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RAPID ANALYSIS WITH SHORT MICRO-PACKED COLUMNS

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SUMMARY

Rapid analysis can be achieved using very short micro-packed columns. Problems of mounting such columns of 0.8 mm I.D. and under one foot in length can be solved simply by use of the separation cassette technique, which allows fast insertion, exchange and back-flushing. To optimize rapid analysis by gas chromatography, the separation number per time unit, valid for the analytical range of interest over carrier-gas pressure and separation number, is an easily understood and useful parameter. Together with the retention index concept, all variables can be calculated in order to decide which short micro-packed column must be used for a given analytical problem and under which conditions of separation.

INTRODUCTION

The length of time available for the solving of problems is a factor which is of common concern to all and its cost is continually increasing. Therefore it is surprising that up till now, optimization of the minimum time necessary for analysis has not been considered to have priority over other factors in routine analytical work. The minimum time for solving a given separation has of course been discussed in detail and good examples of separations have been described for fast analysis by gas chromatography (GC)[1–7], but even the leading manufacturers of gas chromatographs and their data acquisition and processing systems, have not yet constructed acceptable instruments to meet the requirements for the fastest possible analysis by suitable separation systems. This is because the exchangeability of columns, necessary for optimization of the separation system for fast analysis, is poor and is also due to the time constants in detectors, signal cables, amplifiers, integrators, and data storage and processing systems, although modern techniques allow almost any speed of signal treatment at the rates possible in GC. This paper acknowledges that analysts have now realized the importance of the time factor in any one analytical run.

There are a number of reasons for the need for faster chromatography: (i) measurements must be repeated twice in order to obtain the minimum results for any conclusions to be drawn; (ii) three repeated measurements are the minimum for detecting trends or for checking the efficiency of sampling (two samples; two runs with sample one, one run with sample two); and (iii) calibration, the basis of analysis with known systematic errors, needs additional time, non-linear signals being more

widespread than is commonly recognized, and needing more thorough calibration. At least four additional runs are required in order for data to be significant for use in decision making, the ultimate aim of analysis. Making present day GC faster by a factor of five, therefore, would meet practical requirements. This can be done by achieving a higher separating power at the optimum separation conditions needed; higher flow-rates of the mobile phase than are commonly used; use of much shorter columns than is technically possible with present instruments; or by use of faster amplifiers and computer integrators than usual.

For over fifteen years micro-packed columns have been known to offer high separation power per unit length (allowing the use of short separation systems without serious loss of the much-needed separating power); high permeability of the mobile phase (allowing use of high flow-rates at the expense of causing a moderate back-pressure); fast temperature-programming rates and low mechanical mass (allowing the speed of separation to be increased by pressure and temperature programming without serious loss in the separating power caused by the formation of temperature gradients from the column wall to the centre of the packing). Many other aspects, all pertinent to the efficiency of micro-packed columns, have been discussed in the literature[7–10]. Before dealing with the details concerning the length of micro-packed columns and their separating power, as well as the method of installing a 100-mm micro-packed column in any existing GC instrument, the measures necessary in order to optimize, simply, speeded-up chromatographic separations are discussed.

Faster analysis needs very specific information but faster analysis also increases the information flow in bits/sec. Information can be given in bits and depends on some analytical factors:

$$\mathrm{Inf} = a \cdot TZ \frac{T}{t} \cdot \mathrm{ld} \frac{(c_{\mathrm{max.}} - c_{\mathrm{min.}}) \cdot \sqrt{n}}{\sqrt{2\pi e} \cdot s} \qquad (1)$$

where

a	= constant factor
TZ	= separation number, described later
T	= time available for repeated runs
t	= time needed for one run
ld	= *logarithmus dualis* (^{2}log)
$c_{\mathrm{max.}} - c_{\mathrm{min.}}$	= concentration range of the analytical procedure
s	= approximate standard deviation of the procedure
$\sqrt{2\pi e}$	= 4.13273.....
n	= number of runs.

Eqn. 1 combines all the facts important for obtaining accurate analytical information. A minimum of two runs is necessary to obtain data on the basis of which decisions can be made. The main parameter for checking data quality is the repeatability R, which is more informative than the standard deviation, s:

$$R = s \cdot \sqrt{2} \cdot t(P) \qquad (2)$$

where

R = difference between two results obtained in two runs, which fits 95 out of 100 measurements.

$t(P)$ = Student's factor, dependent on the number of repeated runs when measuring the approximate standard deviation of the analytical procedure.

Today, computer integrators have the calculating power to calculate automatically these main quality-control data. Statistical tests are the basis of all decision-making and without their use analytical results cannot be interpreted.

Experimental optimization of separations can easily be achieved by using a quality measure which fits the requirements of practice. Speeding-up the temperature programming as well as pressure programming can do much; therefore, the separation number, TZ, instead of any measure based on the theoretical plate height or plate number concept, is preferred. TZ can be measured in programmed runs, whereas measurements of plate number cannot.

$$TZ = \frac{t_2 - t_1}{b_{0.5(2)} + b_{0.5(1)}} - 1$$

where

t_1, t_2 = non-adjusted retention times

$b_{0.5(1)}$, $b_{0.5(2)}$ = peak widths at half height

TZ must be measured with a pair of homologues that have a "chromatographic distance" of 100 retention-index units.

The retention-index unit concept provides the best treatment of qualitative data in GC, and although many other systems fit special requirements, none of them is as informative as the index system. This is, of course, also true in the field of pesticide or steroid analysis, since calibrations independent of *n*-alkanes can be made and some key compounds can be analyzed relative to *n*-alkanes in the same separation system.

Eqn. 3 combines both the separation number concept and the index concept to explain the minimum separation quality that a column must have to separate compound 1, having retention index I_1, from compound 2, having retention index I_2, to a baseline separation, if the column is not overloaded and the concentration of substance 1 is equal to that of substance 2:

$$TZ_{\text{min.}} = \frac{100}{I_2 - I_1} - 1 \qquad (3)$$

EXPERIMENTAL

Glass columns of 0.8-mm I.D. were packed with 0.25 mm ± 0.02 mm Chromosorb W carrier particles to a length of 250 mm in a separation cassette. Impregnation in cassettes was done simply by pumping a 3% solution of FFAP liquid phase in methylene chloride for 10 h through the cassette, stopping the in-line impregnation and flushing with nitrogen until dry. One column was packed with Carbopack (Supelco, Bellefonte, Pa., U.S.A.) and 0.4% Carbowax 1540, to a length of 100 mm. Insertion of such short micro-packed glass columns in any GC instrument takes only a few seconds when the instrument is equipped with a separation cassette interface, which is a type of frame[11]. Exchange and back-flushing as well as "hinflush", a new

technique similar to a forward flushing technique, can be carried out in a few seconds when the column is cooled to 30–45° (ref. 12).

In order to find a possible speed-up optimum we started at a low carrier-gas pressure. For safety reasons nitrogen was used instead of hydrogen, despite the fact that hydrogen is the optimum carrier gas for rapid analysis, often giving a greater separating power in addition to increased speed. Table I gives the separation number *TZ* as a function of the carrier-gas inlet pressure *p*, the non-adjusted retention time of a peak at retention index 800, and the relation *TZ/t*; *TZ* is measured in the retention index range 700–800.

TABLE I

TZ–TZ/t RELATION AS A FUNCTION OF *p* IN MICRO-PACKED COLUMNS

Maximum = maximum speed for optimum separation.

p (bar)	*TZ*	*t (min)*	*TZ/t*	
Column: 100 × 0.8 mm I.D.; packing, Carbopack A with 0.4% Carbowax 1540; temperature, 120° isothermal.				
0.6	1.85	0.9	2.1	
0.8	1.67	0.7	2.39	
0.9	1.36	0.57	2.39	maximum
1.2	1.45	0.5	2.9	maximum?
1.4	0.72	0.4	1.8*?	maximum
1.6	0.77	0.37	2.1	maximum
1.8	0.77	0.33	2.3	
2.0	0.38	0.32	1.2	
2.2	0.52	0.3	1.7	
Column: 250 × 0.8 mm I.D.; packing, Chromosorb W with 5% Lutensol AP; temperature, 80° isothermal.				
0.2	2.5	3.3	0.75	
0.3	3.3	3.8	0.9	
0.4	3.3	2.6	1.3	
0.5	3.1	2.0	1.6	
0.6	2.8	1.5	1.9	
0.7	2.6	1.3	2.0	
0.8	2.7	1.2	2.2	
1.0	2.1	0.9	2.2	
1.2	1.8	0.8	2.3	
1.4	1.6	0.7	2.4	maximum
1.6	1.5	0.6	2.5	maximum
1.8	1.1	0.53	2.1	maximum
2.0	0.9	0.50	1.9	
2.2	0.9	0.47	1.9	
Column: 250 × 0.8 mm I.D.; packing, Chromosorb W with 5% FFAP; temperature, 80° isothermal; test substance, last peak, index 1200.				
0.2	2.7	3.1	0.9	
0.3	2.6	1.7	1.6	
0.4	2.4	1.2	2.1	
0.6	2.5	0.8	3.2	maximum
0.8	1.8	0.6	2.9	maximum
1.0	1.4	0.5	3.85	maximum
1.2	1.3	0.4	3.3	maximum
1.4	1.0	0.33	3.0	
1.6	0.9	0.3	3.0	
1.8	0.9	0.28	3.2	
2.0	0.46	0.25	1.8	

* *TZ* measurements could be repeated in this fast analysis with a standard deviation of $s = 1.5\%$ rel. at best and $s = 6\%$ rel. under worst conditions.

DISCUSSION

Fig. 1 shows the principle type of function when comparing the *TZ* and *t* data with the speed of mobile phase expressed as inlet pressure; Fig. 2 shows the relation

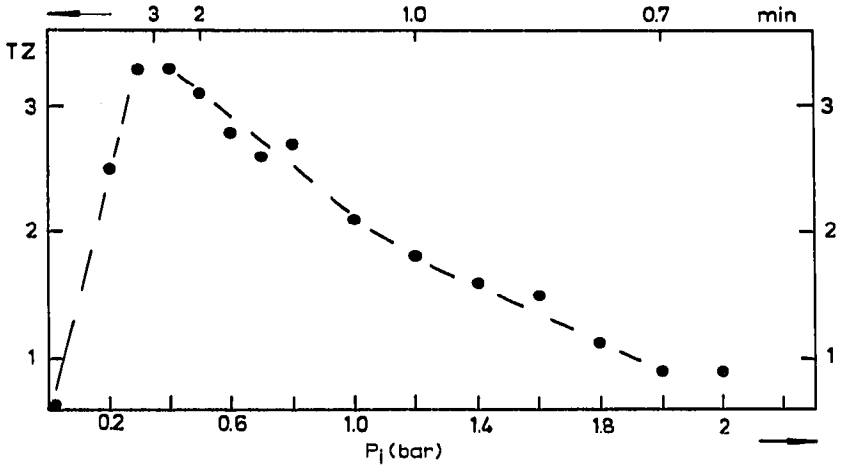

Fig. 1. Separation number, *TZ*, for non-adjusted retention time of the last peak with retention index 1200 and nitrogen inlet pressure p_i (bar). Column, 250 × 0.8 mm I.D.; temperature, 80° isothermal; packing, 5% Lutensol AP on Chromosorb W.

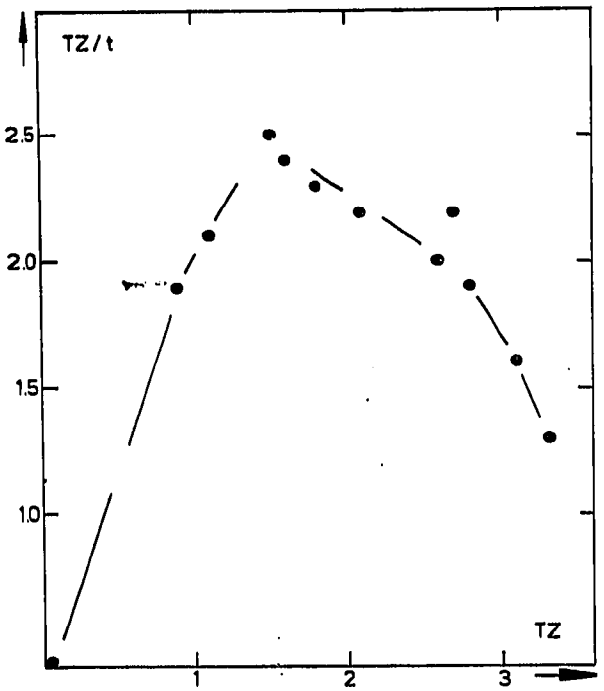

Fig. 2. *TZ/t versus TZ* for the last peak with retention index 1200. Optimum of speed of analysis at 2.5 peaks within 100 index units/min. Column conditions as in Fig. 1.

between TZ/t and TZ, a better measure for practical reasons. As it is necessary to maintain a certain limit on the separation power given as TZ, the limit of speeding-up is simply shown by the minimum TZ that is needed for a given separation problem.

The data in Table I are valid for isothermal separations. Isothermal separations are preferred when rapid analysis is being carried out because of time loss in the cooling period in programmed techniques. Pressure programming or simply back-flushing, if possible, is always better than temperature programming. It is simple to back-flush columns in separation cassettes by taking them out, turning and putting them back in, this being done in a few seconds.

As short open tubular columns very often also show extremely good conditions for fast analysis, and as there is practically no difference whether a short micro-packed column or a somewhat longer but relatively short open tubular column is installed in a separation cassette, this technique, being in addition absolutely free from dead-volume, offers many advantages over the classical technique for speeding up GC.

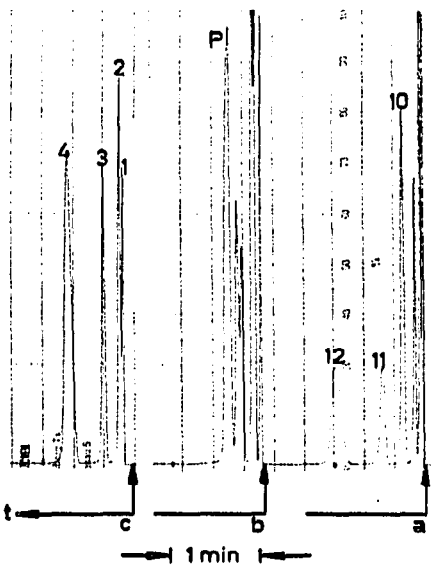

Fig. 3. The use of short micro-packed columns. (a) Hydrocarbons C_6–C_{12} at 80°. Nitrogen inlet pressure, 1 bar; column, 250 × 0.8 mm I.D. packed with 5% Lutensol AP on 80–100 mesh Chromosorb W. 10, 11, 12 = C_{10}, C_{11}, C_{12} *n*-alkanes, respectively. (b) Rohrschneider–McReynolds polarity mixture of benzene–*n*-butanol–methyl propyl ketone–nitropropane–pyridine (P). Conditions as in (a). (c) Methanol = 1, ethanol = 2, propanol = 3, *n*-butanol = 4. Conditions as in (a), except temperature is 70°.

By using a separation phase which permits the lowest separation number to be used, by working at a carrier-gas pressure which just keeps the minimum separation number (cassettes are kept secure to over 1000 p.s.i., as long as the column wall does not break) and by simply back-flushing (in order to elute the heavy ends) one avoids unnecessary waiting time which may otherwise prolong a separation. Fig. 3 gives an example of the appearance of chromatograms on 250-mm micro-packed columns.

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