EVALUATION OF GLASS CAPILLARY COLUMNS FOR GC-MS APPLICATIONS

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Introduction

The combination of gas chromatography (GC), mass spectrometry (MS) and a computer system (COM) is one of the most powerful tools in analytical chemistry. The effective utilization of the expensive equipment involved is essential, so that all parts and techniques should be optimized with regard to versatility and reliable performance.

Gas chromatography, especially using capillary columns, is an utmost efficient technique for the separation of mixtures.Gas chromatography suits to separate the components of highly complex mixtures often consisting of several hundreds of components. The only information for the identification of a separated compound, however, is its retention time, which is dependent on the type of column, its temperature and carrier gas flow rate. Unambiguous identification of the separated compounds merely by their retention times is seldom possible and particularly in case of complex mixtures other analytical techniques may be needed, e.g. NMR, UV, MS.

Mass spectrometry is a very sensitive and powerful analytical technique, which allows the rapid determination of a complete spectrum of a substance in the submicrogram range. Usually the mass spectrum gives clear and unequivocal indication of the structure or identity of the compound measured. Often, however, the mass spectrum alone is not sufficient, and other analytical techniques must be used. Especially with complex mixtures the extreme separation ability of gas chromatography complements the high specificity of the mass spectrometer.

In order to achieve the optimum performance of a GC-MS system the gas chromatographic columns and operating conditions have to be selected with utmost care taking the quality of sample components to be separated into account.

This paper is a report on our results concerning the evaluation of glass capillary columns for GC-MS applications.

Although successful and high-efficiency capillary column separations were

achieved more than 18 years ago, discussion still continues on the versatility and usefulness of capillary columns in practical gas chromatographic analysis.

In the last five years, considerable progress has been made especially in glass capillary column technology and advanced methods for their application [1—10].

The application of capillary columns in GC-MS techniques has received much attention recently, because of some distinct advantages in comparison to packed columns [9, 11—13]. The main advantages of using capillary columns in GC-MS combinations are as follows: 1. Higher separating power, 2. Inert surfaces, 3. Faster analyses, 4. Low flow-rate of carrier gas, 5. Higher sensitivity of the system.

Basic requirements

An ideal glass capillary column is characterized by:

1. Stability of the liquid film at the highest operating temperature (temperature stability)

2. The highest possible resolution for a given column length (Separating power)

3. Minimum rest adsorption on the surface, no irreversible sorption of compounds of different polarities (Quality, inertness)

Different methods and test mixtures for evaluating glass capillary columns have been reported in literature. In the early publications mainly the number of theoretical plates per meter (N/m). the separation number (SN or TZ) and the coating efficiency were used for characterizing the column performance [14—17]. In some cases different columns were evaluated at different operating ranges inhibiting comparison of the results.

Recently more elaborate and complex methods have been suggested for the comparison and evaluation of glass capillary columns [7, 10, 18—21]. The most comprehensive quality test for glass capillary columns has been published by GROB, GROB and GROB [22].

In spite of these efforts there is no standard or generally accepted test method for the comparison and evaluation of glass capillary columns.

Theoretical

Without going into details of theory, some of the fundamental expressions and considerations should be discussed in order to introduce the methods applied for the evaluation of glass capillary columns.

1. Temperature stability

The temperature stability of a capillary column can be assessed by estimating the loss by bleeding of stationary liquid via measurement of the background signal by using a sensitive (generally FID) detector.

The temperature stability of a column is of paramount importance in GC-MS application because the bleeding products leaving the column enter the ion source of the mass spectrometer increasing the background noise and decreasing the sensitivity of the system by deposition on the analyzer.

In addition, column characteristics will deteriorate because of the loss of the stationary liquid which can have an influence on separation efficiency, surface deactivation and polarity of the column.

For the above reasons only very carefully conditioned, well stabilized capillary columns can be used for GC-MS applications. The stability of the column should be checked before use with some sort of bleeding test.

2. Separating power

Column efficiency is generally given by the number of theoretical plates (N). The most widely used expressions for the determination of N are:

$$N = 16\left(\frac{t_R}{w_b}\right)^2 = 5{,}54\left(\frac{t_R}{w_h}\right)^2 = \left(\frac{t_R}{\sigma^2}\right)^2 \qquad (1)$$

where t_R the retention time (or distance) of a given compound

w_b peak width at base
w_h peak width at half height
σ standard deviation ($w_b = 4\,\sigma$)

For characterizing the column the number of theoretical plates per meter N/L are generally used.

The number of theoretical plates gives only the efficiency of a column in terms of the band broadening of a compound during its passage through the column.

The separating power of a column for two adjacent components can be described by the resolution R of the two peaks.

By using the peak characteristics taken from the chromatogram:

$$R = \frac{2(t_{R2} - t_{R1})}{w_{b1} + w_{b2}} \approx \frac{t_{R2} - t_{R1}}{w_{b2}}\,. \qquad (2)$$

In literature also other expressions are used for characterizing the separating power.

Kaiser introduced the "Separation number" (SN) (Trennzahl, TZ):

$$SN = \frac{t_{R2} - t_{R1}}{w_{h1} + w_{h2}} - 1 \tag{3}$$

the number of peaks separated between two consecutive n-alkanes.

The correlation with resolution reads:

$$SN = 0.8495\, R - 1 \tag{4}$$

Hurrel and Perry used the "Effective peak number" EPN:

$$EPN = \frac{2(t_{R2} - t_{R1})}{w_{b2} + w_{b1}} - 1 \tag{5}$$

that is

$$EPN = R - 1. \tag{6}$$

The last two expressions have been demonstrated to be only modified forms of the resolution equation (2) and there is no reason for using them instead of R.

All the better because R can be expressed by using the fundamental equation of chromatography as follows:

$$R = \frac{N^{1/2}}{4}\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{k'}{1 + k'}\right) \tag{7}$$

where α relative retention of the two components investigated

$$\alpha = \frac{k'_2}{k'_1}$$

k' capacity ratio of the more retained component

$$k' = \frac{t_R - t_M}{t_M}$$

where t_M inert peak retention time.

Eq. (7) is the basic and most widely used expression for characterizing and optimizing the column perforance.

The first term of Eq. (7) contains the number of theoretical plates N as a measure of column efficiency i.e. peak broadening.

The second term concerns the selectivity of the given stationary phase. If T = constant, α is constant.

The third term represents the capacity of the column. As this term increases with increasing k', the actual k' value has a great influence on resolution.

Column performance may be characterized by the "Resolution rate" introduced by AVERILL [15]:

$$RR = \frac{R}{t_R}\,\mathrm{min}^{-1} \tag{8}$$

which includes also the necessary time for a given resolution i.e. the rate of analysis.

3 Quality of column, inertness

As regards the quality of column, quite a number of aspects and requirements are found in literature. For this reason a high number of testing procedures and test substances have been suggested.

The most important requirement for the column is exemptness of spots where a pronounced or irreversible sorption of the sample components might occur. On the one hand, such active spots may cause band broadening and distortion of peak shapes decreasing separation efficiency, and on the other hand, certain types of compounds may partially or completely disappear owing to irreversible adsorption.

Peak distortion can be characterized by some asymmetry factor determined from the peak geometry. The most appropriate method seems to be the application of statistical moments. The skew of a Gaussian peak can be given:

$$\text{skew} = \frac{\mu_3}{\mu_2^{3/2}} \tag{9}$$

where μ_2 second moment
μ_3 third moment

The irreversible adsorption can be measured by using a "polarity mixture" containing different types of highly polar compounds and measuring the peak height or peak area relative to one or some reference compounds.

In addition to the above requirements the polarity of a column should also be checked. In case of a given stationary phase column polarity may vary depending on the batch-to-batch quality of the phase, the material and pretreatment of column tubing, film thickness, conditioning of column and so on.

Column polarity can be characterized by the retention indices of different types of compounds or by the relative retentions of the consecutive members of homologue series.

Experimental

Peak widths obtained on high-efficiency capillary columns are in the order of seconds or even less. The source of error of manual evaluation of such sharp peaks on the basis of recorded chromatograms — which was a common practice several years ago — renders it meaningless and makes comparison almost impossible.

For this reason digital data acquisition and computer evaluation are the most favourable means for evaluating of glass capillary columns.

Instrumentation

Gas Chromatograph: Carlo Erba Mod. 2452 (FID)
Injector: Grob-type splitless-split
Carrier gas: Hydrogen
Sample: 0.1—1.0 microliter
Data acquisition: Alpha 16
Data evaluation: Wang 2200 VP

The test mixtures and testing conditions used for column evaluation are given in *Table 1.*

Table 1

Test mixtures and testing conditions

Mixtures	1	2	3	4
	n-Hydrocarbons	*Polyaromatics*	*Fatty acid methyl esters*	*Polarity mixture*
	0.1% n-C_{11} 0.1% n-C_{12} 0.1% n-C_{13} 0.1% n-C_{14} 0.2% n-C_{15} 0.2% n-C_{17} in hexane	0.1% 2,3-DM-naphtalene 0.1% anthracene in mixture 1.	0.1% C_{10} FAME 0.1% C_{11} FAME 0.1% C_{12} FAME 0.2% C_{14} FAME 0.1% n-C_{12} 0.1% n-C_{14} 0.2% n-C_{17} in hexane	n-C_{11} 1-octanol nonanal 2,6-DM-phenol 2,6-DM-aniline C_{10} FAME in acetone
Conditions				
temperature, °C	isotherm 90, 110	isotherm 130	isotherm 130	programmed 50 → 150
carrier flow, cm/s	14.6—35.7	18.9—41.5	18.9—41.5	50

The main characteristics of the capillary columns investigated are summarized in *Table 2.*

The first three columns having been prepared with the same apolar stationary phase (OV-1), but with different surface treatment before coating are shown in Table 2. Column 4 was coated with SE-52, a slightly more polar phase than OV-1, column 5 with Carbowax 20 M, a moderately polar phase.

All the four test mixtures were separated on each column and the peak characteristics were compared.

Table 2

Column characteristics

Number	1	2	3	4	5
Type	WCOT	WCOT	SCOT	WCOT	WCOT
Liquid phase	OV-1	OV-1	SP-2100	SE-52	CW 20 M
L(m)×I. D.(mm)	10×0.30	18.5×0.27	12×0.5	15×0.3	17×0.27
Surface treatment	$BaCO_3$	HCl + HF	porous layer	$BaCO_3$	HCl + HF
Stabilization temperature, °C	270	270	270	250	180
Duration, H	24	24	24	24	24

Before starting sample introduction all columns were carefully conditioned and stabilized under the conditions given in Table 2.

Temperature stability of columns was checked by a simple bleeding test. The column was heated up to the maximum operating temperature. Then heating was switched off and the column was rapidly cooled (in 3—5 minutes) to 40—50 °C. During the cooling step the baseline of the FID detector was continuously recorded. For a well stabilized column the baseline shift during the cooling step must not exced 5—6 mm. If higher shift was observed, conditioning was continued accordingly.

Results and discussion

Separating power

To compare the separating power of the columns investigated, the efficiency and resolution data were calculated from the measurements on test mixtures 1 and 2 for the consecutive members of n-alkanes. The data calculated are summarized in *Table 3.*

Table 3

Efficiency and resolution ($k' = 5$)

Column	1	2	3	4	5
N	15 160	51 208	14 124	23 190	16 014
N/L	1 516	2 768	1 177	1 546	942
R	12.7	23.5	12.1	15.7	11.9
$R/\sqrt{L}$	3.9	5.3	3.4	3.9	2.9
RR	4.1	3.4	3.3	3.7	1.7
$RR/\sqrt{L}$	1.3	0.78	0.95	0.95	0.40
α	1.96	1.95	1.96	1.96	1.82

The number of theoretical plates per meter (N/L) seems to differ considerably, firstly because of the differences in column diameter (0.27—0.5 mm) and secondly because of the different pretreatment techniques and the differences in film thickness.

Resolution R was calculated according to Eq. (7). The relative retention values for the n-alkanes are seen in the table to be the same for the first four columns and somewhat lower for the Carbowax 20 M column. It means that the resolutions obtained for the first four columns can be directly compared.

In *Fig. 1* R values calculated for the column investigated have been plotted as a function of capacity ratio k'. At low k' values R is seen to increase

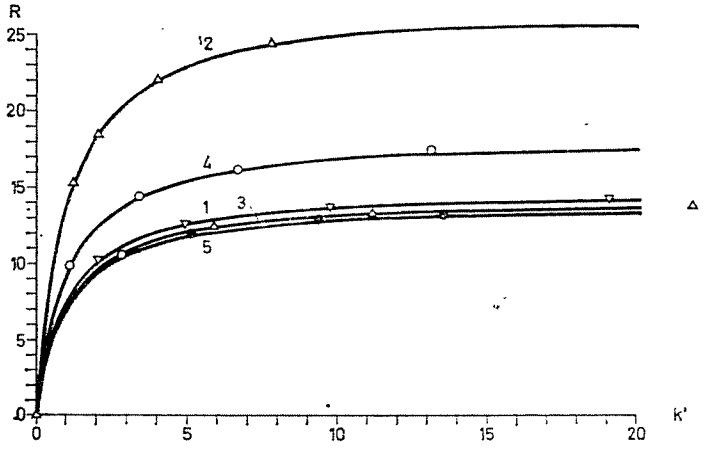

Fig. 1. Resolution of adjacent n-alkanes vs. capacity ratio k'

steeply with increasing k'. At about $k' \geq 5$ the curves will flatten and R changes very slightly with increasing k'. It was a general problem in early publications that columns were compared and evaluated at different k' values rendering a meaningful comparison impossible.

For the above reason column efficiency and separating power should always be measured and evaluated for $k' \geq 5$. Different columns can be compared only at the same capacity ratio k'.

The data given in Table 3 refer to characteristics at $k' = 5$.

An other controversial point in literature is the comparison of columns of different lengths. Resolutions obtained for different columns cannot be directly compared. Since the resolution is proportional to the square root of column length, columns of different lengths should be compared by using the resolution divided by the square root of column length:

$$\frac{R}{\sqrt{L}}. \tag{10}$$

In *Fig. 2* $R/\sqrt{L}$ values have been plotted as a function of capacity ratio k'. The shape of the curves obtained are the same as in Fig. 1, but the relative values are somewhat changed. As regards separating power the best column is Nr. 2. Columns 1 and 4 show the same resolution. Column 3 has the largest diameter (0.5 mm) and consequently the lowest efficiency and separating power.

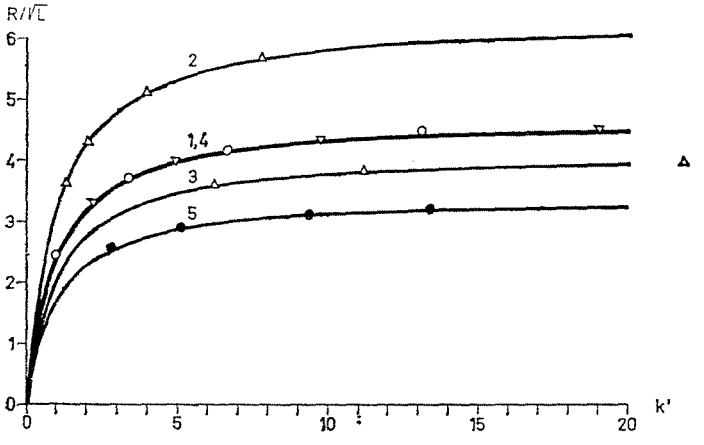

Fig. 2. Resolution of adjacent n-alkanes divided by the square root of column length vs. capacity ratio k'

If the rate of analysis is of some interest, for instance in routine analysis of a high number of samples, column performance can be characterized by resolution rate RR given in Eq. (8) Comparison of columns of different length can be carried out again by using RR divided by the square root of column length:

$$\frac{RR}{\sqrt{L}}. \tag{11}$$

This plot is shown in *Fig. 3* as a function of capacity ratio k', the numerical data are given in Table 3. From the curves of Fig. 3 two conclusions can be drawn. First, the maximum resolution rate, that is the maximum resolution in a given time can be achieved at $k' = 1$. Second, the order of the columns is changed with regard to the maximum resolutions obtained.

Quality of column

In order to characterize peak asymmetry the second and third moments were calculated and the skew values determined according to Eq. (9) from measurements carried out with test mixtures 2 and 3. Measurements were carried out under isothermal conditions because temperature programming has also a considerable influence on peak shape and tailing characteristics.

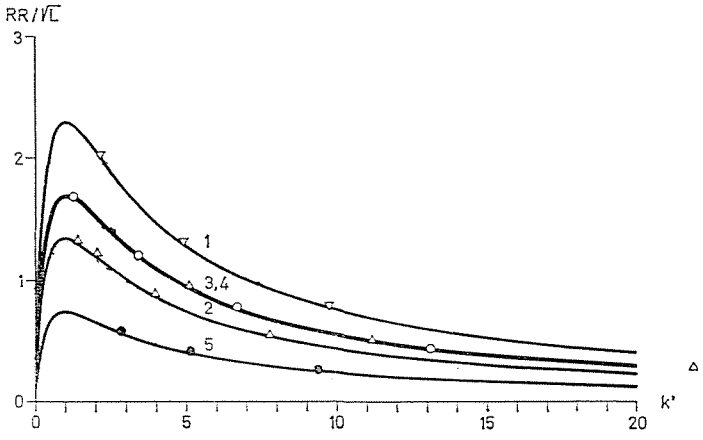

Fig. 3. Resolution rate divided by the square root of column length vs. capacity ratio k'

The aromatics used for the test are sensitive to metal adsorptive spots, the fatty acid methyl esters are generally used for characterizing column quality [6, 8, 22].

The data summarized in *Table 4* can be regarded only as rough approximations of peak distortion. Precision and accuracy of peak shape characterization

Table 4

Peak asymmetry (SKEW)

Column	1	2	3	4	5
n-C_{14}	0.359	−0.028	−0.076	0.210	0.271
DM-naphtalene	0.215	−0.095	0.186	0.470	—
Anthracene	−0.173	−0.335	—	−0.016	—
C_{10} FAME	0.818	0.169	2.006	0.777	−0.070
C_{11} FAME	—	−0.243	1.864	1.097	−0.362
C_{12} FAME	1.325	−0.582	1.622	0.953	−0.185
C_{14} FAME	−0.419	−0.505	1.217	−0.053	—

depends on data acquisition rate. Higher order moments and parameters which are sensitive to peak tailing require about 50 data points per peak for accurate peak shape characterization. For several reasons this high data acquisiton rate was not achieved in our measurements and the skew values calculated are taken as qualitative indications of peak distortion.

Nevertheless, by comparing the skew values for the different columns some very informative conclusions can be drawn.

For aromatics the skew values are practically the same for all columns, there are no significant differences.

For fatty acid methyl esthers the lowest skew values i.e. the most symmetrical peaks were obtained on columns 2 and 5 which have no support layer and were prepared with the same technique, by HCl + HF etching. Next columns 1 and 4 follow prepared by the deposition of a thin $BaCO_3$ layer on the surface. The highest skew values are obtained for column 3 which is a SCOT column prepared with a fairly thick support layer.

These results indicate that the deposition of a solid layer may have an influence on peak symmetry because of pronounced adsorption on some active spots.

The most important requirement of column quality i.e. the inertness of the surface was tested by using the polarity mixture introduced by Grob. The chromatograms were taken with a temperature program according to Grob and coworkers [22].

In order to achieve a uniform evaluation the peak heights were measured and referred to the peak height of n-undecane. The data are given in *Table 5*.

Table 5

Quality test with polarity mixture relative peak height (to n-C_{11})

Column	1	2	3	4	5
n-C_{11}	1.00	1.00	1.00	1.00	1.00
1-octanol	0.33	0.32	0.0	0.12	0.44
Nonanal	—	0.81	0.0	0.69	0.47
2,6-DM-phenol	0.93	0.65	0.57	0.49	0.26
2,6-DM-anilin	0.56	0.07	0.87	0.24	0.26
C_{10} FAME	0.80	0.51	0.58	0.58	0.57

1-octanol and nonanal are seen to completely disappear on column 3 indicating the presence of active siloxyl (or hydrogen bonding) groups and other adsorptive spots. The decrease of the phenol peak height indicates some basicity of the column.

Columns 1 and 4 show the same trends, column 1 giving the better characteristics. Because of the $BaCO_3$ treatment some acidity remained but there are also some basic spots as general with this type of columns.

Column 2 shows good characteristics, only the acidity of the column is too high indicated by the low values of the aniline peak.

Column 5 shows somewhat different characteristics, acidic and basic spots are in nearly equal amounts, but the peak heights are low compared to the best values obtained.

The values in Table 5 suggest that columns should be tested with the type of compounds to be separated because they may have different types of active spots causing irreversible sorption of certain compounds depending on the techniques of pretreatment and preparation of the capillary column.

The polarity of the columns was compared by calculating the retention indices of the aromatics and the fatty acid methyl esters as well as by calculating the relative retentions for adjacent n-alkanes and fatty acid methyl esters.

The data of these calculations are given in *Table 6*. As the first three columns were prepared with the same stationary phase these can be directly compared.

Table 6

Polarity of columns

Column	1	2	3	4	5
	Retention indices (130 °C)				
n-C_{14}	1400.00	1400.00	1400.00	1400.00	1400.00
DM-naphtalene	1416.59	1414.62	1415.33	1447.52	—
Anthracene	1711.22	1709.27	—	1724.26	—
C_{10} FAME	1312.45	1304.48	1315.55	1332.26	1600.48
C_{11} FAME	1410.51	1399.59	1416.49	1427.83	1699.99
C_{12} FAME	1508.32	1502.49	1515.94	1522.02	1800.17
C_{14} FAME	1690.03	1691.76	1714.37	1674.13	—
	Relative retention (α)				
n-C_{14}/n-C_{13}					
90 °C	1.96	1.95	1.96	1.96	1.82
110 °C	1.80	1.81	1.78	1.83	1.71
130 °C	1.70	1.68	1.70	1.71	1.62
C_{12}/C_{11} FAME					
130 °C	1.73	1.70	1.71	1.80	1.62

There is a scatter of retention index values, the greatest difference being 24 index units for C_{14} FAME. In spite of this scatter some definite trends can be observed. The highest index values are obtained for column 3, probably due to the additional effect of support layer. Column 1 ($BaCO_3$ layer) shows intermediate values, the lowest values are obtained on column 2 containing no solid layer.

The selectivities of the columns are practically the same as seen from the relative retentions given.

For column 4 and 5 the retention indices are higher because of the different quality of stationary phases.

The determination of retention index values is of practical importance if literature data are to be used for identifying unknown components. In this case measurements should be carried out with a number of compounds of given types and the literature data should be checked, or if necessary corrected according to the measured values.

Conclusions

Before GC-MS application each capillary column should be carefully tested.

Conditioning and stabilizing of a column should be carried out with utmost care and the baseline shift detected by the bleeding test must be negligible.

Separating power of columns should be measured and compared at the same capacity ratio $k' \geq 5$.

Resolution of columns of different length should be compared by using the resolution divided by the square root of column length.

Quality of columns should be tested by measuring peak distortion and irreversible adsorption for different test substances.

Peak distortion can be characterized by the skew calculated from the second and third moments.

The shape of a peak is not sufficient for detecting adsorption because irreversible adsorption may also occur. The Grob polarity mixture and the measurement of peak heights relative to a completely eluted component furnish relevant information with regard to the type and extent of irreversible adsorption on the column.

The quality of column much depends on the tehniques of pretreatment and preparation. Columns coated with the same stationary phase but with different pretreatment techniques may show quite different characteristics.

The selection of a column for a given GC—MS application has to rely on the tests indicated taking the boiling range and chemical type of substances to be investigated into account.

Summary

A method was described for evaluating glass capillary columns for GC-MS applications. Each column should be tested before GC-MS measurements for temperature stability, separating power and column quality (inertness). Four test mixtures have been used under different operating conditions. Data were collected by digital data acquisition and were evaluated on a Wang 2200 computer. Five columns prepared by different techniques were compared and evaluated. The columns have shown different characteristics depending on the pretreatment applied.

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