CHROM. 14,729

ROUTINE QUANTITATIVE GAS CHROMATOGRAPHIC ANALYSIS OF PESTICIDES FOR QUALITY AND PRODUCTION CONTROL USING CAPILLARY COLUMNS AND ON-COLUMN (SYRINGE) SAMPLING*

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(Received December 28th, 1981)

SUMMARY

Quantitative routine analyses of a technical fungicide have been performed and also an inter-laboratory data comparison was carried out using capillary columns and the on-column (syringe) sampling technique. The precision and accuracy of the data obtained with on-column sampling were compared with those obtained by split sampling. The influence of the various sampling parameters on the precision and accuracy, such as solvent volatility, injector and column temperature and adsorptivity of the column support surface, was studied.

INTRODUCTION

Capillary columns allow high-efficiency and therefore also high-resolution separations. In practice, adequate resolution only is necessary for the separation and reliable determination of all significant components of a mixture including those which are present in minor concentrations. A high column separation efficiency may also help to reduce analysis times and to increase signal-to-noise ratios in the analysis for trace components, because of the steeper peak profiles obtainable.

For the routine chromatographic analysis of industrial samples for quality and production control purposes, the following requirements have to be met: (a) the information about the qualitative composition of a certain product has to be as complete as possible (including the information about the trace components that may, for example, have a high toxicity); (b) the quantitative data about the product composition must be of high, if not of ultimate, precision and accuracy for inter-laboratory comparisons, and also for economic and/or environmental reasons; and (c) the overall analysis times should not be too long, in order for results to be obtained rapidly and for optimal utilization of instrument and manpower capacities.

The samples, the quantitative and qualitative gas chromatographic (GC) analysis of which is considered in this paper, preferably contain a major component or their isomers in high concentrations (between 50 and 100 %). In some instances such com-

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* Dedicated to Professor Dr. mult. Otto Bayer on the occasion of his 80th birthday

pounds may also be diluted in solvents to give concentrations of about 0.5%. Impurities that may also be present in such products, but in much lower concentrations (*e.g.*, 0.1% and less), must first be separated from the main components and subsequently identified and determined. The relative standard deviations of the repeatability of quantitative data on the major components (which may be present in concentrations between 1 and 99%) should be as low as 0.5–1.0%. With packed columns, such low relative standard deviations are considered common, whereas with capillary columns greater difficulties are expected. Indeed, a good knowledge and some experience are necessary with respect to the various steps of such GC analyses in order to achieve high performance and high resolution. Errors may arise as a result of irreversible adsorption on the support surface within the column and inadequate detection and data processing. The total content of the stationary phase in capillary columns of the commonly used length and inner diameter is as low as a few milligrams, depending on the film thickness, which is usually in the range 0.1–1 μm. Both the advantages and the disadvantages of capillary columns are related to this general feature.

Certain pesticides are highly polar, not very volatile and thermally and catalytically unstable in many instances. With such compounds, the lowest possible column temperatures have to be applied in order to avoid decomposition during sampling or in the column during the chromatographic process. Thermally labile compounds have to be analysed by liquid chromatography (LC), provided that sensitive detection and sufficient resolution can be achieved. Low column temperatures in GC can only be applied, however, if capillary columns with a low content of stationary phase are used, otherwise the retention times would become too long. Moreover, non-polar or weakly polar stationary phases, such as alkylpolysiloxanes, are to be preferred in order to decrease intermolecular interactions of the stationary phase with these polar solutes and thus to avoid long residence times of the solute within the column or too high column temperatures.

Sometimes, however, selectivity and also resolution for certain pairs of solutes have to be sacrificed if a non-polar stationary phase is used. Short retention times can be attained by decreasing the column length at the expense of the separation efficiency, which may become insufficient for the minimum resolution required. Short retention times can be obtained by using thin films of the stationary phase and also by using hydrogen as the carrier gas, which gives separation times about three times shorter than those obtained with nitrogen and argon. Helium can also be used, but the retention times are not as short as those with hydrogen. The application of hydrogen may be considered to be an explosion hazard within an industrial environment, and appropriate precautions should be taken.

Non-polar or weakly polar stationary phases, such as alkylpolysiloxanes, have been used for the more polar types of compounds without difficulty only recently. For such columns complete deactivation of the usual support surfaces in glass capillaries is necessary in order to prevent irreversible adsorption, which causes too high losses of the significant solute at very low column loads, of a few nanograms and less. Complete deactivation of such surfaces before the final coating with temperature-stable alkylpolysiloxanes of the gum type is effected by silanization[1,2], PSD* treat-

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* PSD = polysiloxane degradation.

ment[3,4] and related techniques. In addition, the much higher separation efficiency achievable with capillary columns and the effective deactivation of the support surfaces within alkylpolysiloxane columns, low temperatures in the column (and therefore also low sampling temperatures with on-column sampling) can be applied because of the small amount of stationary phase present. This is advantageous for the following reasons: less decomposition of labile sample components, higher selectivity for the resolution of component pairs with similar retentions[5] and less decomposition of the stationary phase, *e.g.*, less bleeding and noise for low detection limits.

Considering the progress described above, we report in this paper on the cooperative work of two laboratories (Bayer and the Max-Planck-Institut), both using capillary columns for routine quantitative analyses of the same technical product at high precision and accuracy. In this connection, different techniques suitable for introducing the sample into the chromatographic system are discussed with special regard to the types of sample to be analysed.

Sampling techniques for quantitative analyses of technical products using capillary columns[6] can be considered as follows. The sample capacity of capillary columns is so low that either the classical split mode or the on-column (direct) mode of introduction[7] of highly diluted samples has to be applied to the type of mixtures specified above. In most instances, in both techniques the original sample has to be diluted with a suitable solvent, either for better manipulation or because the sample is solid or has to be homogenized. With on-column injection, further dilution is necessary in order to achieve a not too high column load in the nanogram range. Volumes lower than 0.2 μl cannot be sampled with high reproducibility when using the usual syringes.

As has been pointed out by Schomburg and co-workers[6,8], Grob and Grob[9,10], Munari and Trestianu[11] and Galli *et al.*[12], various parameters may influence the performance of the sampling procedure with regard to resolution and quantitation. Discrimination of sample components of either low or high volatility and poor precision of relative peak areas are the major problems in quantitation. With split sampling, these parameters are: volatility range of sample components, volatility of solvent or other major components of sample, temperature of injector, temperature of column, splitting ratio and sampling volume, geometry of injector, means of homogenization and type of carrier gas. With on-column sampling, fewer parameters are of importance: temperature of column and the connected inlet valve system, volatility and polarity of solvent or other major components, sampling volume and purity of solvent.

For the comparative assessment of the two sampling techniques, it is also important to realize that many practical samples contain involatile residues. Involatile compounds may also be formed shortly after the injection within the vaporization insert of the splitting device or the column inlet in on-column sampling. With split sampling such deposits remain in the injector insert and do not enter the column. They can be easily removed by exchange or cleaning of the insert after a certain series of injections. Nevertheless, in many instances numerous repeated injections can be executed before cleaning of the vaporization insert becomes necessary. This possibility does not exist with on-column sampling, because the sample enters the column directly. The column inlet could be cleaned by rinsing with solvent if the new cross-linked ("chemically bonded") types of columns are used, but in most instances the

first part of the columns has to be broken off and removed to provide for a fresh, uncontaminated column inlet.

EXPERIMENTAL AND RESULTS

For comparative purposes, a typical sample from the Bayer laboratories in Wuppertal was selected. The active ingredient of the product Baycor® (proposed common name Bitertanol) is a fungicide of the triazole type with the following structure:
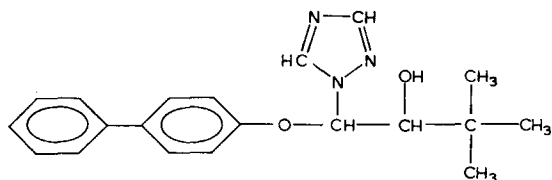
Its low volatility requires a column temperature of about 260°C, even with capillary columns. In our experiments, the sample was diluted in three solvents of different volatility and diisooctyl phthalate was added to the solution as an internal standard to calculate the "absolute" content of the two significant (isomeric) species in the technical product and to determine the relative response factors of these components for calibration purposes.
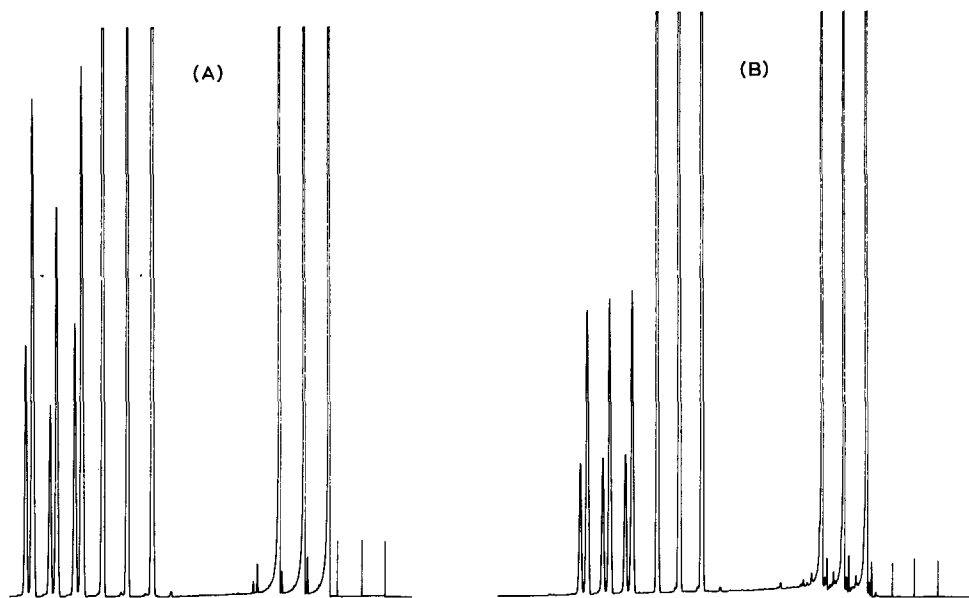
Fig. 1. Isothermal separation of Baycor with split sampling. Solvents: (A), acetone, b.p. 56°C; (B) methyl laurate, b.p. 262°C. Sample: 3 × 0.2 μl each. Column: 30 m OV-101, dealkalinized, PSD treated, soft glass. Temperatures: column, 260°C isothermal; injector, 280°C; detector, 320°C. Carrier gas: 0.78 bar He. Analysis time: 10 min. Solutions: (A) 37.365 mg Baycor, 44.480 mg diisooctyl phthalate, 1058.1 mg acetone; (B) 21.270 mg Baycor, 40.350 mg diisooctyl phthalate, 1304.9 mg methyl laurate.
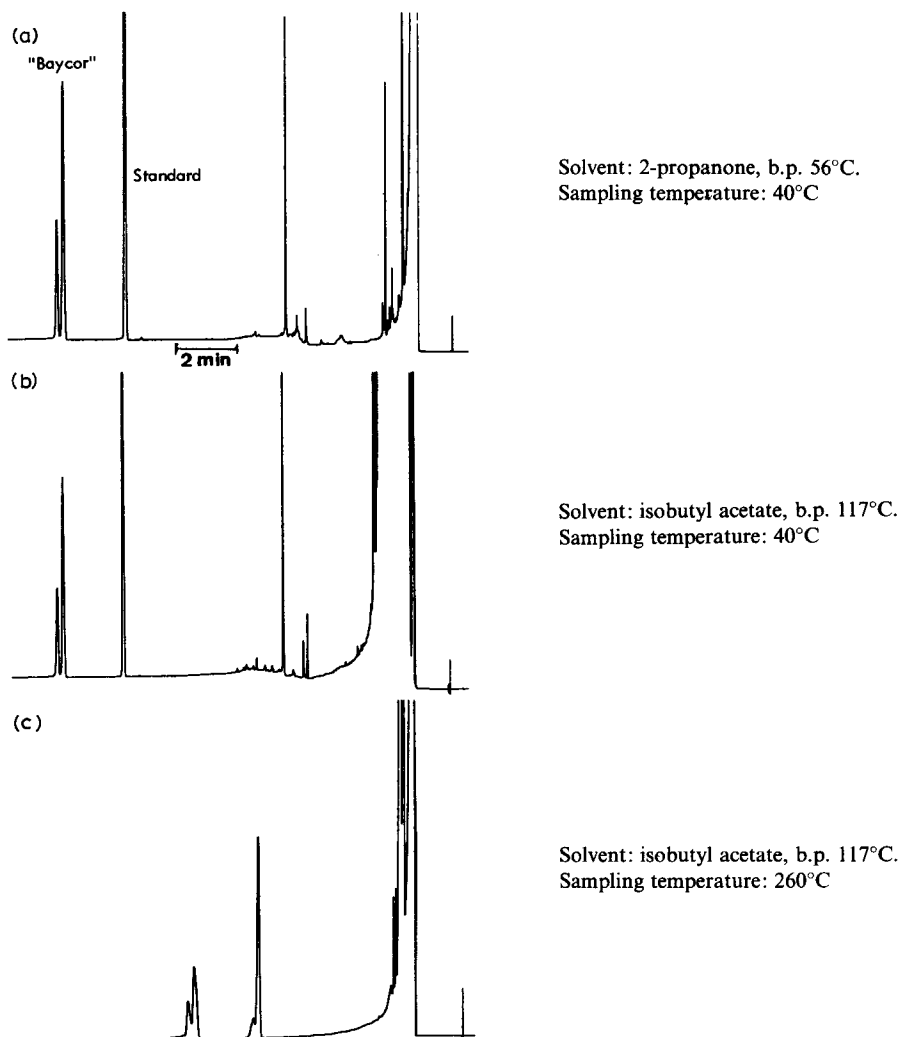
Fig. 2. Influence of solvent volatility and column temperature on resolution with on-column (syringe) sampling. Sample: 0.2 μl *ca.* 4 % Baycor and *ca.* 4 % diisooctyl phthalate (diluted 1:400). Column: 30 m × 0.27 mm I.D. OV-1, dealkalinized alkali glass. Carrier gas: 0.78 bar He, flow-rate 27 cm/sec. (a) Temperature, 40–260°C at > 40°C/min; solvent, 2-propanone (b.p. 56°C). (b) Temperature, 40–260°C at > 40°C/min; solvent, isobutyl acetate (b.p. 117°C). (c) Temperature, 260°C, isothermal; solvent, isobutyl acetate.

Optimization of the chromatographic separation was achieved as follows. With split sampling and using isothermal column operation, an injector temperature slightly higher (280°C) than the column temperature (260°C) was applied. The detector temperature was 320°C and flame-ionization detection was used exclusively. The column dimensions were 25–30 m (length) and 0.27 m (I.D.). A smaller inner diameter could not be used because on-column sampling was also to be applied in these series of experiments. The non-polar stationary phase methylpolysiloxane OV-1 was select-
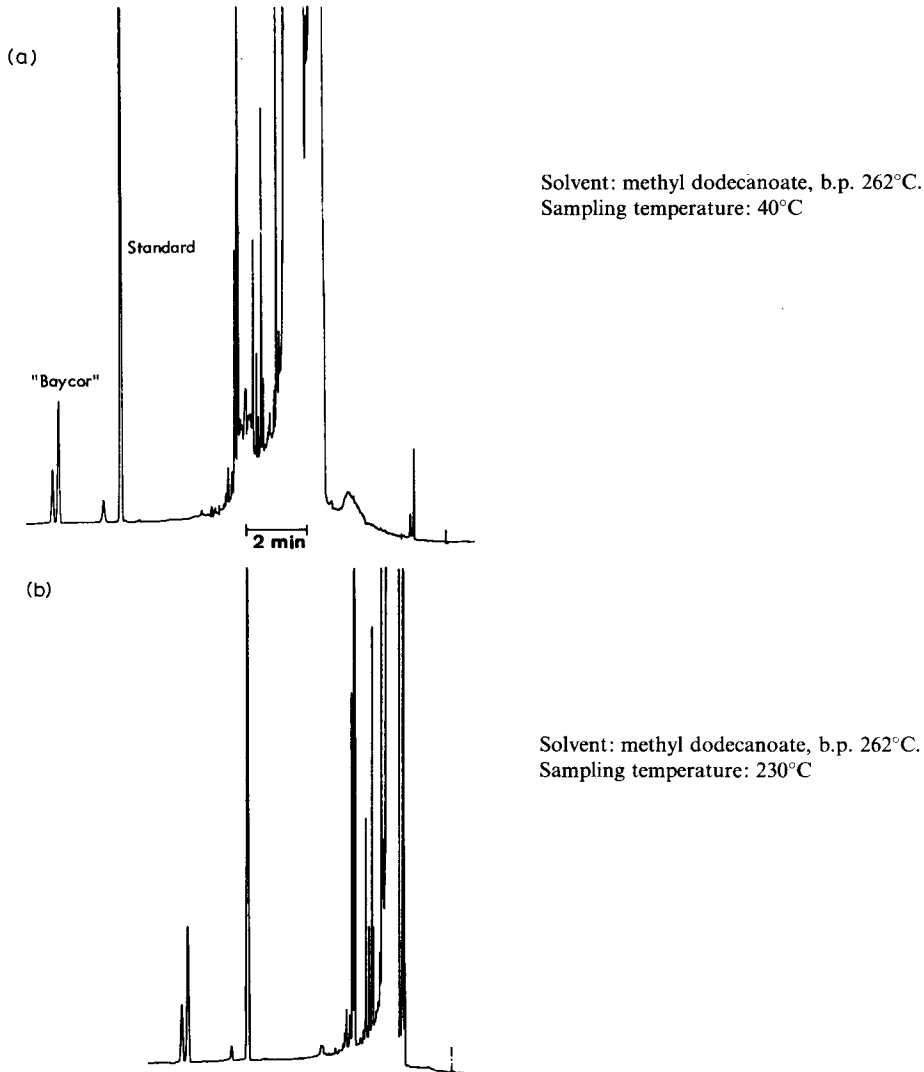
(a)

Standard

"Baycor"

2 min

Solvent: methyl dodecanoate, b.p. 262°C.
Sampling temperature: 40°C

(b)

Solvent: methyl dodecanoate, b.p. 262°C.
Sampling temperature: 230°C

Fig. 3. Influence of solvent volatility and column temperature on resolution with on-column (syringe) sampling. Sample: 0.2 μl *ca.* 3 % Baycor and *ca.* 4 % diisooctyl phthalate (diluted 1:400). Column: 30 m × 0.27 mm I.D. OV-1, dealkalinized alkali glass. Carrier gas: 0.78 bar He, flow-rate 27 cm/sec. (a) Temperature, 40–260°C at 40°C/min; solvent, methyl dodecanoate (b.p. 262°C). (b) Temperature, 230–260°C at 40°C/min; solvent, methyl dodecanoate (b.p. 262°C).

ed for the reasons mentioned above. The splitting ratio for the already diluted sample (4–5 % in solvent) was adjusted to 1:280. With on-column sampling, the initial column temperature was 40–50°C (*i.e.* the lowest temperature achievable in the column oven without additional cooling) when the syringe needle was introduced into the column inlet. Such low sampling temperatures are essential, especially when solvents such as the volatile acetone are used. After injecting the sample, the column temperature was increased as quickly as possible (*ca.* 40°C/min) up to the final level of 260°C.

For on-column sampling, the sample had to be further diluted (1:400) in order to achieve the optimal column load because lower volumes, such as 0.1–0.2 μl, cannot be sampled reproducibly enough by the syringe technique, using a Hamilton 701 SN syringe.

The chromatograms obtained with split and on-column sampling are given in Figs. 1–3. Three different solvents were used with both techniques. Fig. 1 shows the chromatograms of two series of three consecutive injections of an acetone and a methyl dodecanoate solution. Complete resolution of the isomers was attained and the solvent peaks showed only slight tailing. The chromatograms in Figs. 2 and 3 illustrate the influence of the solvent volatility and column temperature on the resolution and the peak shape with on-column injection. With isobutyl acetate (b.p. 117°C) as the solvent a column temperature of 260°C proved to be too high, as a lower resolution and irregular peak shapes were observed, whereas with methyl dodecanoate at 230°C (i.e., 30°C below the boiling point of the solvent) the same performance was achieved as at ambient temperature.

The chromatograms obtained with on-column injection also illustrate how many impurities are present in the usually available "pure" solvents. Difficulties with overlapping of the peaks of significant sample components with those of solvent impurities may occur at the very high dilutions applied. Solvents with low boiling points usually have higher purities, and are therefore to be preferred in routine GC analysis, although sampling of mixtures containing large amounts of volatile solvents may become a source of error in quantitation.

Table I gives the operating parameters for the optimized GC separations from which the results in Table II were obtained. Table II gives results for the quantitation of the two Baycor isomers, obtained with both split and on-column injection. The syringe technique of Grob and Grob[9,10] and Galli et al.[12] was used. The relative response factors (internal standard: diisooctyl phthalate), the isomer ratio and the corresponding relative standard deviations (calculated from five measurements) were determined.

With split sampling the following conclusions can be drawn from the results. The relative response factors are only slightly dependent on the type (volatility) of solvent, the average value being 1.28 for both techniques. The relative standard deviations of the relative response factors are strongly dependent on the type of solvent, decreasing from a high value of 4.5% for the volatile acetone to 0.7% for the less volatile methyl dodecanoate. The relative standard deviations of the isomer ratio, for which no influence of discrimination is to be expected because of their structural similarity, are about 1% and are independent of the type of solvent used.

With on-column injection using a sampling temperature of 40–50°C, relative standard deviations of the relative response factor always lower than 1%, independent of solvent volatility, are attained.

Inter-laboratory comparison of quantitative data obtained in analyses of a pure Baycor and a technical product of lower purity

A test mixture containing pure (ca. 100%) Baycor and acetone of standard purity (98.8%) was analysed at the Max-Planck-Institut für Kohlenforschung in two different non-polar methylpolysiloxane (OV-1) columns, borosilicate glass (Duran) and fused silica (FS) being the capillary (support) material. With both columns, the

TABLE I

QUANTITATIVE GC ANALYSIS WITH HIGH PRECISION AND ACCURACY USING GLASS CAPILLARY COLUMNS; SPLIT *VERSUS* ON-COLUMN* (SYRINGE) SAMPLING

The GC parameters listed gave the results reported in Table II.

Parameter	Conditions	
	Split sampling	On-column sampling
Column	30 m × 0.27 mm I.D. methylpolysiloxane OV-1, HCl-dealkalinized alkali glass, PSD treated (polysiloxane degradation)	
Temperatures:		
Injector	280°C	
Column	260°C	40°C → 260°C (rapid increase to column temperature after sampling)
Detector	320°C	320°C
Sampling	0.2 µl at a splitting ratio of 1:280	0.2 µl of same sample diluted 1:400
Sample	About 2–4 % of each Baycor and internal standard in specified solvent	

* Carlo Erba Model 4160.

TABLE II

INFLUENCE OF SOLVENT VOLATILITY AND SAMPLING TECHNIQUE ON PRECISION AND ACCURACY

Results obtained using the conditions specified in Table I.

Sampling mode	Solvent	Boiling point (°C)	RRF*	RSD (%)**	Isomer peak-area ratio	RSD (%)**	Sample component weight (mg)		
							Baycor	Standard	Solvent
Split	Acetone	56.2	1.248	4.47	2.182	1.18	37.365	44.480	1058.1
	Isobutyl acetate	117	1.296	1.26	2.191	0.67	40.686	39.116	1288.8
	Methyl dodecanoate	262	1.274	0.71	2.167	1.09	21.270	40.350	1304.9
On-column (syringe)***	Acetone	56.2	1.293	0.65	2.180	1.25	37.365	44.480	1058.1
	Isobutyl acetate	117	1.278	0.39	2.186	0.89	40.687	39.116	1288.8

$\star$ RRF = relative response factor = $\dfrac{\text{area of standard} \times \text{weight of substance}}{\text{area of substance} \times \text{weight of standard}}$.

$\star\star$ RSD = Relative standard deviation (>5 injections).

$\star\star\star$ Sample diluted 1:400 in solvent.

TABLE III

INTER-LABORATORY COMPARISON OF RELATIVE RESPONSE FACTORS (RRF) OF THE FUNGICIDE BAYCOR AS A FUNCTION OF COLUMN LOAD AND TYPE OF COLUMN

The weights used were as follows. Calibration: 0.2037 g Baycor (100%), 0.2090 g internal standard, *ca.* 6 g acetone. Analysis: 0.2070 g Baycor (sample), 0.2081 g internal standard, *ca.* 6 g acetone.

Parameter	MPI*	Bayer**
Column	A: 25 m × 0.27 mm I.D. methylpolysiloxane OV-1, Duran, HF, PSD treated B: 23 m × 0.32 mm I.D. methylpolysiloxane OV-1, fused silica, PSD treated	C: 30 m × 0.27 mm I.D. methylpolysiloxane SE-30, alkali glass, silanized
Carrier gas	0.52 bar H_2	He
Temperature (°C)	50–260°C at 30°C/min	50–260°C at 30°C/min

Dilution	Column load (ng)	RRF (100% Baycor) MPI (column A)	RRF (100% Baycor) Bayer (column C)	RSD (%)*** MPI	RSD (%)*** Bayer	Baycor (sample) concentration (%) MPI (column A)	Baycor (sample) concentration (%) Bayer (column C)	RSD (%)*** MPI	RSD (%)*** Bayer
1:40	250	1.129	1.148	0.21	0.17	96.62	96.6	0.30	0.34
1:80	125	1.142	1.160	0.12	0.14	96.50	96.9	0.25	0.18
1:160	62.5	1.159	1.140	0.19	0.22	96.42	96.6	0.43	0.25
1:250	40	—	1.159	—	0.31	—	—	—	—
1:480	20	—	—	—	—	98.77	—	0.37	—
1:960	10	1.208	1.178	1.14	1.14	—	—	—	—
		(column B)							
1:2000	5	1.172	—	0.36	—				
1:20,000	0.5	1.251	—	0.67	—				

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** Bayer AG, Wuppertal, G.F.R.

*** RSD = Relative standard deviation.

column load was decreased from 250 ng of Baycor to 10 ng (Duran column) or from 5 to 0.5 ng (FS column) (see Table III). With the Duran (borosilicate) column, for which (in spite of proper deactivation) a more adsorptive support surface can be presumed, an increase in the relative response factor was already observed with a column load of 10 ng (1.21 compared with 1.15). The relative standard deviation also increased considerably (1.1 % in comparison with 0.2 %) for several reasons, such as increased adsorption and impeded peak area determination because of too low signal-to-noise ratios. With the more inert fused silica column, an appreciable increase in the relative response factor was observed with a column load of only 500 pg, although the relative standard deviation did not increase higher than 0.67 %. The influence of the column adsorptivity on the relative response factors also depends on the difference in the polarities of the solute (Baycor) and the internal standard used. With a less polar internal standard such as an alkane, an even greater increase in the relative response factors will be observed.

A technical Baycor sample of unknown purity was analysed under the same conditions using the previously determined relative response factors. In measurements with three different column loads between 480 and 40 ng, a purity of about 96.50 % was obtained (see Table III). The relative standard deviations for the three series of experiments were about 0.4 % on average. At the Bayer laboratories Baycor contents of about 96.7 % and relative standard deviations of about 0.3 % were found, which can be considered excellent for the analysis of such types of sample.

CONCLUSION

The results show that with the application of modern types of capillary columns, together with on-column (syringe) injection, quantitative data of high precision (with relative standard deviations as low as 0.5 % and less) and accuracy can be achieved. Such low relative standard deviations of the relative peak areas can also be attained with split sampling if all of the parameters are properly optimized. With split sampling it is of advantage that sample constituents of low volatility are deposited in the vaporization insert and do not contaminate the column. Low-boiling solvents cannot be applied if high injector temperatures are necessary owing to the low volatility of the significant sample components itself; increased relative standard deviations are the consequence. The "cold injection" technique described recently by Poy et al.[13] and Schomburg[14] may overcome some of these difficulties.

With on-column sampling the column temperature has to be adapted to the volatility of the sample itself and also to the solvent used because otherwise difficulties arise in the period of introduction of the syringe needle into the column. Disadvantages of on-column sampling are the possible contamination of the column inlet by involatile compounds originating from the sample and the necessary high dilution of "concentrated" samples with solvents as pure as possible in order to attain appropriate column loads. Pure solvents with varying volatility are not always available, however. In any other respect, on-column sampling is the superior technique.

It has also been shown again that capillary columns can be used with great advantage for quantitative analyses of high precision and accuracy. High resolution and high signal-to-noise ratios in trace analysis can be easily obtained. Low column temperatures are characteristic of the capillary GC of less volatile samples.

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