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EVALUATION OF MULTI-STAGE GAS CHROMATOGRAPHY IN QUANTITATIVE CHEMICAL ANALYSIS

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SUMMARY

Two-stage gas chromatography has proved to be a powerful means for the isolation of trace components from complex mixtures. The application of this technique in quantitative analysis has not yet been evaluated and is considered in this paper. The influence of the size and position of the transferred fraction on the quality of the analytical data was investigated. A compromise between low peak interference and high transfer efficiency has to be found. It is shown that the same ultimate precision and accuracy can be obtained with column switching as in single-column operation provided that a high-performance switching device and appropriate fractionation are used.

INTRODUCTION

In order to separate a complex mixture by chromatography, the first requirement is to achieve a separation performance that is at least as large as the number of components to be separated by a given minimum resolution. In practice, the separation performance should be significantly larger than the number of components of the sample. The separation performance, n_{Rt}, is defined as the maximum number of components, n, that can be separated with a given resolution, R, in a given time, t. The separation performance depends on the average theoretical plate number, $\bar{N}$, of the column and is given by the following equation[1,2]:

$$n_{Rt} = \frac{\log (t_{Rn}/t_{R0})}{\log (1 + R/\sqrt{\bar{N}})} = \frac{\log (1 + \kappa_n)}{\log (1 + R/\sqrt{\bar{N}})} \tag{1}$$

where t_{Ri} = retention time of component, i; $i = 0,1,\ldots, n$.

The subscript zero indicates a non-retarded component with capacity factor $\kappa_i = 0$; the subscript n, indicates the last eluting component with the highest capacity factor, $\kappa_n = $ maximum. From eqn. 1, it can be seen that the separation performance

depends on the retention range, $t_{Rn}/t_{R0} = 1 + \kappa_n$, as well as on the average theoretical plate number of the column, $\bar{N}$. The individual theoretical plate number, N_i, of the components, i, depends not only on the chromatographic column but also on the sample component, as the capacity factor, κ_i, and the diffusion coefficients, D_{mi} and D_{si}, in the mobile phase and in the stationary phase, respectively, occur in the equation of the theoretical plate number[3,4].

In order to obtain a value for the dispersion characteristic of the column that is dependent only on the system and independent of the sample, it is necessary to estimate the average of the theoretical plate number, $\bar{N}$, from the measured individual values. N_i, of a number of components. The best average value, $\bar{N}$, is obtained by linear regression of the peak variances and the squares of the retention times according to

$$\sigma_{ti}^2 = \bar{N} \, t_{Ri}^2 \tag{2}$$

where σ_{ti}^2 is the variance of the elution peak of component i. The slope, $\bar{N}$, of the linear regression line of σ_{ti}^2 versus t_{Ri}^2 depends only slightly on the choice of the test compounds if the diffusion coefficients and therefore the individual values, N_i, of these compounds do not vary too much.

If the first requirement to make the separation performance for a given time interval sufficiently large compared with the number of components to be separated, $n_{Rt} \geqslant n_S$, is fulfilled, then the second requirement is to utilize fully this separation performance. For this purpose the elution sequence of the components has to be adjusted within the given time interval via the selectivity of the chromatographic phase system. The aim is to arrange the components in such an order that all of them are separated at least according to the required resolution. It is obvious that this target can be reached more easily the larger the separation performance is, compared with the number of components to be separated, $n_{Rt} \gg n_S$.

Often it is not possible, however, to find a phase system that is sufficiently selective for all components of the mixture and the required minimum resolution cannot be obtained for all components. In such instances the required degree of separation can be achieved by gradual adjustment of the column selectivity. The separation is carried out in several stages on different columns. This multi-stage chromatography can be executed on-line by column switching where the effluent of a column of a given stage is divided into fractions which are transferred to the detector or to the columns of the next stage by means of a switching device. If columns with different retention characteristics are used in the individual stages of a separation path the multi-stage technique is called multi-dimensional chromatography.

Multi-stage chromatography can also be used with advantage if columns with the same retention characteristics are used in each stage. In this technique, a given component is enriched relative to interfering constituents of the sample by fractionation. An effluent fraction containing the analyte component and as little as possible interfering components is transferred from the first to the second column on which the separation of the fraction can be completed owing to the more favourable peak size ratio. This relative enrichment has the result that the analytical information parameters (retention time, peak area) can be determined with a higher accuracy than

in a single-stage separation giving the same resolution. Correspondingly, a lower resolution is required to obtain a given accuracy.

Multi-stage gas chromatography was applied first as a relative enrichment technique (heart cutting)[5] and later as a multi-dimensional method[6-10]. The performance of multi-stage gas chromatography in quantitative analysis has never been evaluated. It was our intention to investigate the quality of the analytical data in multi-stage gas chromatography compared with single-column operation using advanced instrumentation.

EXPERIMENTAL

Chemicals

All chemicals used were of analytical-reagent grade from E. Merck (Darmstadt, G.F.R.), except decahydronaphthalenes, which were obtained from EGA-Chemie (Steinheim, G.F.R.). *Cis-* and *trans*-decahydronaphthalene diluted with toluene to a content of 10% (v/v) with addition of *n*-tridecane as internal standard and a commercially available lavender oil diluted to 10% (v/v) with 2,2,4-trimethylpentane were used as test samples. The carrier gas used in gas chromatography was nitrogen of 99.995% (v/v) purity (Messer-Griesheim, Düsseldorf, G.F.R.).

The polydimethylsiloxane OV-101 (E. Merck) and the aromatic polyether Marlophen (Hüls, Marl, G.F.R.) were used as stationary phases.

Apparatus

A gas chromatograph equipped with two flame-ionization detectors and a switching device was used (Model L402, Siemens, Karlsruhe, G.F.R.). The switching device is described later. Two capillary columns were used in the switching operation. The chromatograms were recorded by means of a two-channel potentiometric line recorder (Siemens, Kompensograph X–T). Retention-times, peak-areas and switching intervals were measured with the aid of a computing integrator (Model Autolab, System I, Spectra Physics, Santa Clara, CA, U.S.A.).

The following chromatographic conditions were used for the determination of the precision and accuracy of the two-stage switching procedure depending on the size and position of the fraction: two open-tubular columns, coated with OV-101, lengths 18 and 23 m, I.D. 0.3 mm; column temperature, 95 and 130°C, respectively; temperature of injector and detector, 200°C; splitting ratio, 1:100.

The average efficiencies of the columns were measured as discussed in the Introduction and gave values of $\bar{N} = 55,000$ for the 18-m column and $\bar{N} = 125,000$ for the two columns (18 + 23 m) in series.

In the application of the relative enrichment procedure to the analysis of trace components in lavender oil, the gas chromatographic conditions were as follows: two open-tubular columns, both coated with Marlophen, lengths 18 and 23 m, I.D. 0.3 mm; $\bar{N} = 57,000$ for the 18-m column, $\bar{N} = 98,000$ for the two columns (18 + 23 m) in series; splitting ratio, 1:100; temperature programme, linear gradient from 60 to 160°C at the rate of 5°C min^{-1}; injector and detector temperature, 220°C.

The carrier gas flow-rate, measured at the outlet of the second column at ambient temperature and pressure, was 2 ml min^{-1} in both instances, with $p_0 = 1.8$ bar and $p_B = 1.6$ bar.

The switching device was an improved and optimized version of the valveless design as proposed by Deans[5], and is shown schematically in Fig. 1. The interface is described in detail elsewhere[11]. It consists of a double T-piece with tube connections for the adjustment of variable pressures and a platinum capillary (2.4 cm × 0.1 mm I.D.) for the connection of the two capillary columns.
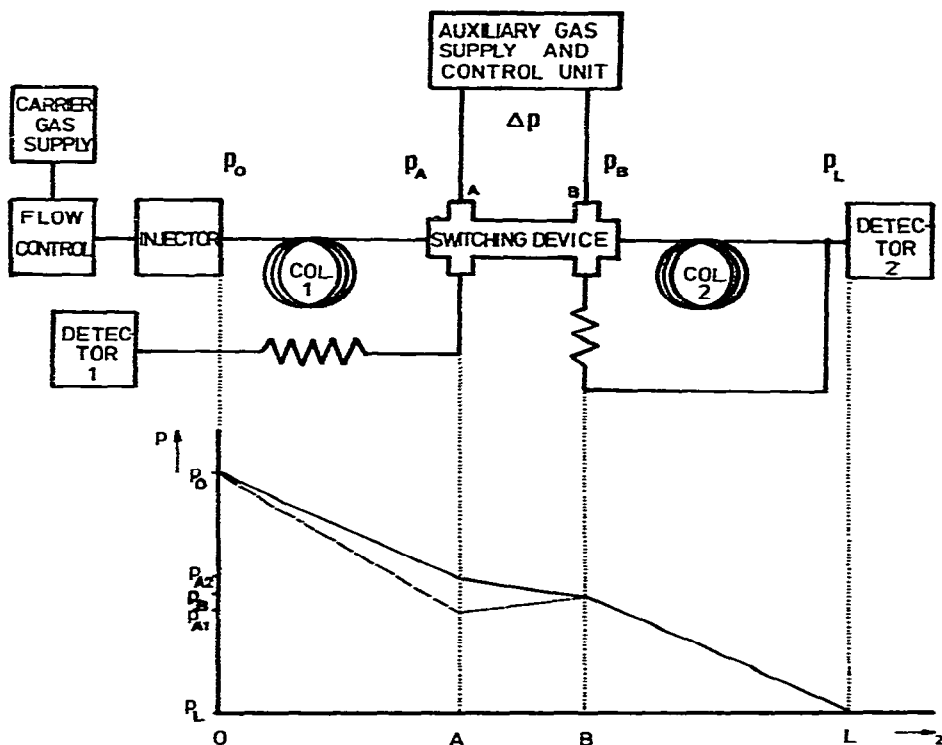

Fig. 1. Schematic diagram of a gas chromatograph for column switching. p = pressure; z = length; p_0 = pressure at the inlet of the first column ($z = 0$); p_A, p_B = pressures at points A and B, respectively, of the switching device; p_L = pressure at the outlet of the second column ($z = L$). The pressure difference, Δp, is adjusted with the aid of the auxiliary gas supply unit and equals $p_{A1} - p_B$ for single-stage operation and $p_{A2} - p_B$ for two-stage operation. For details, see Experimental.

The direction of flow in the connecting tube between the two columns is controlled by variable pressure levels. The pressure, p_0, at the inlet of the first column is kept constant and adjusted by a pressure controller. The pressure, p_B, at the outlet of the interface is identical with the inlet pressure of the second column. It is kept constant and is also adjusted with the aid of a pressure controller. In order to direct the flow from the first column either to the first detector or to the second column and the second detector the pressure, p_A, at the inlet of the interface is varied between two levels, p_{A1} and p_{A2}, by means of a pressure controller.

In the single column mode, where the effluent from the first column is directed to the first detector, a pressure drop, Δp, is adjusted with the aid of the pressure controller unit, which sets point A of the interface to a pressure, p_{A1}, a few millibars lower than the pressure at point B. The pressure drop Δp is measured by means of a digital pressure meter. Owing to the pressure difference, the effluent from column 1 is

hindered from entering column 2 and is forced to flow to the monitoring detector, over a constriction capillary.

In order to cut out a fraction of the effluent from column 1, which is recorded on detector 1, the pressure at point A is rapidly increased to a value, p_{A2}, allowing flow to the second column. After transferring the fraction of interest into the second column, the pressure at point A is set back to the lower value, interrupting the flow of the effluent to the second column. As the flow of carrier gas in none of the columns is interrupted, the elution proceeds continuously.

The great advantage of the switching device is the negligible dead volume, the very high speed of switching and the absence of mechanical valves in the flow path of the sample.

RESULTS AND DISCUSSION

The information parameters in quantitative chemical analysis by means of chromatography are peak area or peak height. The quality of these data in two-stage gas chromatography applying relative analyte enrichment by column switching was investigated. This technique was applied to the determination of minor components in a matrix of interfering major components. A system of two columns of the same type connected by a switching valve was used, the effluent from each column being led to a separate detector. The sample was injected into the first column where the analytes were partially separated from interfering matrix components. The fraction containing the analytes was transferred to the second column, where the separation was completed. A complete separation can only be achieved by this two-stage operation and not by a single column with the total length of the two columns. The superior separation effect by the two-stage operation is caused by the fact that in the second stage the separation is continued with a significantly improved ratio of the peak height of the analyte to those of the overlapping matrix components. The resolution in both modes of operation remains the same but the peak-height ratio is significantly improved in two-stage operation, leading to a better degree of separation of the analytes. In general, several analytes can be isolated in a single run. For the determination of many components several runs are required.

It can be expected that both the accuracy and the precision of the analytical data in column switching will depend on three factors: the technical characteristics of the switching device, the size of the fraction and the position of the fraction. The effects of these three factors on the precision and accuracy of peak area and peak height are discussed below for both isolated peaks and interfering peaks.

Fractionation of isolated peaks
The influence of the fraction size on the analytical results in two-stage gas chromatography was first investigated for the transfer of a single, isolated peak in order to define the optimum switching conditions and to test the performance of the switching device. The peak maximum of component 1, eluting first from the first column, was used as a marker for the measurement of the switching time. The second eluting component 2 was fractionated by means of a switching valve. The fraction was transferred from the first to the second column and measured in its effluent by the second detector. Quantitation of this compound was carried out based on the peak-
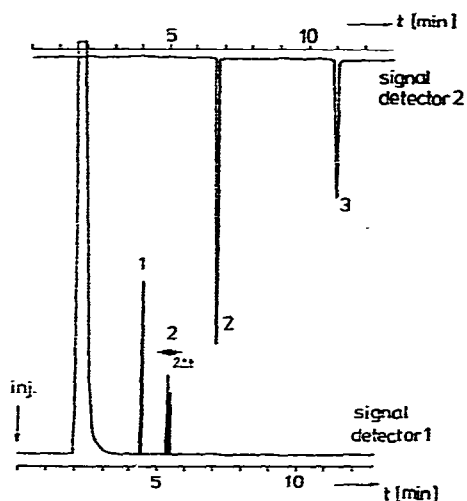
Fig. 2. Chromatogram from a two-stage gas chromatograph with two-channel detection. Compound 1 (*trans*-decahydronaphthalene) was used as a marker substance for the measurement of the switching time, $2\Delta t$, for the transfer of fractions of compound 2 (*cis*-decahydronaphthalene) from column 1 to column 2. Compound 3 (tridecane), used as reference substance in the quantitation of compound 2, was totally transferred to column 2. t = time.

area measurement obtained from the second detector. The third eluting component 3 was transferred to the second column without fractionation and was used as a reference in the quantitation of the second component. The precision and accuracy of the area of the peaks occurring in the chromatogram from the second detector were determined relative to the area of the totally transferred third peak. An example of such a two-channel chromatogram is shown in Fig. 2, and the results for precision and accuracy are given in Table I and are shown graphically in Fig. 3.

TABLE I

DATA ON THE PRECISION AND ACCURACY OF COLUMN SWITCHING IN GAS CHROMATOGRAPHY FOR ISOLATED PEAKS

Switching was carried out for half-intervals of $\Delta t = n\sigma_t$ symmetrical to the expected position of the peak maximum. σ_t = peak standard deviation in time units; n = 1,2,3...

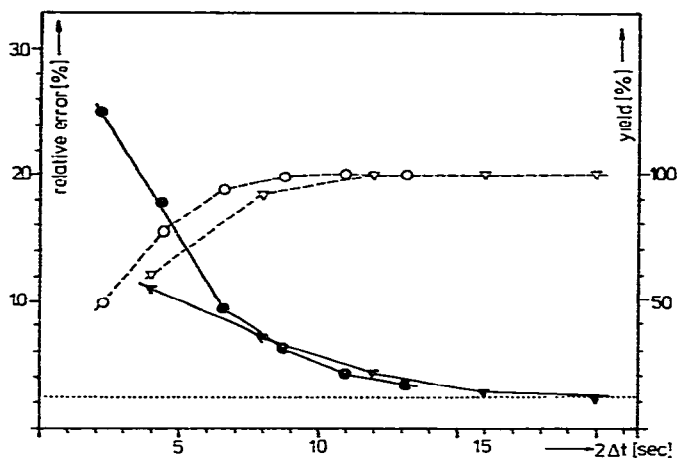| Peak standard deviation σ_t (sec)	Total switching interval $2\Delta t$ (sec)	Relative switching half-interval $n = \Delta t/\sigma_t$	No. of measurements	Relative statistical error, s (%)	Yield (% of total peak area)
1.1	2.2	1	9	2.50	48.7
	4.4	2	8	1.78	77.7
	6.6	3	6	0.94	93.6
	8.8	4	5	0.63	98.6
	11.0	5	6	0.42	100.2
	13.2	6	8	0.33	100.1
2.0	4.0	1	6	1.10	59.5
	8.0	2	5	0.75	92.4
	12.0	3	6	0.42	99.6
	16.0	4	5	0.28	99.8
	20.0	5	4	0.21	99.8

Fig. 3. Precision and accuracy of column switching in gas chromatography for an isolated peak. Measurements were carried out for peaks with standard deviations of 1.1 sec (●, ○) and 2.0 sec (▼, ▽). $2\Delta t$ = total switching time, with half-intervals of Δt sec symmetrical to peak maximum. Dotted line: limiting value of the relative error measured in single-stage operation. Closed symbols, relative error; open symbols, yield.

It can be seen that for a peak standard deviation of 2 sec the systematic error due to the switching operation reduces to below -0.5% if the switched fraction exceeds six times the peak standard deviation and has a symmetrical position to the centre of the peak. With a fraction size of ten times the peak standard deviation the transfer yield is 99.8%. For a peak standard deviation of 1.1 sec the systematic error is -1.4% for a fraction size corresponding to eight times the peak standard deviation.

It can also be seen in Fig. 3 that the statistical error decreases together with the systematic error and approaches a limiting value of 0.24% for a sufficiently large fraction size. The same precision was also found for single stage operation without switching.

The results indicate that the switching device has no effect on the precision and accuracy of the data in quantitative analyses, assuming that the fraction size is large enough. We recognize, however, that the precision depends primarily on the absolute fraction size in seconds. It seems that the minimum time interval for column switching without loss in precision is about 1 sec.

Fractionation of overlapped peaks

The choice of the size and position of a fraction becomes extremely important if an analyte is overlapped by matrix components. In such a case a compromise has to be found for the fractionation step after the first column. On the one hand, the analyte should be transferred to the second column by means of the switching device as completely as possible in order to achieve a high precision; on the other hand, the amount of overlapping matrix components should be reduced as much as possible in order to achieve a high accuracy. The first aim requires a large and the second a small fraction size. This situation requires the optimization of the fraction size depending on the sample.

Such an optimization will be demonstrated in the determination of a minor constituent in lavender oil. Even with high-efficiency open-tubular column gas chro-

matography this component appears in the chromatogram as a shoulder on a large peak. It can be assumed that the minor component can be isolated from the interfering large component by a two-stage operation applying relative enrichment. In order to combine high accuracy and high precision in the determination of this component, the optimum fraction size has to be used in the transfer from the first to the second separation stage.

In Fig. 4 the chromatograms obtained with different fraction sizes are shown, assuming a constant end-point of the fraction. It can be seen that the overlapping by the large peak can be reduced to an insignificant effect and practically complete isolation of the analyte peak can be achieved. It can also be seen that, as expected, the peak height of the analyte peak increases with decreasing fraction size, approaching a maximum value. Below the fraction size corresponding to the total amount of analyte the peak height starts to decrease, as only part of the analyte is transferred and detected. The effect of the overlapping is already neglegible for the fraction size corresponding to Fig. 4C, as can be concluded from the comparison of the peak heights in Fig. 4C and D.

The precision of the system and the relative independence of the results of slight variations in the position and size of fraction are shown in Fig. 5. We recognize the very good reproducibility of the peak height for the analyte although the patterns
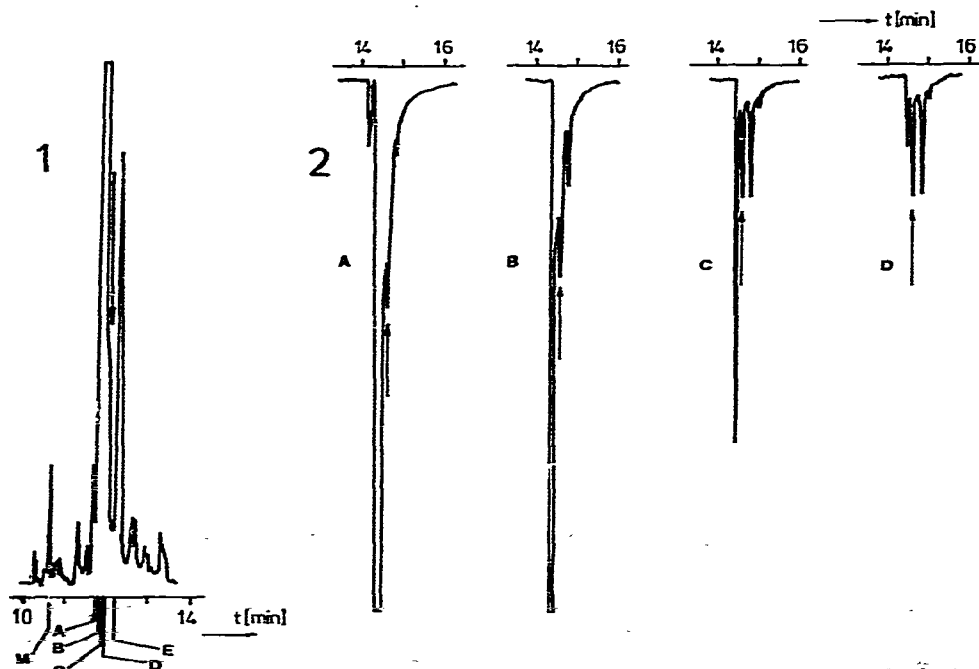

Fig. 4. Influence of the fraction size in column switching on the peak interference in the second stage. A minor component in lavender oil, indicated by arrows, appears as a shoulder in the chromatogram of column 1. The time, E, of the end of the switching interval was held constant at 97.1 sec, measured from the maximum of the marker peak, M. The start, A, B, C and D, of the switching interval was varied: A = 62.1 sec; B = 72.1 sec; C = 73.1 sec; D = 75.1 sec; measured from the peak maximum of M. 1, Part of the chromatogram from column 1, recorded on detector 1; 2, chromatograms of the different fractions, A–E, B–E, C–E and D–E, switched from column 1 to column 2 and recorded on detector 2. *t* = time.
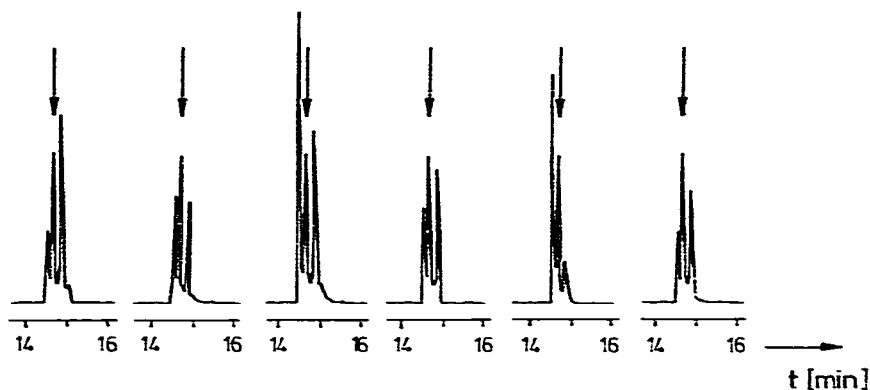
Fig. 5. Reproducibility of column switching in gas chromatography with open-tubular columns. The minor component, as shown in Fig. 4. was switched to column 2 with an average switching time interval of 23 sec. The position and size, respectively, of the switching interval were varied in the range of 2 sec. t = time.

of the chromatograms vary significantly owing to variations in the position of the fractions.

The evaluation of a large number of data leads to the conclusion that high precision and high accuracy can be obtained by choosing the optimum fraction size and position. An example of the choice of the optimum fraction is shown in Fig. 4, where it can be seen that in this instance the optimum switching interval is 23 sec, giving a precision of 1.2 % and an accuracy of 99.8 % for the relative peak height. The absolute values of precision and accuracy are less favourable owing to the sampling error.

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