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PRECISION OF CONTEMPORARY LIQUID CHROMATOGRAPHIC MEASUREMENTS

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SUMMARY

The precision of chromatographic measurements including retention time, peak width, peak height, and peak area determinations made manually and by computer acquisition are compared. The standard deviations of the data are given together with some of their derivative properties, such as column efficiency, peak asymmetry, sample analysis by peak height and by peak area. The minimum difference in temperature and in solvent composition necessary to attain a given precision are determined, and the effect of mass of solute on retention times and column efficiencies examined. The effect of computer data acquisition frequency, data point averaging, threshold signal level and noise filtering on the precision of chromatographic measurement is discussed, and an optimal system for the computer liquid chromatograph concerned is suggested. The optimized system is used to demonstrate that retention times can be measured with a standard deviation of the difference between duplicate samples, determined on the same day or on different days, at a level of about 0.1% or less.

INTRODUCTION

The technique of liquid chromatography (LC) has developed rapidly over the past decade and is now being used, not only as a separation technique, but also for qualitative and quantitative analysis. It follows that the precision with which chromatographic measurements can be made has become exceedingly important. In the production of chromatographic data, there are two areas that can affect the precision of the measurements, namely, the method of measurement itself, and the conditions under which the chromatographic analysis is carried out. In this paper, both these aspects are examined, and the level of precision that can be expected from the methods and equipment presently available is determined. The work described is in three parts, firstly the relative precision of data measured manually and by computer data acquisition are compared, secondly the operating conditions that affect the precision of measured data are identified and specifications given to provide a required precision; finally, the precision attainable under the controlled specified conditions is determined.

THE COMPARISON OF THE PRECISION OF LIQUID CHROMATOGRAPHIC MEASUREMENTS MADE MANUALLY AND BY COMPUTER DATA ACQUISITION

Experimental

The solvent, contained in a closed vessel, passed directly to a Waters Assoc. Model 6000A pump and then through a heat exchanger to a Valco high-pressure sample valve (sample volume, 1 μl). The heat exchanger consisted of a 6 ft. $\times$ 1/16 in. O.D. $\times$ 1 mm I.D., stainless-steel tubing situated in a thermostated water bath having a temperature control of $\pm$0.05 °C. The sampling valve was automatically operated by a pneumatic actuator and was connected directly to the column. The column was 25 cm $\times$ 1/4 in. O.D. $\times$ 4.6 mm I.D. and was packed with Silarex 1 silica gel having a particle diameter of 10 μm. The detector employed was a LDC/UV monitor operated at a wavelength of 254 nm. The eluent from the detector passed directly to a 50-ml Grade A burette, the top of which was connected to the solvent reservoir to eliminate evaporation losses. The output from the detector was fed both to a 10-mV potentiometric recorder and to an analog-to-digital (A/D) convertor. The A/D convertor accepted the total signal from the attenuator output network of the detector electronics and the digitized signal was fed directly to a time sharing computer. The unique software employed was developed by Computer Inquiry Systems who utilized a Hewlett-Packard HP1214A computer incorporating a HP1296A disc system.

Method

The mobile phase employed was a 44% (w/v) mixture of butylchloride in heptane and the composition (w/v) of the test mixture was as follows: solute 1, chlorobenzene 0.016%; solute 2, *p*-chlorophenetol 0.14%; solute 3, 2-ethoxynaphthalene 0.16%; solute 4, *o*-dinitrobenzene 0.15%.

Twelve replicate samples of the mixture, each 1 μl in volume, were placed on the column over a period of about 8 h and during this time, the bath temperature and the room temperature remained sensibly constant at 24.7 °C and 22.8 °C, respectively. The flow-rate was determined over the total period of each chromatogram by measuring the time elapsed for a volume of 15 ml of mobile phase to pass through the column. The time period was measured to the nearest 1 sec. At the instant of injection, the burette reading was taken, a mark was made on the recorder chart (chart speed 1 in./min) and computer data collection initiated. A burette reading was then taken at the maximum of each peak (visually identified) and the retention volume taken as the difference between the reading at the peak maximum and the burette reading at injection. When the recorder peak had returned to the base line after the last peak was eluted, data collection was halted and the data processed. An example of a chromatogram obtained from one of the replicate analyses is shown in Fig. 1.

Retention distances were measured manually on the recorder chart with a good quality plastic rule, all distances being estimated to the nearest 0.1 mm. The base line under each peak was constructed using a sharp pencil and the peak height taken as the distance between the constructed base line and the center of the recorder trace at the peak maximum. The peak width was taken as the distance between the inside edge of the recorder trace on the leading edge of the peak to the outside edge of the recorder trace on the trailing edge of the peak at 0.6065 of the peak height.

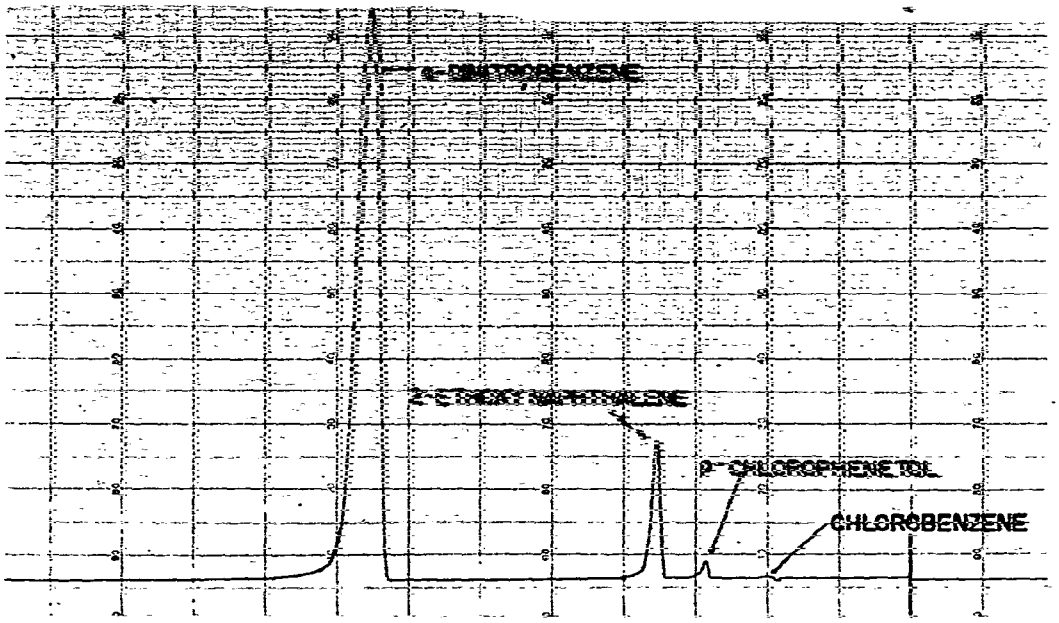

Fig. 1. Sample chromatogram of mixture used for replicate analyses. Solvent: 44.0% (w/v) butyl chloride in *n*-heptane.

The peak widths were measured by means of a 3× comparator and graticule which was calibrated in units of 0.1 mm. Peak widths were estimated to the nearest 0.1 mm. The peak area was taken as the product of the peak height and the peak width.

The computer data sampling rates available were 750, 375 and 187.5 samples per min. The rate employed in this work was 750 data sample points per min. Data sample points could be averaged over any chosen time period, but in this experiment, no averaging was employed and thus the computer received an average of 12.5 data points per sec for processing. The reading threshold was set at 15 μV and the peak was assumed to be sensed with the signal exceeded 125 μV. The peak maxima was sensed as the first recorded maximum signal. As each data point during the signal represented the signal value at that point, the peak area was taken as the integral of all the data points with respect to time by summing the successive data point readings over the period of the peak duration. Other more sophisticated data processing systems were available for unresolved peaks, peak shoulders, baseline drift, etc., but these were not pertinent to the separations obtained.

Results and discussion

Retention times and peak widths. The data obtained for the twelve samples is shown in Tables A1 and A2 in the Appendix and are summarized in Table I. The standard deviation (S.D.) for the two methods of measuring retention data are seen to be mutually commensurate, except possibly for the first peak which, from Fig. 2, is seen to be very small, and the position of its maximum difficult to visually determine. The peak widths, as determined by the computer, however, have a significantly lower S.D. than those measured manually and only at a k' value of about 5 do the

TABLE I

PRECISION OF RETENTION AND PEAK WIDTH MEASUREMENTS MADE MANUALLY AND BY COMPUTER FROM TWELVE REPLICATE SAMPLES

Parameter	*Peak*			
	1	*2*	*3*	*4*
k' Value	0.22	0.94	1.50	5.21
Retention data				
Mean (cm)	10.17	16.0	20.59	51.14
S.D. (% of mean, manual measurement)	0.85	0.24	0.19	0.15
Mean	3.97	6.27	8.11	20.14
S.D. (% of mean, computer measurement)	0.31	0.20	0.17	0.33
Peak width				
Mean		0.465	0.602	1.968
S.D. (% of mean, manual measurement)		5.30	1.84	1.16
Mean	0.247	0.177	0.236	0.774
S.D. (% of mean, computer measurement)	8.23	1.16	0.59	1.13

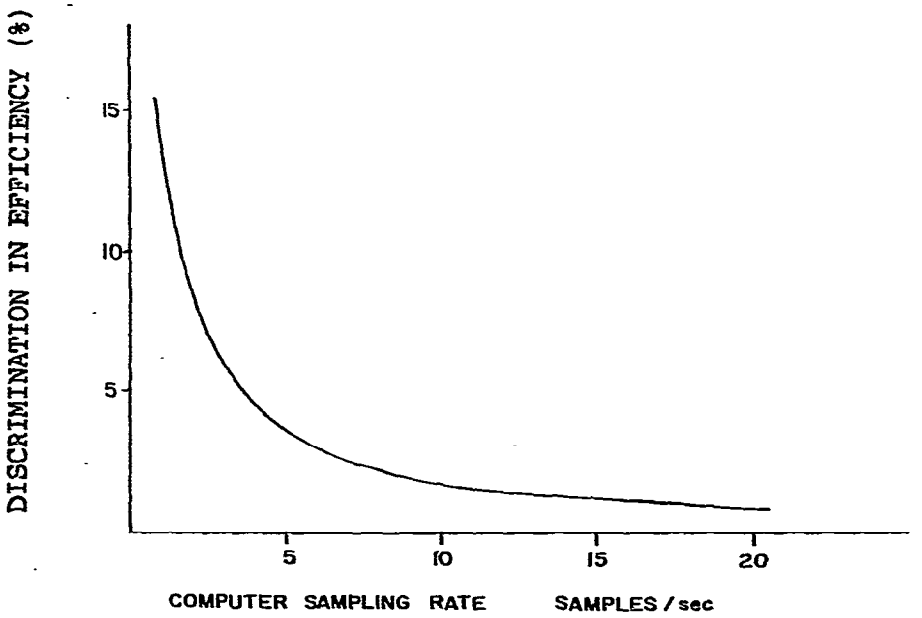

Fig. 2. Graph of minimum efficiency discrimination (%) against computer data sampling rate. Column, 25 cm × 4.6 mm I.D.; dead volume, 3 ml; flow-rate, 1 ml/min; k' of solute, 1.0; column efficiency, 5000 theoretical plates.

two methods give commensurate precision. It is also interesting to note that the computer could provide peak width data for the first peak, whereas it was impossible to measure the peak width manually.

Efficiency and retention volumes. The data determined for the twelve replicate samples is given in Tables A3 and A4 in the Appendix and are summarized in Table II. It is seen that the computer provides the more precise results for column efficiency

TABLE II

PRECISION OF EFFICIENCY AND RETENTION VOLUME MEASUREMENTS MADE MANUALLY AND BY COMPUTER FROM TWELVE REPLICATE SAMPLES

Parameter	*Peak*			
	1	*2*	*3*	*4*
k' Value	0.22	0.44	1.50	5.21
Efficiency				
Mean		4770	4688	2703
S.D. (% of mean, manual measurement)		10.8	3.6	2.5
Mean	1053	5029	4707	2704
S.D. (% of mean, computer measurement)	17.9	2.2	1.2	2.3
Retention volume				
Mean	3.86	6.09	7.86	19.45
S.D. (% of mean, manual measurement)	0.71	0.27	0.20	0.14
Mean	3.83	6.06	7.83	19.46
S.D. (% of mean, computer measurement)	0.28	0.21	0.19	0.37

as a result of the greater precision of measurement by the computer for peak widths. There appears little to choose between the standard deviations observed for retention volume measurement, except, perhaps, for the first minor peak, where, as already mentioned, the position of the peak maximum was difficult to determine. The small standard deviations realized for both retention measurements and retention volumes indicate that the Waters 6000A pump is an extremely precise instrument.

Peak height and peak area measurement. The data obtained for the twelve replicate samples is given in Tables A5 and A6 in the Appendix and are summarized in Table III. It is seen that, except for peaks eluted at a k' value of 5 or more, the computer gives more precise results than those from manual measurements. In general, the peak height data is more precise than the peak area data obtained either by computer data processing or by manual measurement. Considering the excellent precision

TABLE III

PRECISION OF PEAK HEIGHT AND PEAK AREA MEASUREMENTS MADE MANUALLY AND BY COMPUTER FROM TWELVE REPLICATE SAMPLES

Parameter	*Peak*			
	1	*2*	*3*	*4*
k' Value	0.22	0.94	1.50	5.21
Peak height data				
Mean (cm)		0.603	4.97	19.19
S.D. (% of mean, manual measurement)		3.48	1.12	1.19
Mean	1499	6966	53740	207700
S.D. (% of mean, computer measurement)	6.33	0.86	0.72	1.16
Peak area data				
Mean		0.281	2.99	37.75
S.D. (% of mean, manual measurement)		5.80	2.2	2.1
Mean	184000	626000	6320000	73600000
S.D. (% of mean, computer measurement)	10.6	1.35	0.45	2.1

achieved by the pump, this result is surprising and must indicate that precision of the sampling valve with respect to the initial sample band width may be rather poor. The precision of the sample volume injected by the valve, however, appears reasonable from the data given in the last column of Table A6 in the Appendix. An S.D. in sample volume of 2.0% is not unreasonable, and as the sample volume is of minor importance relative to the proportion of each solute that is in the sample, the variability of sample volume cannot account for the greater precision achieved by peak height analyses. Another explanation might be that, as a result of noise, the position of the start of the peak was ill defined and, thus, the area calculated would vary with the position and extent of such noise.

Analyses by normalization of computer data. The data obtained for the twelve replicates is given in Table A7 in the Appendix and are summarized in Table IV. It again seems that analysis by normalization of peak heights is, in general, more precise than analysis by peak area. It is seen that in a mixture containing component ratios of over 100, by peak height analysis, the component at a level of 0.56% can be determined at a precision of $\pm 0.04\%$ (actual) and, in the same mixture, a component present at a level of 76.95% can be determined at a precision of $\pm 0.27\%$ (actual).

TABLE IV

PRECISION FOR THE ANALYSES OF MIXTURE BY NORMALIZATION OF PEAK HEIGHTS AND PEAK AREAS MEASURED BY THE COMPUTER FROM TWELVE REPLICATE SAMPLES

Parameter	*Peak*			
	1	*2*	*3*	*4*
k' Value	0.22	0.94	1.50	5.21
Composition of mixture by normalization of peak heights				
Mean value (%)	0.556	2.582	19.916	76.947
S.D.	0.038	0.029	0.227	0.267
S.D. (% of the mean)	6.9	1.1	1.1	0.35
Composition of mixture by normalization of peak areas				
Mean value (%)	0.229	0.777	7.831	91.163
S.D.	0.027	0.021	0.153	0.188
S.D. (% of the mean)	11.7	2.64	1.95	0.21

FACTORS THAT CONTROL THE PRECISION OF LIQUID CHROMATOGRAPHIC MEASUREMENTS

The Computer

There are two general methods of data acquisition by a computer; in the first, the output from the detector is sampled, a limited amount of data is temporarily stored; the data is processed and the processed data in the form of retention times, efficiencies, peak areas, etc., is permanently stored; in the second method, the data is sampled and each data point in the chromatogram is permanently stored, the chromatographic information required is obtained by subsequently processing the stored

data. The former method is inflexible and, as the raw data is discarded, reprocessing by an alternative procedure is not possible, and a reconstructed chromatogram cannot be obtained. The second method is far more flexible, the data can be processed by any chosen method or by a number of different methods if required and as the raw chromatographic data is permanently available, a reconstructed chromatogram, in whole or in part, can be obtained on any chosen scale and presented on a cathode ray tube (CRT) screen or plotter. The stored data can also be statistically tested and any points representing outliers can be rejected. The computer system employed for the work described in this paper employed the second method of data acquisition.

There are two main factors in the acquisition of data that can affect the precision of the chromatographic results obtained; they are the data sampling rate and signal noise level. Minicomputers that are employed for chromatographic data acquisition have, in general, a maximum sampling rate of 240 samples per sec, which has to be shared between the number of stations involved in the time sharing system, and there will be a maximum sampling rate for any one station of 60 samples per sec. The limit of 240 samples per sec is imposed on the computer by the auto ranging amplifiers associated with the A/D convertor, that is necessary to utilize the complete linear dynamic range of the detector. If a disc storage system is used in conjunction with the computer, the time of data transfer to the disc will also limit the sampling rate of the computer. The 60 samples per sec limit for each station results from the fact that a sampling rate in excess of the 60-Hz frequency tends to permit mains induced signals into the cable which results in unacceptable noise. All sampling rates should be simple factors of 60 Hz to ensure induced noise is minimal. Thus, if there are 10 stations from which data is to be simultaneously acquired, at the maximum sampling rate, that rate for each station will be 24 data points per sec. The fact that a finite data acquisition rate exists causes a discrimination limit to be imposed on any chromatographic results obtained unless the data is processed from the disc using special software. Consider a peak having a time width of 10.3 sec sampled at a rate of 5 samples per sec, then a value of either 10.2 or 10.4 will be taken by the computer as the peak width. Thus, assuming the peak is eluted at a retention time of 360 sec,

$$N_1 = 4(360/102)^2 = 4983 \qquad \text{and} \qquad N_2 = 4(360/104)^2 = 4793$$

Thus, the sampling rate will permit a discrimination of 190 theoretical plates in 4983, equivalent to 3.0% and this precision of measurement, due to the sample acquisition rate being 5 samples per sec, cannot be improved. In Fig. 2, the maximum discrimination in efficiency, expressed as a percentage of the actual column efficiency, is plotted against data acquisition rate for a 25 cm × 4.6 mm I.D. column operated at a flow-rate of 1 ml/min and having a dead volume of 3 ml eluting a solute of $k' = 1$ with an efficiency of 5,000 theoretical plates. It is seen that, irrespective of the control over chromatographic conditions, a precision of 1% cannot be realized unless the data acquisition rate is greater than 20 samples per sec.

The noise level of the signal that is digitized can also significantly affect the overall precision of the chromatographic data obtained, and an example of this can be taken from the results previously discussed. In Table A1 in the Appendix, the first and last replicate values for the retention time of peak 4 have the greatest divergence.

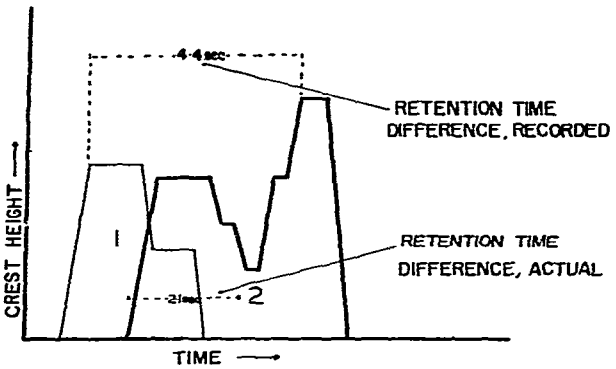

Fig. 3. Peak crests reconstructed by the computer (99.9–100% peak height).

The crests of these two peaks were taken from the computer data and expanded on a CRT screen and the resulting traces are shown in Fig. 3. It is seen that, although the differences in retention time between the two peaks is only 2.1 sec, as a result of a noise spike on the front of the crest of the peak from replicate one and the noise spike on the back of the peak crest from replicate 12, the measured difference in retention times is 4.4 sec. It follows that, to attain the highest precision, the noise has to be significantly reduced or, if possible, eliminated from the detector signal. There are several ways of reducing noise. The first and obvious method is to average a number of points and take the average value for data acquisition. This procedure is frequently used and is sometimes called the "slice" method of data acquisition. This method has the advantage of reducing noise without distorting the peak but at the same time may reduce the data acquisition rate which is also undesirable. Another method of reducing noise is to use an "on the fly" exponential smoothing procedure. This procedure is very effective, does not reduce the data acquisition rate, but tends to distort the peak. If all data points are stored on disc, very sophisticated smoothing procedures are possible, including the rejection of outliers, furthermore, this procedure in no way affects the rate of data acquisition. An alternate procedure which was employed in this work was to interpose a filter circuit between the detector output and the A/D convertor. This procedure again does not affect the rate of data acquisition, but, if not designed correctly, can produce peak dispersion and asymmetry.

The effect of a time constant is to combine two functions, an exponential function and a Gaussian function. As these two functions describe physical phenomena that are not interacting in the sense that they proceed independently of one another, the variance of the combined function is equivalent to the sum of the variances of each individual function. The time variances of an exponential function of the form

$$\exp(-t/T_1)$$

is T_1^2, and that of a Gaussian function of the form

$$\exp(-t^2/2T_t)$$

is T_t^2. It follows that

$$T_1^2 + T_t^2 = T^2$$

where T^2 is the variance of the resulting peak.

For a 25 cm × 4.6 mm I.D. column having an efficiency of 5000 theoretical plates and a dead volume of 3 ml operated at a flow-rate of 1 ml/min, the retention time of a solute eluted at a k' value of 1 will be 360 sec. Thus:

$$5000 = 4\left(\frac{360}{T_t}\right)^2 \text{ and } T_t = 10.18 \text{ sec}$$

It follows that, if 0.10% increase in band width is to be tolerated,

$$T_1^2 + (10.18)^2 = (10.19)^2$$

or $T_1 = 0.45$ sec. Thus, a time constant of 0.45 sec in the form of a passive filter was introduced between the detector output and the A/D convertor with a view to reducing the noise and increasing the precision of the chromatographic data produced. It should be pointed out that an active filter would be more effective than the passive filter employed as the active filter would have a much sharper frequency cut-off and, thus, provide more efficient noise rejection.

The liquid chromatograph and solvent system

Solvent composition. The composition of the solvent used as the mobile phase can have a profound effect on solute retention and is used as an operating variable to control the retention of the solutes in a given mixture. It follows that, if retention times are required to have a precision of 0.1% then the solvent composition must be maintained sufficiently constant to maintain the required precision. In Fig. 4, curves relating the corrected retention times of three solutes are plotted against solvent composition. In fact, it has been shown[1] that the reciprocal of the retention volume is linearly related to the solvent composition, but over a small solvent composition range V' can be taken to be linearly related to solvent composition and the points in Fig. 5 are force fitted to a linear function. The results are summarized in Table V. It is seen that, to achieve a precision of 0.1%, the solvent concentration must be maintained to within 0.02% (w/v). This level of constancy of solvent composition is fairly easy to maintain providing a closed solvent system is employed but it is extremely difficult, if not impossible, to make up a solution to this accuracy using volatile solvents. It is, therefore, recommended that large bulks of solvent are made up if precise results are required, and each new solvent checked by chromatographing a standard solute. Appropriate correction factors can then be calculated and employed where retention times are to be compared with previously used solvents.

The solvent pump. The Waters 6000A pump gave a flow of mobile phase over a period of about 12 h with a standard deviation of 0.07%. This performance was considered amazingly good, but such precision can only be maintained if the pump is operated with the necessary precautions. The majority of chromatographers (and it must be said that until recently, the authors of this paper are to be included) treat

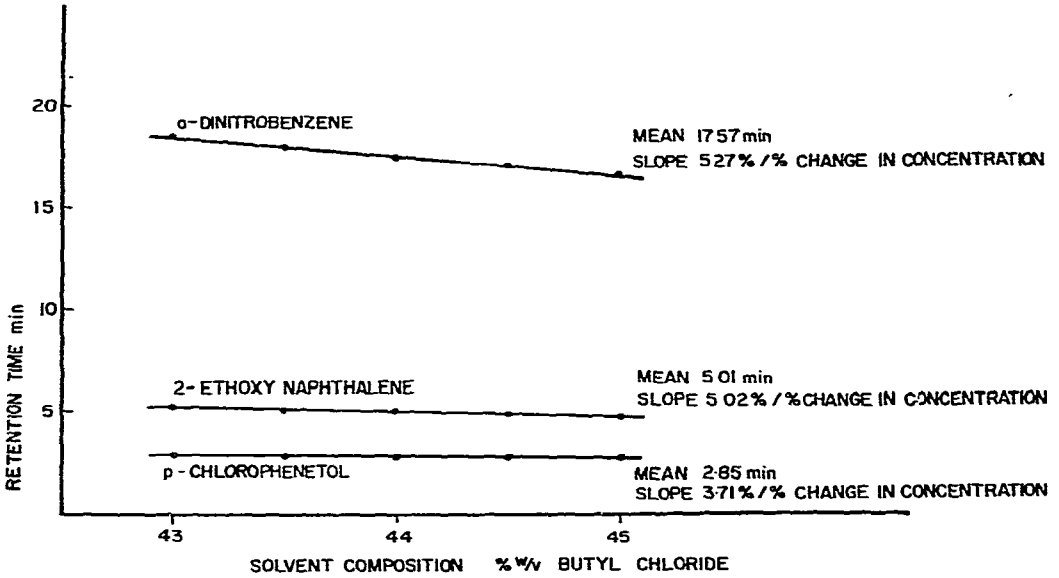

Fig. 4. Graphs of corrected retention time against solvent composition for three solutes.

TABLE V

SOLUTE CONCENTRATION TOLERANCES FOR RETENTION TIME PRECISION

Solute	*Retention time at 44% of butyl chloride in heptane*	*Tolerance for retention time precision*	
		Concn. tolerance for 1% precision (% w/v)	*Concn. tolerance for 0.1% precision (% w/v)*
p-Chlorophenetol	2.85	±0.14	±0.014
2-Ethoxynaphthalene	5.01	±0.10	±0.010
o-Dinitrobenzene	17.27	±0.10	±0.010

precision LC pumps as just another piece of pumping hardware, whereas they should be treated with the care and respect given to an analytical balance. It was found that to maintain the precision, the following procedures needed to be taken. The pump should never be allowed to run dry otherwise abrasion between piston and cylinder produces small leaks. Any mobile phase used should be filtered through a 0.2-μm Millipore filter particularly if the solvent had been dried over activated silica gel or alumina. The usual method of filtering by a filter paper was found to be inadequate. The filter contained in the pump should be regularly changed and the inlet tubes to the pump should have as large a bore as possible (2 mm I.D.) to prevent the pump being starved of solvent. The pump should never be subjected to a back pressure greater than 6,000 p.s.i., the rated maximum for the Waters Assoc. 6000A pump.

The mobile phase is brought to a constant and fixed temperature, and, thus, constant density, prior to entering the column and if the volume flow-rate through the column is to be maintained constant, then the pump must deliver a constant mass flow-rate to the column. As the pump is designed to provide a constant volume flow-

rate, then it must be supplied with solvent at a constant density and its displaced volume must also remain constant. If a precision of 0.10% is required, then the displaced volume of the pump and the solvent density must also be maintained constant to within this level of precision. It is almost impossible to thermostat the pump so the ambient temperature of the pump and solvent reservoir must be controlled and this means that the temperature of the room in which the apparatus is situated must be controlled. Most pumps are made of stainless steel which has a coefficient of cubical expansion of about $1.3 \cdot 10^{-5}$/°C (*i.e.*, 0.0013%/°C) and thus, the effect of ambient temperature changes on the pump volume will be negligible. The cubical expansion of solvents, however, is much higher and for heptane is $1.25 \cdot 10^{-3}$/°C (*i.e.*, 0.125%/°C). Thus, to maintain the solvent density of 0.1%, the ambient temperature must be maintained constant ±0.4 °C. This control of ambient temperature is not unreasonable in normal heat-controlled and air-conditioned laboratories, but has to be maintained if the required precision is to be achieved.

Column temperature. It is well known that the retention volume and retention time of a solute varies considerably with temperature and in Fig. 5, the retention volume of the three solutes determined over a narrow temperature range is shown plotted against temperature. The relationship between retention volume and temperature is, in fact, logarithmic, but over the small range of temperatures concerned, it is approximately linear, so a linear function was force-fitted to the results for the three solutes. A summary of the results obtained from the regression analysis of the data used in Fig. 5 is shown in Table VI. It is seen that, to attain a precision of 0.1%, the temperature of the solvent and column must be maintained to within ±0.04%. It was not found difficult to maintain this level of temperature control with the thermostat bath, but it was found extremely difficult to return to a given temperature to within ±0.04% (after prior change). It should also be pointed out that column

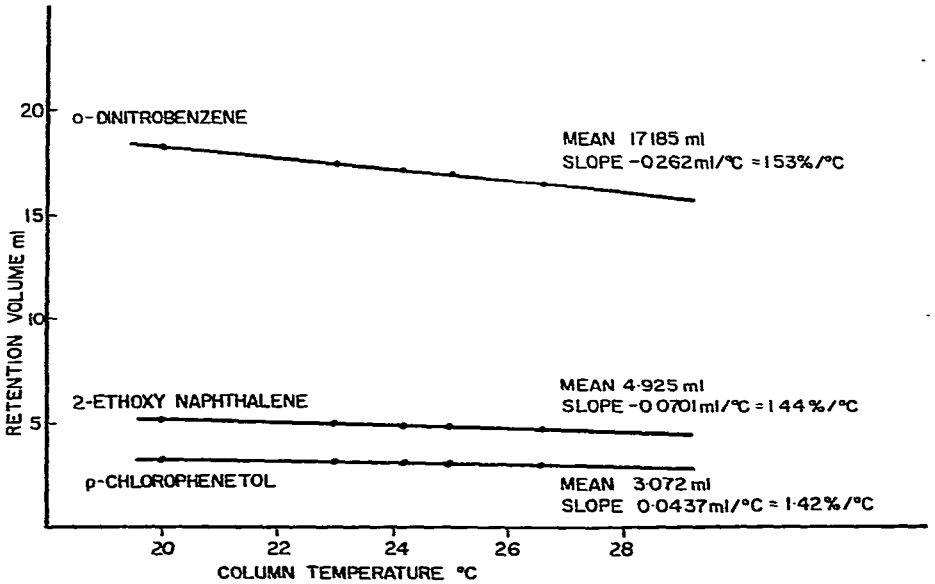

Fig. 5. Graphs of corrected retention volume against column temperature for three solutes.

TABLE VI

TEMPERATURE TOLERANCES FOR RETENTION TIME PRECISION

Solute	*V' at 23.8 °C (ml)*	*k'*	*Temperature control for 1% precision (°C)*	*Temperature control for 0.1% precision (°C)*
p-Chlorophenetol	3.072	0.945	±0.35	±0.04
2-Ethoxynaphthalene	4.925	1.519	±0.35	±0.04
o-Dinitrobenzene	17.185	5.301	±0.33	±0.03

temperature control to ±0.04% would be extremely difficult if not impossible to obtain, if an air bath was employed. For example, due to the relatively low thermal capacity and specific heat of air, local variations of 1 °C can usually be found in gas chromatograph hot air ovens. Thus, for precise work, a liquid is recommended as the thermostating medium for LC columns.

Sample load. The mass of the sample injected onto a LC column can significantly affect both the solute retention time and column efficiency[2,3]. In Fig. 6, the

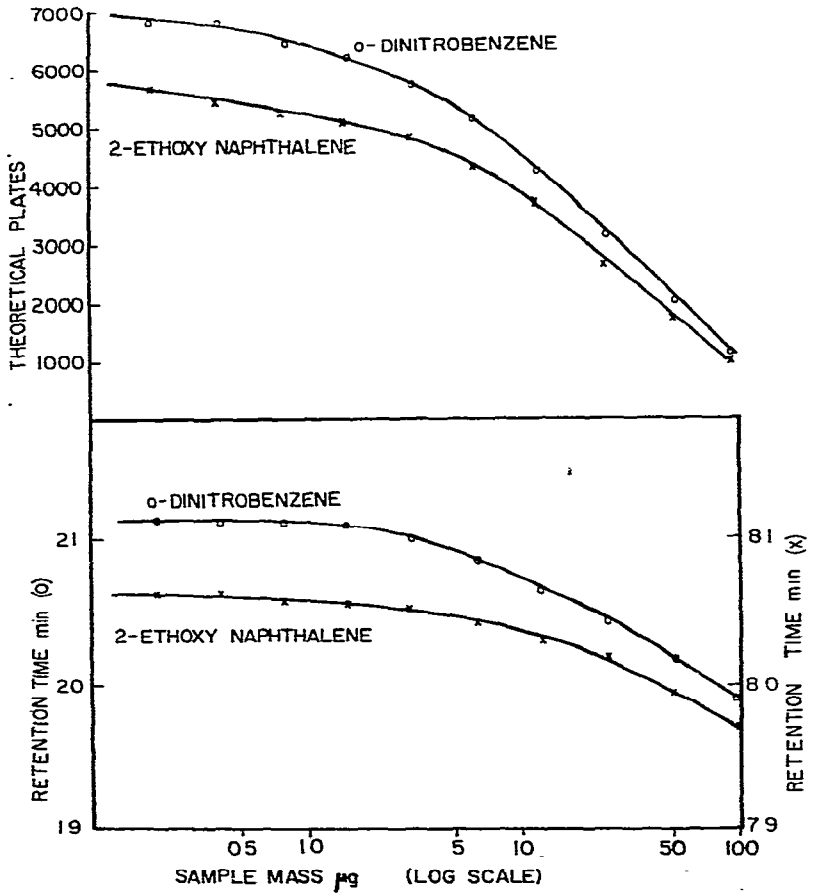

Fig. 6. Graphs of retention time and column efficiency against sample mass for two solutes.

retention time and efficiency obtained from a column for the solutes *o*-dinitrobenzene and 2-ethoxy naphthalene are shown, plotted against mass of solute injected into the column. It is seen that for precise comparative work, either the mass of sample injected must be kept constant, or the total mass of each solute maintained at a level below 0.1 μg. It is also seen that column efficiency is far more sensitive to sample load than retention time.

THE PRECISION OF CHROMATOGRAPHIC DATA UNDER OPTIMIZED CONDITIONS

Method

A passive filter circuit having a time constant of 0.45 sec was interposed between the detector and the A/D convertor. The computer data acquisition rate was again set at 750 samples per min, and no point averaging was employed. The reading threshold was 0.3 μV, but in these experiments, the peak was assumed to be sensed when the signal exceeded 50 μV. Twelve replicate samples of the same mixture were injected, but the first minor peak for chlorobenzene was ignored.

Results and discussion

Retention time and peak widths. The results obtained for the twelve replicate samples are shown in Table A8 in the Appendix and are summarized in Table VII. It is seen, comparing the results given in Table VII with those in Table I, that the introduction of the passive filter has decreased the S.D. of retention times significantly. The retention time of the last peak (20.421 min) has an S.D. of only 0.46 sec (0.04% of the mean) and this represents scatter of only 6 data points. The improvement in the precision of peak widths measurement is, however, marginal, the S.D. of the first peak increasing by a factor of two and that for the last peak reduced by a factor of two.

Efficiency and peak asymmetry. The results obtained are shown in Table A9 in the Appendix and are summarized in Table VIII. Comparing the results for column efficiency in Table VIII with those in Table II, it is seen that the precision of measurement of the first peak is less with the passive filter but significantly improved in the

TABLE VII

PRECISION OF RETENTION TIME AND PEAK WIDTH MEASUREMENTS BY COMPUTER WITH AN INPUT TIME CONSTANT OF 0.5 sec

	Peak		
	1	*2*	*3*
k' Value	0.94	1.50	5.21
Retention time			
Mean (min)	6.283	8.119	20.421
S.D. (sec)	0.38	0.20	0.46
S.D. (% of the mean)	0.10	0.04	0.04
Peak width			
Mean (min)	0.1691	0.2251	0.5753
S.D. (sec)	0.24	0.06	0.19
S.D. (% of the mean)	2.38	0.45	0.55

TABLE VIII

PRECISION OF EFFICIENCY AND PEAK ASYMMETRY MEASUREMENTS BY COMPUTER WITH A 0.5-sec INPUT TIME CONSTANT

	Peak		
	2	*3*	*4*
k' Value	0.94	1.50	5.21
Column efficiency			
Mean (theoretical plates)	5525	5201	5025
S.D.	274	48	41
S.D. (% of the mean)	5.0	0.93	0.82
Asymmetry ratio			
Mean	1.246	1.241	1.436
S.D.	0.195	0.041	0.023
S.D. (% of the mean)	15.6	3.3	1.6

second and third peaks. The large S.D. in the asymmetry factor for the first peak results from the peak being only 10 sec wide and the peak width having an S.D. of 0.26 sec.

Peak heights and peak areas. The results obtained are shown in Table A10 in the Appendix and are summarized in Table IX. Comparing the results previously obtained and shown in Table III with those shown in Table IX, it is seen that the differences in precision between peak height measurements and peak area measurements is less significant when a passive filter is employed. In general, the precision in peak height measurement has deteriorated when a passive filter is present and has only improved for the last peak in the area measurements. However, the precision shown here is more a reflection of the precision of sampling than the precision of the chromatographic data.

Peak height and peak area analysis. The results obtained are shown in Table A11 in the Appendix and are summarized in Table X. It is seen by comparing the re-

TABLE IX

PRECISION OF PEAK HEIGHT AND PEAK AREA MEASUREMENTS BY COMPUTER WITH A 0.5-sec INPUT TIME CONSTANT

Parameter	*Peak*		
	2	*3*	*4*
k' Value	0.94	1.50	5.21
Peak height measurements			
Mean (arbitrary units)	1785	15191	75140
S.D.	63	319	1183
S.D. (% of the mean)	3.5	2.1	1.6
Peak area measurements			
Mean (arbitrary units)	282929	3344011	41042092
S.D.	17301	64503	583521
S.D. (% of the mean)	6.11	1.93	1.42

TABLE X

PRECISION FOR THE ANALYSES OF THE MIXTURE OF NORMALIZATION BY PEAK HEIGHTS AND PEAK AREAS MEASURED BY COMPUTER WITH A 0.5-sec INPUT TIME CONSTANT

Parameter	*Peak*		
	1	*2*	*3*
k' Value	0.94	1.50	5.21
Analysis by peak height			
Mean	1.937	16.491	81.574
S.D.	0.0465	0.121	0.148
S.D. (% of the mean)	2.46	0.736	0.18
Analysis by peak area			
Mean	0.633	7.486	91.884
S.D.	0.032	0.072	0.0823
S.D. (% of the mean)	5.071	0.97	0.09

sults in Table X with those in Table IV that both in peak area analyses and peak height analyses, the precision has improved for the last two peaks but for the first peak, the precision is less. It is also seen that only for the most retained peak is the S.D. for analysis by peak area below that by peak height. However, the determination of a solute present at the 80–90% level with an S.D. of $\pm 0.08\%$ (actual) is from an analytical point of view highly satisfactory.

Repeatability. The repeatability of retention times, peak height analyses, and peak area analysis was determined under the same conditions. Three replicate analyses were carried out every day consecutively over 4 days, and the results obtained are shown in Table A12 in the Appendix and are summarized in Table XI. It is seen from Table XI that there is little difference between the S.D. within a day and between days for retention time data. For solutes having retention times ranging between 6 and 20 min, the repeatability appears to be of the order of 0.1%. It is seen again that the S.D. for peak height analyses is less than that for peak area analyses, both within days and between days. In general, the S.D. of analyses made between days is significantly greater than those made within the same day. Employing peak height analyses, between days, components present at a level of about 80% will have S.D.s of about $\pm 0.22\%$ (absolute); components present at a level of 16% will have a S.D. of about $\pm 0.20\%$ (absolute) and components present at a level of 2.0% will have an S.D. of about 0.08% (absolute).

PRECISION AS AN ALTERNATIVE TO RESOLUTION

Providing retention times can be measured with high precision then retention times can be used to determine the composition of a mixture of two substances that, although having finite differences in retention times, are eluted as a single peak by the column employed. This can only be achieved if the S.D. of the measured retention time is small, compared with the retention time difference of the two solutes.

Consider two solutes eluted close together such that a single composite peak

TABLE XI

REPEATABILITY OF RETENTION TIMES, PEAK HEIGHT, AND PEAK AREA ANALYSES FOR TWELVE REPLICATE SAMPLES TAKEN OVER A 4-DAY PERIOD

T_t = S.D. of the difference between two determinations on the same day; T_d = S.D. of the difference between two determinations on different days.

	Peak		
	1	*2*	*3*
k' Value	0.94	1.50	5.21
Retention data			
Mean retention time (min)	6.286	8.121	20.929
T_t (min)	0.0093	0.0074	0.0186
T_t (%)	0.15	0.09	0.08
T_d (min)	0.0096	0.0091	0.0246
T_d (%)	0.15	0.11	0.12
Peak height analysis			
Mean	1.958	16.484	81.558
T_t	0.0986	0.0883	0.1453
T_t (%)	5.04	0.54	0.18
T_d	0.0802	0.2065	0.2264
T_d (%)	4.09	1.25	0.25
Peak area analysis			
Mean	0.6340	7.457	91.890
T_t	0.0508	0.0546	0.0626
T_t (%)	8.0	0.73	0.07
T_d	0.0438	0.1549	0.156
T_d (%)	6.90	2.08	0.17

is produced. From the plate theory, the concentration profile of such a peak can be described by the following equation:

$$X_{AB} = \frac{X_A}{\sqrt{2\pi n_A}} \cdot \exp\left[-(v_A - n_A)^2/2n_A\right] + \frac{X_B}{\sqrt{2\pi n_B}} \cdot \exp\left[-(v_B - n_B)^2/2n_B\right] \quad (1)$$

when X_{AB} is the concentration of solutes A and B at any point in the composite peak, X_A is the initial concentration of solute A, X_B is the initial concentration of solute B, n_A is the column efficiency for solute A, n_B is the column efficiency for solute B, v_A is the volume of mobile phase passed through the column in units of plate volumes of solute A, and v_B is the volume of mobile phase passed through the column in units of plate volumes of solute B.

If t_A and t_B are the retention times of solutes A and B eqn. 1 can be transformed into:

$$X_{AB} = \frac{X_A}{\sqrt{2\pi n_A}} \cdot \exp\left[-(n_A/2)\,(t/t_A - 1)^2\right] + \frac{X_B}{\sqrt{2\pi n_B}} \cdot \exp\left[-(n_B/2)\,(t/t_B - 1)^2\right] \quad (2)$$

where the variable v is now replaced by variable t, the elapsed time. It is seen from eqn. 2, that when only solute A is present, the function will exhibit a maximum at $t = t_A$ and, if only solute B is present, it will exhibit a maximum at $t = t_B$. It follows

that the composite curve will give a range of maxima between $t = t_A$ and $t = t_B$ for different ratios of X_A to X_B and thus from the value of t at the maxima of the composite peak X_A/X_B can be determined.

For closely eluted peaks $n_A = n_B$ and thus, as the function $2n_A$ is in effect an average dilution factor resulting from the dispersion, they can be replaced by a constant. The efficiencies n_A and n_B in the exponent function, however, can only be considered equal if the peak is symmetrical as, in the part of the composite peak that determines its maximum, the rear part of the first peak is combined with the front part of the second peak. In liquid–solid chromatography, the concentration profiles of eluted peaks are rarely symmetrical, and, thus, n_A must represent the efficiency of the rear half of the peak for solute A. Similarly, n_B must represent the efficiency of the front half of solute B. Further, the detector response to solutes A and B must be taken into account. Thus, if D is the detector signal then eqn. 2 can be put into the form

$$D = C\{\alpha X_A \cdot \exp[-(n/2)\,(t/t_A - 1)^2] + \beta X_B \cdot \exp[-(n/2)\,(t/t_B - 1)^2]\} \qquad (3)$$

where C is a constant, α is the response factor of the detector to solute A, and β is the response factor of the detector to solute B.

The system was examined employing nitrobenzene and fully deuterated nitrobenzene as the solutes. Their elution times were 8.927 and 9.061 min, respectively, thus having a retention difference of 8.04 sec. The separation ratio of the two solutes was 1.023, and the efficiencies of the front and rear portions of the peaks were 5908 and 3670 theoretical plates, respectively. The detector was found to have the same response for both solutes, *i.e.* $\alpha = \beta$. Thus, inserting these values in eqn. 3

$$D = C\{(X_A \cdot \exp[(-3670/2)\,(t/8.927 - 1)^2] + X_B \cdot \exp[(-5908/2)\,(t/9.061 - 1)^2]\} \qquad (4)$$

Employing a range of values for X_A/X_B the retention time of the composite peak was calculated from eqn. 4 by means of a computer. The curve relating the composition of the mixture to retention time is shown in Fig. 7.

A series of mixtures of nitrobenzene and deuterated nitrobenzene were made to a known concentration ratio and the retention time of the composite peak were determined experimentally. The retention time of each mixture was determined in triplicate, and the average for each mixture is represented as plotted points in Fig. 7. It is seen that close agreement is obtained between the experimental points and the theoretical curve.

Employing very precise methods of measuring retention times as a means of determining the composition of unresolvable binary solute mixtures would be extremely valuable in the analyses of configurational isomers. Providing the retention time of a known mixture of the two components is available (in most instances one pure isomer and a 50% (w/v) mixture of one isomer in the other can be obtained) a calibration curve can be calculated theoretically. The asymmetry ratio of the peak for one pure component and the column efficiency for that component is usually the only further information required as the detector response factors for configurational isomers are generally identical.

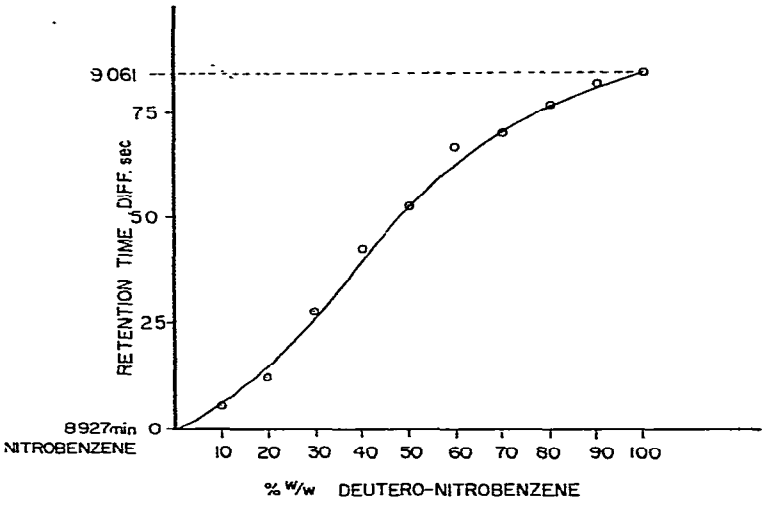

Fig. 7. Graph of retention time difference against sample composition for mixtures of nitrobenzene and deutero-nitrobenzene.

CONCLUSIONS

In LC, retention times for peaks eluted at a k' value of 1.5 or more can be measured with a standard deviation of better than 0.1%. There is little difference between the S.D. of retention times measured within one day or between days. Peak width for solutes eluted at a k' of greater than 1.5 have S.D.s of about 0.5%, which means that column efficiencies can be measured with a precision of about $\pm 1.0\%$. The S.D. of asymmetry ratios for peaks of the same k' range lies between 2 and 3%. For all types of retention measurements the computer can give more precise results than those obtained by manual measurement. In general, analysis by normalization of peak heights give more precise results than normalization of peak areas except possibly for broad peaks that exhibit some asymmetry. Employing peak height analysis, components present at levels from 2.0–16% (w/w) have an S.D. of about 0.1% (absolute) and at a level of 80% (w/w) an S.D. of 0.2% (absolute). This precision of measurement can only be achieved under the following controlled conditions:

(1) A pump is employed that controls the column flow-rate to $\pm 0.07\%$.

(2) The solvent composition is maintained constant to $\pm 0.02\%$ (w/v).

(3) The temperature of the mobile phase and column is maintained constant to ± 0.02 °C.

(4) The ambient temperature or the temperature of the pump and mobile phase supply must be maintained constant to ± 0.4 °C.

(5) The charge on the column for any one solute must be less than 0.1 μg.

(6) The rate of data acquisition must be greater than 10 samples per sec and appropriate noise elimination procedures must be employed that do not distort the peak or produce band dispersion of an unacceptable level.

Under some conditions, where a pair of solutes is completely unresolved, the composition of the mixture can be determined from retention time measurements, if the chromatographic system can provide retention data with sufficiently high precision.

APPENDIX

TABLE A1

RETENTION DATA FOR TWELVE REPLICATE SAMPLES

Chromatogram	*Retention distance measured from chart (cm)*				*Retention time determined by computer (min)*				*Flow-rate (ml/min)*
	Peak 1	*Peak 2*	*Peak 3*	*Peak 4*	*Peak 1*	*Peak 2*	*Peak 3*	*Peak 4*	
1	10.42	15.98	20.52	51.02	3.969	6.301	8.141	20.337	0.9664
2	10.11	15.97	20.56	51.06	3.944	6.256	8.080	20.091	0.9670
3	10.19	16.04	20.60	51.12	3.965	6.261	8.099	20.120	0.9661
4	10.16	16.02	20.54	51.13	3.957	6.272	8.107	20.157	0.9653
5	10.20	16.02	20.64	51.23	3.963	6.269	8.099	20.091	0.9657
6	10.16	16.04	20.59	51.07	3.976	6.280	8.112	20.123	0.9651
7	10.13	15.93	20.55	51.09	3.963	6.277	8.107	20.152	0.9654
8	10.09	15.97	20.61	51.16	3.960	6.269	8.112	20.128	0.9658
9	10.12	15.97	20.63	51.22	3.955	6.272	8.107	20.128	0.9667
10	10.12	15.96	20.63	51.19	3.976	6.283	8.104	20.165	0.9664
11	10.18	16.05	20.61	51.28	3.992	6.283	8.101	20.107	0.9654
12	10.21	16.00	20.59	51.10	3.971	6.261	8.096	20.083	0.9657
Mean	10.17	16.00	20.59	51.14	3.966	6.274	8.105	20.140	0.9660
S.D.	0.0864	0.0380	0.0387	0.0782	0.0123	0.0123	0.0141	0.0674	0.000653
S.D. (%)	0.85	0.24	0.19	0.15	0.31	0.20	0.17	0.33	0.068

TABLE A2

PEAK WIDTHS FOR TWELVE REPLICATE SAMPLES

Chromatogram	*Peak width measured from chart (mm)*				*Peak width determined by computer (min)*			
	Peak 1	*Peak 2*	*Peak 3*	*Peak 4*	*Peak 1*	*Peak 2*	*Peak 3*	*Peak 4*
1		0.45	0.60	1.99	0.2630	0.1750	0.2345	0.7839
2		0.45	0.58	1.95	0.2958	0.1731	0.2372	0.7841
3		0.51	0.60	1.98	0.2382	0.1768	0.2353	0.7883
4		0.48	0.59	2.01	0.2708	0.1788	0.2368	0.7807
5		0.48	0.61	1.93	0.2474	0.1789	0.2348	0.7610
6		0.45	0.60	1.98	0.2811	0.1769	0.2355	0.7738
7		0.44	0.62	1.99	0.2396	0.1739	0.2387	0.7750
8		0.49	0.60	1.96	0.2600	0.1775	0.2359	0.7674
9		0.46	0.60	1.96	0.2338	0.1744	0.2357	0.7685
10		0.47	0.62	1.96	0.2451	0.1782	0.2352	0.7705
11		0.42	0.60	1.96	0.2054	0.1776	0.2381	0.7638
12		0.48	0.60	1.94	0.2289	0.1752	0.2344	0.7690
Mean		0.465	0.602	1.968	0.2466	0.1768	0.2360	0.7738
S.D.		0.247	0.0111	0.0230	0.0203	0.00205	0.00140	0.00871
S.D. (%)		5.3	1.84	1.16	8.23	1.16	0.59	1.13

TABLE A3

RETENTION VOLUME FOR TWELVE REPLICATE SAMPLES

Chromatogram	*Retention volume measured by burette (ml)*				*Retention volume from the product of retention time and flow-rate*			
	Peak 1	*Peak 2*	*Peak 3*	*Peak 4*	*Peak 1*	*Peak 2*	*Peak 3*	*Peak 4*
1	3.79	6.04	7.83	19.41	3.833	6.092	7.872	19.664
2	3.87	6.08	7.87	19.47	3.814	6.050	7.813	19.428
3	3.90	6.09	7.86	19.42	3.831	6.049	7.824	19.438
4	3.85	6.10	7.88	19.46	3.820	6.056	7.826	19.458
5	3.88	6.09	7.87	19.42	3.827	6.054	7.821	19.402
6	3.85	6.08	7.84	19.46	3.837	6.061	7.829	19.421
7	3.86	6.09	7.85	19.48	3.826	6.060	7.827	19.455
8	3.86	6.10	7.88	19.46	3.825	6.055	7.835	19.440
9	3.84	6.09	7.87	19.46	3.823	6.063	7.837	19.458
10	3.87	6.08	7.86	19.49	3.842	6.072	7.832	19.487
11	3.88	6.10	7.88	19.46	3.854	6.066	7.821	19.411
12	3.87	6.08	7.86	19.41	3.835	6.046	7.818	19.394
Mean	3.86	6.085	7.863	19.45	3.831	6.060	7.830	19.455
S.D.	0.0273	0.0162	0.0160	0.0276	0.0107	0.0125	0.0151	0.0711
S.D. (%)	0.71	0.27	0.20	0.14	0.28	0.21	0.19	0.37

TABLE A4

EFFICIENCY VALUES FOR TWELVE REPLICATE SAMPLES

Chromatogram	*Efficiencies calculated from chart data*				*Efficiencies calculated from computer data*			
	Peak 1	*Peak 2*	*Peak 3*	*Peak 4*	*Peak 1*	*Peak 2*	*Peak 3*	*Peak 4*
1		5044	4678	2629	900	5113	4746	2626
2		5038	5026	2743	1028	5217	4636	2625
3		3957	4715	2666	1108	5011	4729	2604
4		4456	4848	2588	853	4917	4684	2605
5		4456	4580	2818	1025	4906	4756	2787
6		5082	4711	2661	799	5034	4741	2703
7		5243	4394	2637	1093	5210	4609	2703
8		4249	4720	2725	927	4987	4723	2751
9		4821	4729	2732	1143	4886	4725	2743
10		4612	4429	2729	1051	4966	4743	2739
11		5841	4720	2738	1509	4998	4624	2770
12		4444	4711	2775	1202	5101	4767	2727
Mean		4770	4688	2703	1053	5029	4707	2704
S.D.		516	168	67	188	111	55	61
S.D. (%)		10.8	3.6	2.5	17.9	2.2	1.2	2.3

TABLE A5

PEAK AREAS FOR TWELVE REPLICATE SAMPLES

Chromatogram	*Peak area (peak height × peak width) from chart (cm^2)*				*Peak area from computer (total counts)*				*Total area*
	Peak 1	*Peak 2*	*Peak 3*	*Peak 4*	*Peak 1*	*Peak 2*	*Peak 3*	*Peak 4*	
1		0.266	2.922	38.626	171284	607303	6265688	75294144	82338419
2		0.266	2.877	38.084	193953	621526	6369017	75398064	82582560
3		0.296	2.988	38.531	181078	630209	6314873	75563904	82690064
4		0.288	2.944	38.939	186727	631570	6317336	75165984	82301617
5		0.288	3.056	36.381	184582	635102	6332275	71561536	78713495
6		0.252	3.006	38.155	160555	632422	6304906	73894800	80831645
7		0.268	3.038	38.148	170436	623447	6361052	73320896	80475831
8		0.304	2.988	37.495	166090	634427	6301794	73929696	80532007
9		0.285	2.970	37.358	197971	620944	6296811	73040336	80156062
10		0.287	3.112	37.083	186004	618610	6316502	72068000	79189116
11		0.265	2.934	36.907	235252	630818	6318854	71123776	78308700
12		0.302	3.030	37.287	178404	638505	6338860	73283360	80439129
Mean		0.281	2.989	37.749	184361	626324	6319789	73595375	80738138
S.D.		0.0167	0.0650	0.779	19510	9723	28128	1527132	1583570
S.D. (%)		5.8	2.2	2.1	10.6	1.55	0.45	2.1	2.0

TABLE A6

PEAK HEIGHT FOR TWELVE REPLICATE SAMPLES

Chromatogram	*Peak height measured from chart (cm)*				*Peak height determined by computer (arbitrary units)*			
	Peak 1	*Peak 2*	*Peak 3*	*Peak 4*	*Peak 1*	*Peak 2*	*Peak 3*	*Peak 4*
1		0.59	4.87	19.41	1413	6851	52846	209597
2		0.59	4.96	19.53	1552	7018	53538	211260
3		0.58	4.98	19.46	1450	6942	53760	210494
4		0.60	4.99	19.37	1476	6973	54011	209677
5		0.60	5.01	18.85	1515	7030	54136	204107
6		0.56	5.01	19.27	1392	6980	53858	208629
7		0.61	4.90	19.17	1444	7058	53561	207290
8		0.62	4.98	19.13	1454	6957	53805	207089
9		0.62	4.95	19.06	1561	6944	53677	206377
10		0.61	5.02	18.92	1495	6905	53860	205076
11		0.63	4.89	18.83	1755	6915	53426	204036
12		0.63	5.05	19.22	1477	7023	54399	208144
Mean		0.603	4.97	19.19	1499	6966	53740	207696
S.D.		0.021	0.0559	0.236	95	60	390	2422
S.D. (%)		3.48	1.12	1.19	6.33	0.86	0.72	1.16

TABLE A7

PERCENT COMPOSITION OF MIXTURE BY NORMALIZATION OF PEAK HEIGHT AND PEAK AREAS FROM COMPUTER DATA

Chromatogram	*Composition by peak height*				*Composition by peak areas*			
	Peak 1	*Peak 2*	*Peak 3*	*Peak 4*	*Peak 1*	*Peak 2*	*Peak 3*	*Peak 4*
1	0.522	2.531	19.521	77.426	0.208	0.738	7.610	91.445
2	0.568	2.567	19.585	77.281	0.235	0.753	7.712	91.300
3	0.532	2.546	19.718	77.204	0.219	0.762	7.637	91.382
4	0.542	2.562	19.847	77.048	0.227	0.767	7.676	91.330
5	0.568	2.635	20.242	76.506	0.234	0.807	8.045	90.919
6	0.514	2.577	19.884	77.025	0.198	0.781	7.784	91.237
7	0.536	2.620	19.885	76.959	0.212	0.775	7.904	91.109
8	0.540	2.583	19.979	76.898	0.206	0.788	7.825	91.181
9	0.581	2.586	19.987	76.846	0.247	0.775	7.856	91.123
10	0.559	2.583	20.147	76.711	0.235	0.781	7.976	91.007
11	0.659	2.598	20.075	76.667	0.300	0.806	8.069	90.825
12	0.545	2.591	20.072	76.791	0.222	0.794	7.880	91.104
Mean	0.556	2.582	19.916	76.947	0.229	0.777	7.831	91.163
S.D.	0.0381	0.0288	0.227	0.268	0.0267	0.0205	0.153	0.188
S.D. (%)	6.9	1.1	1.1	0.35	11.7	2.64	1.95	0.21

TABLE A8

CHROMATOGRAPHIC DATA FROM COMPUTER WITH 0.5-sec INPUT TIME CONSTANT

Chromatogram	*Retention time (min)*				*Peak width (min)*			
	Peak 1	*Peak 2*	*Peak 3*	*Peak 4*	*Peak 1*	*Peak 2*	*Peak 3*	*Peak 4*
1		6.288	8.120	20.428		0.1613	0.2255	0.5763
2		6.280	8.117	20.416		0.1736	0.2264	0.5806
3		6.291	8.115	20.416		0.1646	0.2260	0.5781
4		6.289	8.123	20.419		0.1665	0.2240	0.5763
5		6.289	8.116	20.417		0.1736	0.2253	0.5768
6		6.283	8.124	20.415		0.1678	0.2259	0.5783
7		6.275	8.117	20.412		0.1742	0.2265	0.5759
8		6.277	8.116	20.413		0.1706	0.2246	0.5734
9		6.281	8.117	20.423		0.1705	0.2245	0.5746
10		6.285	8.121	20.433		0.1695	0.2234	0.5744
11		6.284	8.123	20.433		0.1655	0.2244	0.5746
12		6.272	8.116	20.429		0.1712	0.2241	0.5724
Mean		6.283	8.119	20.421		0.1691	0.2251	0.5753
S.D.		0.00628	0.00325	0.00772		0.00402	0.00102	0.00319
		(0.38 sec)	(0.2 sec)	(0.46 sec)		(0.24 sec)	(0.06 sec)	(0.19 sec)
S.D. (%)		0.10	0.04	0.04		2.38	0.45	0.55

TABLE A9

CHROMATOGRAPHIC DATA FROM COMPUTER WITH 0.5-sec INPUT TIME CONSTANT

Chromatogram	*Efficiency (theoretical plates)*				*Asymmetry ratio*			
	Peak 1	*Peak 2*	*Peak 3*	*Peak 4*	*Peak 1*	*Peak 2*	*Peak 3*	*Peak 4*
1		6069	5183	5023		1.198	1.251	1.425
2		5224	5134	4992		1.315	1.332	1.481
3		5832	5153	4985		1.048	1.244	1.427
4		5701	5254	5018		1.118	1.181	1.438
5		5245	5187	5010		1.092	1.224	1.421
6		5597	5168	4982		1.333	1.215	1.440
7		5183	5134	5022		1.528	1.238	1.427
8		5408	5216	5066		1.207	1.202	1.456
9		5422	5224	5052		1.286	1.263	1.421
10		5487	5280	5059		1.184	1.216	1.436
11		5773	5233	5057		1.098	1.233	1.463
12		5362	5243	5085		1.650	1.298	1.391
Mean		5525	5201	5025		1.246	1.241	1.436
S.D.		274	48	41		0.1950	0.041	0.023
S.D. (%)		5.0	0.93	0.82		15.6	3.3	1.6

TABLE A10

CHROMATOGRAPHIC DATA FROM COMPUTER WITH 0.5-sec INPUT TIME CONSTANT

Chromatogram	*Peak height (total counts)*				*Peak area (total counts)*				*Total area*
	Peak 1	*Peak 2*	*Peak 3*	*Peak 4*	*Peak 1*	*Peak 2*	*Peak 3*	*Peak 4*	
1		1705	14522	72853		252247	3194109	39726312	43172669
2		1771	15045	75913		292686	3313712	41636792	45243190
3		1695	15081	75149		257191	3356568	41130664	44744423
4		1782	15179	74872		279136	3329446	40842080	44450661
5		1674	14784	73156		263395	3269428	40184664	43717487
6		1784	15115	74484		286522	3345522	40848384	44530428
7		1845	15149	74830		307398	3355625	41189152	44852175
8		1840	15575	76443		293322	3431460	41525896	45250678
9		1850	15552	76264		297769	3425088	41556904	45279761
10		1846	15460	76252		298472	3363609	41602392	45264473
11		1811	15341	75483		280724	3362814	41022200	44665738
12		1817	15495	75981		286291	3380753	41189664	44599046
Mean		1785	15191	75140		282929	3344011	41042092	44647561
S.D.		63	319	1183		17301	64503	583521	649863
S.D. (%)		3.5	2.1	1.6		6.11	1.93	1.42	1.46

TABLE A11

CHROMATOGRAPHIC DATA FROM COMPUTER WITH 0.5-sec INPUT TIME CONSTANT

Chromatogram	*Peak height analysis*				*Peak area analysis*			
	Peak 1	*Peak 2*	*Peak 3*	*Peak 4*	*Peak 1*	*Peak 2*	*Peak 3*	*Peak 4*
1		1.914	16.302	81.784		0.584	7.398	92.017
2		1.910	16.224	81.866		0.647	7.324	92.029
3		1.844	16.406	81.750		0.575	7.502	91.924
4		1.940	16.529	81.531		0.628	7.490	91.882
5		1.868	16.498	81.635		0.602	7.479	91.919
6		1.953	16.539	81.508		0.643	7.513	91.884
7		2.009	16.498	81.493		0.685	7.482	91.833
8		1.960	16.594	81.446		0.648	7.592	91.769
9		1.975	16.604	81.421		0.658	7.562	91.778
10		1.973	16.525	81.502		0.659	7.431	91.910
11		1.955	16.561	81.484		0.628	7.529	91.843
12		1.948	16.609	81.443		0.638	7.537	91.825
Mean		1.937	16.491	81.574		0.633	7.486	91.884
S.D.		0.0465	0.121	0.148		0.032	0.072	0.0823
S.D. (%)		2.46	0.736	0.18		5.071	0.97	0.09

TABLE A12

CHROMATOGRAPHIC DATA FOR THREE REPLICATES TAKEN ON FOUR CONSECUTIVE DAYS

σ = S.D. of the difference between two determinations on the same day; σ_d = S.D. of the differences between two determinations on different days.

Date		*Retention time*			*Peak height analysis*			*Peak area analysis*		
		Peak 1	*Peak 2*	*Peak 3*	*Peak 1*	*Peak 2*	*Peak 3*	*Peak 1*	*Peak 2*	*Peak 3*
11/19/76	1	6.281	8.123	20.417	1.921	16.651	81.428	0.625	7.627	91.749
	2	6.289	8.120	20.416	1.929	16.586	81.485	0.659	7.551	91.790
	3	6.293	8.132	20.441	1.943	16.643	81.414	0.594	7.581	91.825
11/20/76	1	6.283	8.115	20.439	2.014	16.584	81.397	0.661	7.527	91.811
	2	6.281	8.120	20.439	1.968	16.547	81.485	0.644	7.477	91.879
	3	6.293	8.128	20.445	1.993	16.571	81.437	0.645	7.547	91.808
11/21/76	1	6.288	8.123	20.445	1.942	16.433	81.625	0.637	7.426	91.437
	2	6.289	8.123	20.436	1.939	16.317	81.744	0.647	7.351	92.002
	3	6.285	8.123	20.437	1.940	16.408	81.592	0.630	7.430	91.950
11/22/76	1	6.272	8.108	20.391	2.113	16.316	81.570	0.702	7.328	91.969
	2	6.283	8.117	20.416	1.954	16.429	81.617	0.629	7.396	91.975
	3	6.291	8.117	20.420	1.840	16.257	81.903	0.577	7.347	92.076
Mean		6.286	8.121	20.429	1.958	16.484	81.558	0.634	7.457	91.898
σ		0.0093	0.0074	0.0156	0.0986	0.0883	0.1453	0.0508	0.0546	0.0626
σ (%)		0.148	0.091	0.076	5.04	0.54	0.18	8.0	0.73	0.07
σ_d		0.0096	0.0091	0.0246	0.0802	0.2065	0.2264	0.0438	0.1549	0.156
σ_d (%)		0.153	0.112	0.121	4.09	1.25	0.25	6.9	2.08	0.17

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