

TABLE II
AVERAGE RETENTION VOLUMES AND CALCULATED COLUMN HOLD-UP VOLUMES
FOR BENZENE AND SIX *n*-ALKYLBENZENES

Conditions as in Table I.

Sample component	Retention volume (cm ³)
Benzene	4.52 ± 0.04
Toluene	5.19 ± 0.04
Ethylbenzene	5.94 ± 0.06
<i>n</i> -Propylbenzene	7.30 ± 0.08
<i>n</i> -Butylbenzene	9.3 ± 0.1
<i>n</i> -Pentylbenzene	12.4 ± 0.2
<i>n</i> -Hexylbenzene	17.1 ± 0.2
Uracil	3.27 ± 0.01

Parameter	Alkyl group				
	C ₀ -C ₆	C ₁ -C ₆	C ₂ -C ₅	C ₂ -C ₆	C ₃ -C ₆
V ₀	3.33	3.81	3.13	3.26	3.37
σ(V ₀)	0.02	0.04	0.02	0.02	0.06
<i>b</i>	0.411	0.449	0.412	0.416	0.427
<i>δ</i>	0.398	0.399	0.409	0.411	0.412

TABLE III

MATHEMATICAL DEAD TIMES AND SLOPES OF LOGARITHMIC RETENTION PLOTS CALCULATED FROM RETENTION DATA FOR *n*-ALKYLBENZENES

Parameter	Retention time (min)			
	SE-30 column	OV-7 column	SILAR 5CP column	SILAR 9CP column
<i>t_d</i> (<i>n</i> -alkanes)	1.43 (C ₆ -C ₁₀)	1.22 (C ₆ -C ₁₀)	1.48 (C ₈ -C ₁₁)	1.57 (C ₈ -C ₁₁)
<i>t_d</i> (C ₀ -C ₆)	1.27	1.02	1.35	1.50
<i>t_d</i> (C ₁ -C ₆)	1.63	1.38	1.60	1.94
<i>t_d</i> (C ₂ -C ₆)	1.90	1.76	2.06	2.47
<i>t_d</i> (C ₃ -C ₆)	1.27	0.94	0.93	0.82
<i>t_d</i> (C ₄ -C ₆)	1.96	1.44	1.15	1.36
<i>b</i> (C ₀ -C ₆)	0.577	0.585	0.479	0.405
<i>b</i> (C ₁ -C ₆)	0.578	0.602	0.498	0.432
<i>b</i> (C ₂ -C ₆)	0.589	0.619	0.528	0.462
<i>b</i> (C ₃ -C ₆)	0.568	0.592	0.470	0.391
<i>b</i> (C ₄ -C ₆)	0.586	0.604	0.478	0.408
<i>δ</i> (C ₀ -C ₆)	0.564	0.592	0.487	0.405
<i>δ</i> (C ₁ -C ₆)	0.561	0.588	0.483	0.403
<i>δ</i> (C ₂ -C ₆)	0.566	0.593	0.486	0.409
<i>δ</i> (C ₃ -C ₆)	0.574	0.602	0.499	0.423
<i>δ</i> (C ₄ -C ₆)	0.572	0.598	0.492	0.415

the logarithmic rule very closely. However, the fact that the estimate of V_0 obtained from the C_3 - C_6 homologues increases by approximately 3% above that obtained from the C_2 - C_6 compounds suggests that the semilogarithmic behaviour of these compounds is not sufficiently linear for the application of this technique.

We then decided to investigate the suitability of the *n*-alkylbenzenes for the determination of the dead time of a GC system equipped with columns of varying polar character. The results thus obtained were compared with the estimate of dead time derived from chromatographing a series of *n*-alkanes. The results, which are summarized in Table III, show considerable differences between the retention times calculated for the two types of homologous series. These differences are as large as *ca.* $\pm 50\%$ of the value obtained using the *n*-alkanes. This shows that the non-linearity is significantly greater than in liquid chromatography. The degree of non-linearity is not apparent from the values of *b* and \hat{b} in Table III. In fact, when logarithms of the corrected retention times are plotted against carbon number, the resulting plots appear to be reasonably linear for all the alkylbenzenes, with only slight departures from the linear relationship for benzene, toluene and ethylbenzene. Clearly, then, the *n*-alkylbenzenes are very poor estimators of the dead time of a GC system. Further, the mathematical dead time is seen to give a far more sensitive estimate of linearity of the logarithmic retention plot for a homologous series than does the slope of the line, *b*.

CONCLUSIONS

The series of *n*-alkylbenzene homologues was found to be an inappropriate choice for GC dead time studies; a possible reason for this observation may lie in the structure of these compounds, with an aromatic nucleus appended to relatively short alkane chains. The interaction between the aromatic nucleus and the stationary phase may restrict the effect of additional methylene groups. If steric effects vary the retention behaviour of these compounds in GC, perhaps their use in RPLC is also unwise.

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