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"DIRECT" (ON-COLUMN) SAMPLING INTO GLASS CAPILLARY COLUMNS

COMPARATIVE INVESTIGATIONS ON SPLIT, SPLITLESS AND ON-COLUMN SAMPLING

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SUMMARY

On-column (direct) sampling is superior to other techniques of sampling into capillary columns, especially for analyses with quantitative objectives, for example the precise and accurate determination of relative response factors. Discrimination by volatility of sample constituents is minimal and only slightly dependent on solvent volatility and column inlet temperature, but is independent of the type of carrier gas. Prior heating of the sample for vaporization before entering the column is avoided and decomposition of labile sample components is reduced to a minimum. The performances of two different on-column sampling techniques for quantitative analysis were compared. A new construction of an on-column sampling device for very low sample volumes (column loads) is described, which allows for the introduction of volumes as low as a few nanolitres of samples into capillary columns.

INTRODUCTION

With "direct" or on-column sampling the liquid sample can be introduced into narrow-bore (0.20–0.35 mm I.D.) capillary columns without any additional heating of either the sampling device or the column inlet itself, thus avoiding thermal or catalytic decomposition of sensitive sample constituents. No volatilization of the sample into the carrier gas before entering the column is carried out and no splitting in the gas phase, which could give rise to changes in composition of that part of the sample which enters the column, is applied. Moreover, compounds of very low volatility and high polarity enter the column directly and without losses or retardation caused by condensation and/or adsorption in insufficiently heated regions such as connecting tubing. For on-column sampling several techniques for sample introduction and/or volume adjustment have been described. Two different techniques have been previously proposed by the present authors for either small (< 100 nl) or large (500–2000 nl) sample volumes[1]. As an alternative, Grob and co-workers[2,3] proposed the use of a special device for on-column introduction through a microsyringe. Temporary additional cooling of the sampling device including the column

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inlet during the introduction of the syringe needle into the column was applied by Galli *et al.*[4] in order to prevent selective vaporization of the sample, *i.e.*, discrimination.

The macroversion of the Max-Planck-Institut (MPI) technique described earlier[1] makes use of a small crucible in which the sample is placed. The column inlet dips into the liquid sample during the sampling procedure, the liquid being transferred into the column by carrier gas pressure. The sample volume can be adjusted with a reproducibility of only about 5% relative. Volumes as low as 500 nl cannot be easily sampled by this technique. In the microversion technique a narrow-bore glass or metallic capillary pipette is introduced into the capillary column in a similar manner as the syringe needle technique proposed by Grob and Grob, Jr.[2]. Small volumes of 5–20 nl can be sampled with this technique, but the sample volume cannot be adjusted as easily as with a syringe. Micropipettes of different volumes have to be used in order to achieve variable sample volumes.

Considering the requirements for the practical analysis of various kinds of samples and the capabilities and features of classical sampling techniques (of both the split and splitless types), it would be desirable if the following requirements for versatile on-column sampling techniques be met:

(1) For the separation of highly dilute solutions of the species of analytical interest in volatile solvents, maximal sample volumes of up to 2 μl have to be introduced into (glass) capillary columns with a diameter between 0.25 and 0.35 mm. The sample volume should be easily adjustable at least in the range between 0.5 and 2 μl.

(2) For less dilute samples the minimal volumes that have to be introduced should be as low as 10–50 nl in order to attain optimal column loads even when using capillary columns with extremely thin films of the liquid stationary phase ($\leqslant$100 nm).

(3) Generally good precision (low standard deviation of repeatability) and accuracy of gas chromatographic (GC) analysis via peak area percentages as well as a reasonable adjustability of the sample volume are desirable.

In this paper we also describe a new construction of the microversion of our on-column injection device that involves splitting of the liquid sample at the column inlet, which allows for the introduction of sample volumes (column loads) of less than 200 μl.

COMPARATIVE MEASUREMENTS OF THE QUANTITATIVE PERFORMANCE OF ON-COLUMN SAMPLING

On-column sampling: crucible technique, MPI versus Grob's syringe technique with additional cooling (Carlo Erba)

We determined response factors of methyl octanoate (b.p. 192.9°C) relative to *n*-dodecane (b.p. 216.3°C) as the standard compound comparing the two on-column sampling techniques. The MPI macroversion (crucible) was coupled with a Varian 2700 instrument, whereas the Carlo Erba (CE), (Milan, Italy) on-column injector was part of a CE 4160 instrument. A 1-μl volume of a mixture of methyl octanoate and *n*-dodecane in cyclohexane was injected in both instances into a 20-m polypropylene glycol column (I.D. 0.28 m) at a column temperature of 80°C, which was increased to 130°C at a programming rate of 10°C/min. The results of these measurements are given in Table I.

Using the crucible technique, a relative standard deviation of the absolute

TABLE I

DETERMINATION OF RELATIVE RESPONSE FACTORS (RRF) OF METHYL OCTANOATE

Determined using on-column (crucible) and on-column (syringe) sampling. Sample: 1 μl of methyl octanoate + *n*-dodecane in cyclohexane; column: PPG, 20 m × 0.28 mm I.D.; temperature: 80–130°C, 10°C/min; carrier gas: N_2, 0.25 bar; instruments: on-column (MPI), Varian 2700; on-column (CE), Carlo Erba 4160.

Material	*Crucible*		*Syringe*	
	Peak area	*Relative S.D. (%)*	*Peak area*	*Relative S.D. (%)*
n-Dodecane	27.747	2.46	27.550	0.56
Methyl octanoate	19.342	2.60	20.293	0.81
RRF	1.551	0.25	1.468	0.14

peak areas of both components of about 2.5% was observed on repetitive injection. The ratio of peak area to grams of substance (relative response factor by weight) for the ester and the standard was found to be 1.55 with a relative standard deviation of 0.25%. For the CE sampling unit (which was equipped with a device for column inlet cooling) the average relative standard deviation of the "absolute" peak areas was 0.7%; the response factor was 1.47 with a relative standard deviation of only 0.14%. The difference in the relative response factors obtained is due to the influence of the different detector types on the response in the instruments used.

Even for the macroversion the standard deviations of the absolute and relative peak areas are surprisingly low. The syringe technique is superior to the technically simple crucible technique with regard to the standard deviation of the absolute peak areas (3–4 times lower) and also with regard to the adjustability of variable sample volumes.

On-column sampling (CE, syringe) versus split sampling using a standard splitter. Influence of parameters with split and splitless sampling

A C_{18}–C_{36} alkane mixture containing the 10 even-numbered alkanes in about the same concentrations, diluted in *n*-octane (b.p. 126°C), was used for discrimination measurements. A 1-μl volume of this sample was injected into the split injector at a splitting ratio of about 1:35, the injector temperature being 300°C. Hydrogen was used as the carrier gas and the column temperature was 120°C in each instance. With sampling parameters such as an injector temperature of 300°C, a boiling point of the solvent (*n*-octane) of 126°C and hydrogen as the carrier gas, strong discrimination is to be expected, as already pointed out previously[1,5].

The mode of injection and the speed of extrusion of the liquid sample from the syringe needle into the vaporization insert also influence the discrimination under the specified conditions (see also Grob, Jr. and Neukom[6]). By using "fast" rather than "slow" injection less discrimination was found with both split and the splitless sampling especially if not too narrow bore insert tubes were used.

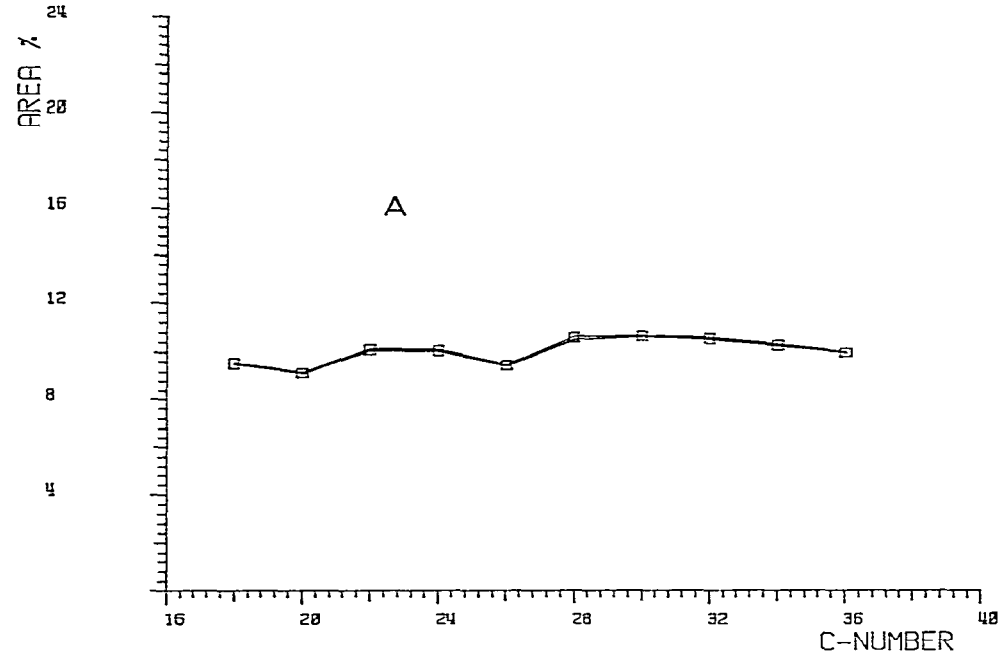
For the experiments on the performance of the on-column sampling the original mixture was diluted a further 35-fold with *n*-octane. Thus the same column load for the C_{18}–C_{36} alkanes as in split sampling at a splitting ratio of 1:35 was achieved. A 1-μl volume was injected in both instances with a 10-μl syringe. The results of these measurements are given in Table II and Fig. 1.

TABLE II

COMPARATIVE MEASUREMENTS OF DISCRIMINATION WITH ON-COLUNN AND SPLIT SAMPLING

Peak areas (%) of C_{18}–C_{36} alkane test mixture.

Sampling	*Parameter*	C_{18}	C_{20}	C_{22}	C_{24}	C_{26}	C_{28}	C_{30}	C_{32}	C_{34}	C_{36}	*Rel. peak area ratio,* C_{18}/C_{36} *(%)*
Split sampling with vaporization insert	$\bar{M}$	9.760	9.048	9.695	9.427	9.039	10.140	10.658	10.905	10.822	10.507	0.93
80 × 3.9 mm I.D.	S.D.	0.312	0.251	0.190	0.103	0.120	0.173	0.159	0.141	0.128	0.119	
glass-wool packing	Rel. S.D. (%)	3.19	2.77	1.96	1.09	1.32	1.70	1.50	1.29	1.18	1.13	
Split sampling with vaporization insert	$\bar{M}$	21.068	16.588	14.623	11.351	8.564	7.569	6.351	5.355	4.561	3.971	5.31
80 × 1.7 mm I.D.,	S.D.	1.505	0.997	0.548	0.334	0.408	0.526	0.554	0.563	0.548	0.554	
glass-wool packing	Rel. S.D. (%)	7.14	6.01	3.75	2.94	4.76	6.96	8.72	10.52	12.00	13.95	
On-column	$\bar{M}$	9.502	9.115	10.073	10.038	9.408	10.582	10.606	10.519	10.226	9.933	0.96
	S.D.	0.026	0.013	0.022	0.030	0.018	0.063	0.026	0.027	0.039	0.031	
	Rel. S.D. (%)	0.27	0.14	0.22	0.30	0.19	0.60	0.24	0.25	0.38	0.31	



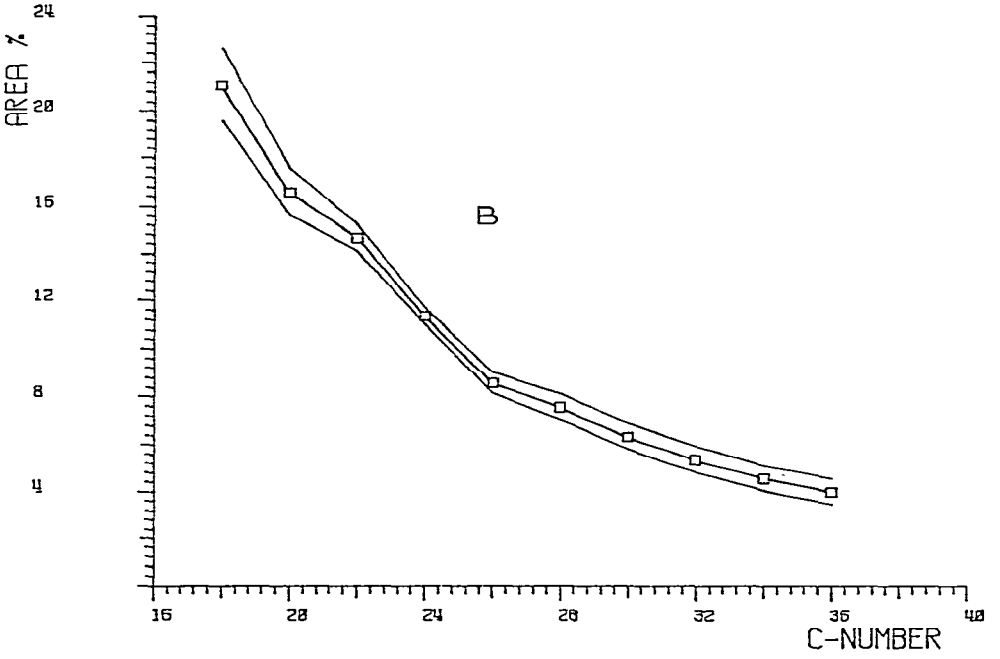


Fig. 1.

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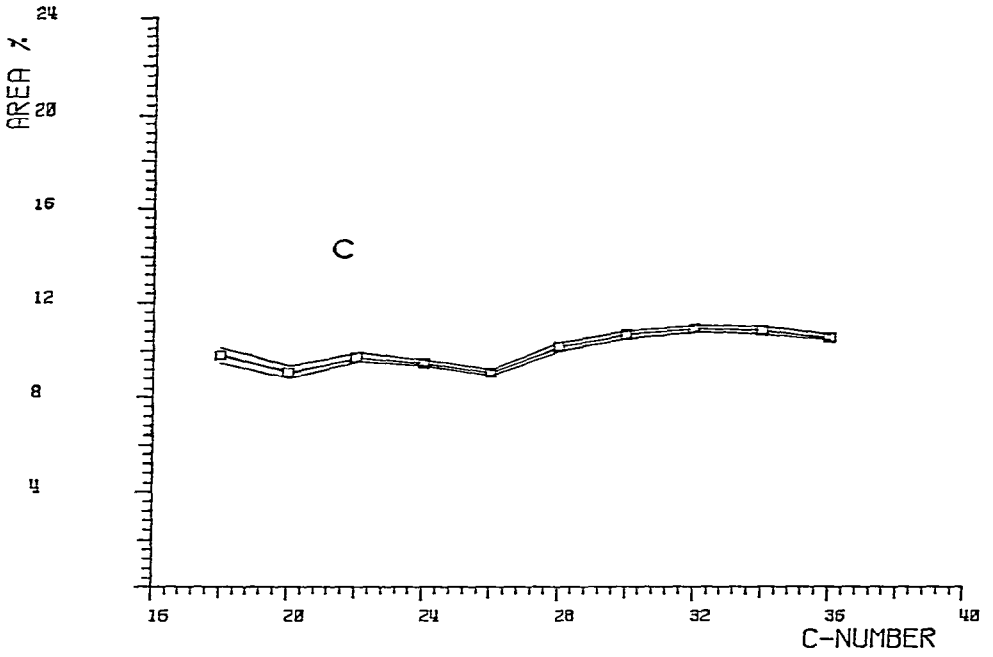

Fig. 1. Comparative measurements of discrimination with on-column and split sampling. (A) On-column (CE) sampling (sampling at column temperature). (B) Split sampling (narrow bore insert, 1.7 mm), glass-wool packing, "fast" injection. (C) Split sampling (wide bore insert, 3.9 mm), "fast" injection. Sample: 1 μl C_{18}–C_{36} alkanes, diluted in *n*-octane (b.p. 126°C); column: methylpolysiloxane (SE-30), I.D. 0.28 mm; temperatures: injector, 300°C; column, 120–300°C, 10°C/min; carrier gas: H_2, 0.65 bar, $\bar{u}$ = 50 cm/sec; split flow-rate: 80 ml/min; column flow-rate: 2.5 ml/min; instrument: Carlo Erba 4160 including sampling device.

The comparison of the relative peak areas normalized to 100% and of the corresponding standard deviations (S.D.) obtained with the different techniques allows the following conclusions to be drawn. With on-column injection of the original sample, the expected normalized peak areas of about 10% for each component were found, the relative standard deviation for repetitive injection being as low as 0.3% on average for the ten components. No appreciable dependence on solvent volatility or type of carrier gas could be observed.

Using the split mode of sampling with a narrow-bore vaporization tube packed with glass-wool, extremely high discrimination was observed under the given conditions, in spite of the fast mode of sample injection that had been applied. The ratio of the C_{18} and C_{36} alkane peak areas was 5.3 compared with 0.96 with on-column injection and the corresponding standard deviations for repetitive sampling were as high as 8% on average. The standard deviations may also be assessed from Fig. 1, in which the parallel lines indicate the positive and negative deviations.

This extremely strong discrimination is due to the high sampling temperature in the injector (300°C), the carrier gas used (hydrogen) and the low boiling point of the solvent, *n*-octane (126°C). In a previous publication[5], we demonstrated that by decreasing the splitter temperature and by increasing the boiling point of the solvent

discrimination can be completely eliminated. We also found that the discrimination in nitrogen as the carrier gas is considerably lower than in hydrogen. Discrimination can therefore also be avoided by using nitrogen in the sample introduction procedure only. After a few minutes hydrogen was used as the carrier gas for the completion of the separation by switching of the carrier gas inlet to a supply of hydrogen. The influence of the various parameters of split sampling can be assessed from the chromatograms in Fig. 2.

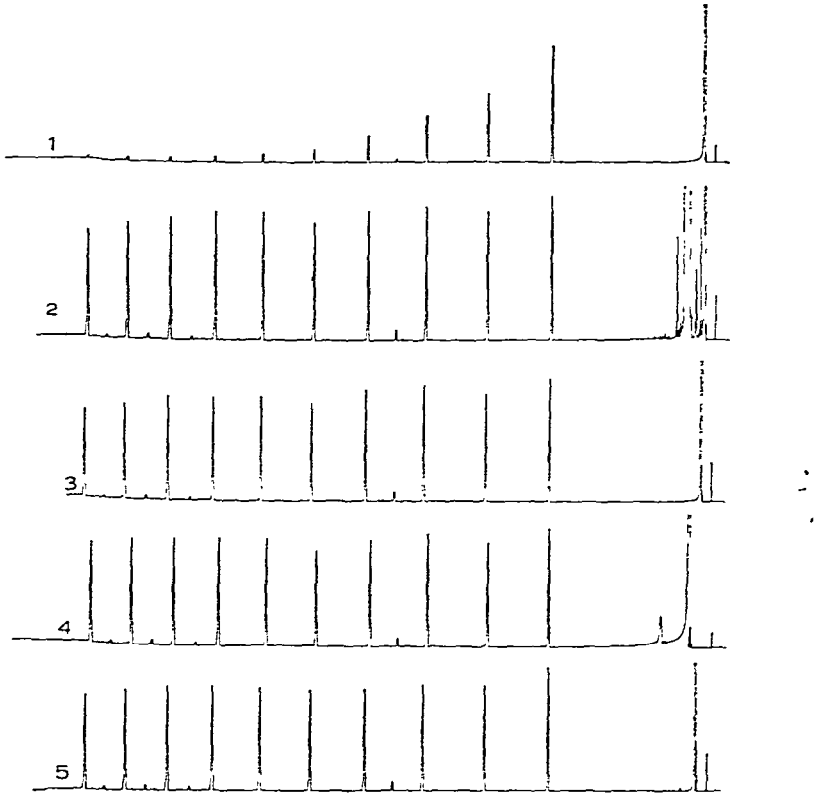

Fig. 2. Discrimination with split sampling of diluted even-numbered C_{18}–C_{36} *n*-alkanes (1). Influence of solvent volatility (2), injection temperature (3), type of carrier gas in sampling (4) and speed of injection (5). Sample: 1 μl of solution in *n*-octane or *n*-dodecane; column: 20-m methylpolysiloxane OV-101, 0.27 mm I.D.; temperature: 120–300°C, 8°C/min.

	1	*2*	*3*	*4*	*5*
Solvent	*n*-Octane	*n*-Dodecane	*n*-Octane	*n*-Octane	*n*-Octane
Injector temperature (°C)	300	300	210	300	300
Injector speed (sec)	2–3	2–3	2–3	2–3	<1
Carrier gas	H_2	H_2	H_2	$N_2 \rightarrow H_2$	H_2

In a second series of measurements using a wide-bore insert tube of I.D. 3.9 mm and length 80 mm we found a significant difference in discrimination when adopting either a fast or a slow mode of injection. With fast injection the discrimination was nearly negligible (compared with the discrimination-free on-column sampling

mode), whereas with slow injection the same poor results as with the narrow-bore insert were obtained. The data in this series of experiments are given in Table II and Fig. 1. The discrimination and the standard deviation of the normalized peak area percentages are much lower than when the narrow-bore insert tube was used. The ratio of the C_{18} and C_{36} peak areas was 0.93, which is similar to that found with on-column (syringe) sampling. The relative standard deviations of the normalized peak area percentages were 1.7% on average for the ten alkanes, which is still about six times higher than with on-column sampling, however.

For comparison we also investigated again the splitless sampling mode under similar conditions, using the same diluted sample as for the on-column sampling. Regarding discrimination, the performance of splitless sampling, applying the same devices (with a wide-bore insert tube) and the fast mode of injection, was higher than with the split mode of sampling but could also be considerably reduced by using a solvent of lower volatility (higher boiling point). No significant improvement could be achieved by changing either the column or injector temperature or by using nitrogen instead of hydrogen as the carrier gas. The results are shown in the chromatograms in Fig. 3.

The conclusion from the results of the two series of experiments on discrimination by volatility with split and splitless sampling is that the main source of error in the introduction of the sample into heated sampling units with a syringe is heat transfer from the vaporization insert on to the syringe needle and the selective vaporization of samples with a wide volatility range from the needle tip. This conclusion is in agreement with the results of Galli *et al.*[4].

According to the results of the experiments, discrimination is increased by the following:

(a) too high a vaporization insert temperature in general;

(b) contact of the needle with the insert walls (especially with too narrow bore insert tubing);

(c) fast heat transfer from the insert walls to the needle by a carrier gas of high thermal conductivity (hydrogen);

(d) too volatile solvents or major constituents of the sample and with samples of high average volatility in general;

(e) too "slow" sample introduction with the syringe (long residence time of the syringe needle within the heated insert).

The most effective means of decreasing the discrimination is to use either a solvent with higher boiling point and/or not too high injector temperatures.

The performance of sample introduction with the on-column (direct) technique is much less influenced by such adverse factors because no additional heating or vaporization of the sample before entering the column is carried out.

On-column (crucible) versus split sampling (standard splitter with narrow-bore glass-wool packed insert) in relative response factor determination using standard compounds with varying boiling points

For methyl pentanoate (b.p. 126.5°C) and heptanoate (b.p. 172°C) relative response factors by weight were determined, using C_8, C_{10}, C_{11} and C_{12} *n*-alkanes as standards (see Fig. 4). The fairly volatile *n*-pentane was used as the solvent.

With on-column injection the relative response factor for methyl pentanoate

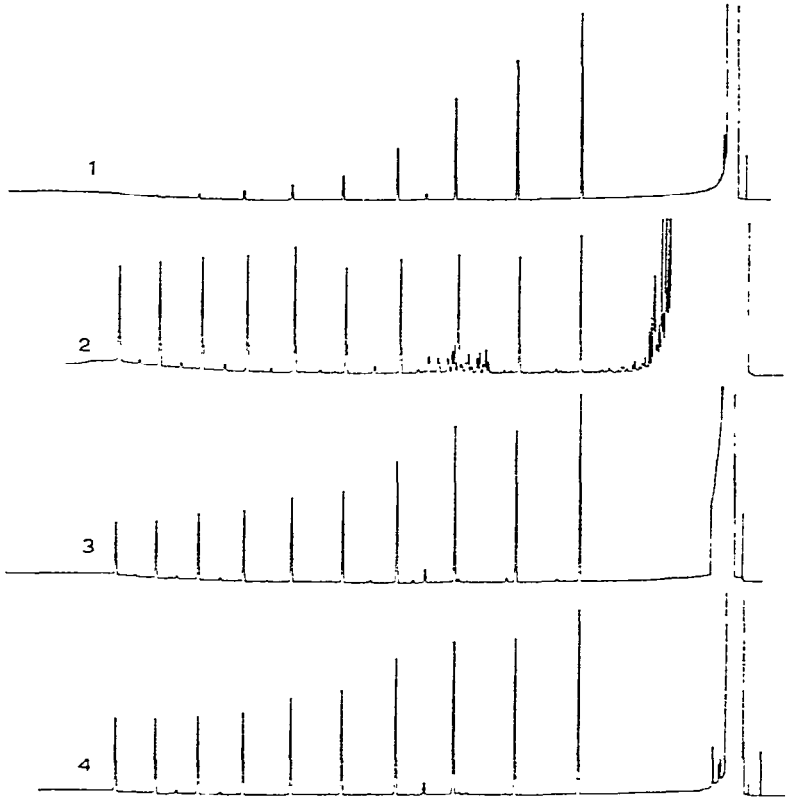

Fig. 3. Discrimination with splitless sampling of diluted even numbered C_{18}–C_{36} *n*-alkanes (1). Influence of: solvent volatility (2), injection temperature (3) and type of carrier gas (4). Sample: 1 μl of solute in *n*-octane or *n*-dodecane; column: 20-m methylpolysiloxane OV-101, 0.27 mm I.D.; temperature: 120–300°C, 8°C/min.

	1	*2*	*3*	*4*
Solvent	*n*-Octane	*n*-Dodecane	*n*-Octane	*n*-Octane
Injection temperature (°C)	210	300	300	300
Carrier gas	H_2	H_2	H_2	$N_2 \rightarrow H_2$

was nearly independent of the volatility of the standards, between 1.78 and 1.80 for all four standard alkanes, although the boiling points of these varied between 126°C (*n*-octane) and 215°C (*n*-dodecane). With split sampling, using a standard splitter with a glass-wool packed narrow-bore (80 × 2.0 mm I.D.) insert, the response factor of methyl pentanoate decreased from 1.80 for *n*-octane as standard to 1.67 for the high-boiling *n*-dodecane as standard. By avoiding the use of a very low-boiling solvent the discrimination at split sampling can be decreased considerably.

The same relative response factors were found with both sampling techniques only if in split sampling a standard *n*-alkane with a boiling point similar to or identical with that of the ester was used.

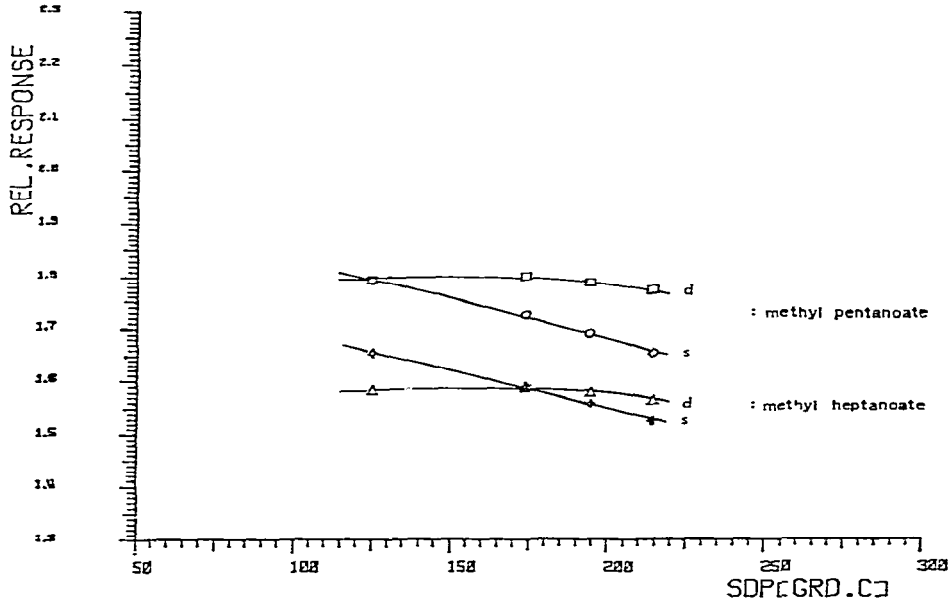

Fig. 4. Influence of sampling technique on discrimination in response factor determination using *n*-alkanes with increasing boiling points as standards. Column: 75-m Emulphor O, I.D. 0.27 mm; sample: C_5, C_7 methyl ester + *n*-C_8, C_{10}, C_{11}, C_{12}; s = split, 1 μl wtih 1:100 splitting ratio; d = direct, 1 μl with diluted sample 1:100 in *n*-pentane; temperatures: split, injector 150°C, column 110°C isothermal; direct, 60°C, column 60 to 120°C at 10°C/min; carrier gas: N_2, 1.1 bar, $\bar{u}$ = 14 cm/sec; instrument: MPI standard splitter (2.0 mm I.D.), glass-wool packing; MPI on-column (crucible) sampling.

CONCLUSION ON COMPARATIVE SAMPLING MEASUREMENTS

For quantitative measurements of mixtures with a wide volatility range and for response factor determinations that require high precision and accuracy, the on-column sampling technique is superior to the classical split and splitless sampling techniques with regard to discrimination and to the standard deviation of the relative peak areas. The performance of any kind of quantitative measurements with the on-column technique is less dependent on the various sampling parameters and on the type of calibration mixtures used regarding volatility range, etc.

The standard compounds used in response factor determinations must have similar volatilities (boiling points) to those of the species to be examined if split sampling is to be applied. It is not important for the standard compound to have the same or a similar retention, however, because the performance of the sampling method determines the accuracy of the calibration.

Comparing the different techniques of on-column injection, we conclude that the proper adjustment of the volume and the reproducibility of its introduction are optimal with the syringe technique. The crucible method is technically simpler and the adjustability of sample volume and the standard deviations of absolute (column load) and relative peak areas are sufficient for practical work. We consider that additional column inlet cooling as used in the CE technique may be of an advantage

to all on-column techniques, but makes the devices more sophisticated. If the solvent volatility and the column inlet temperature are chosen properly, however, additional cooling may not be necessary.

Nevertheless, there is still a need for new devices for on-column sampling techniques that allow especially for the achievement of adequate column loads, as for example in the case of undiluted samples that arise in industrial product control. Further, there is an urgent need for automated techniques of on-column injection.

NEW CONSTRUCTION FOR ON-COLUMN SAMPLING OF LOW VOLUMES

In an earlier paper on on-column sampling[1] with capillary columns, we also described a microversion technique that avoids the use of the septum and also possibly a syringe. The sample is introduced into the capillary column by means of a thin capillary of defined volume, which is directed into the correct position inside the capillary column by a special device; a similar one was used later also by Grob in his syringe technique. The volume of the sample can be varied by the application of micropipettes of different volumes, whereas a syringe can be used more universally for a certain range of variable sample volumes.

The lowest volume that can be sampled with the special syringes used with the CE (Grob) technique may be about 100 nl, which is still too high for the sampling of undiluted samples. With our previously described (micro) technique we have been able to sample volumes of about 20–50 nl reliably.

We have now investigated whether the advantages of the macro version can be combined with those of the micro version and whether it is possible to construct a device that allows for "liquid splitting" of the sample. We used a metallic micropipette newly designed according to the same principles as our previously described micro version device, as can be seen from Fig. 5.

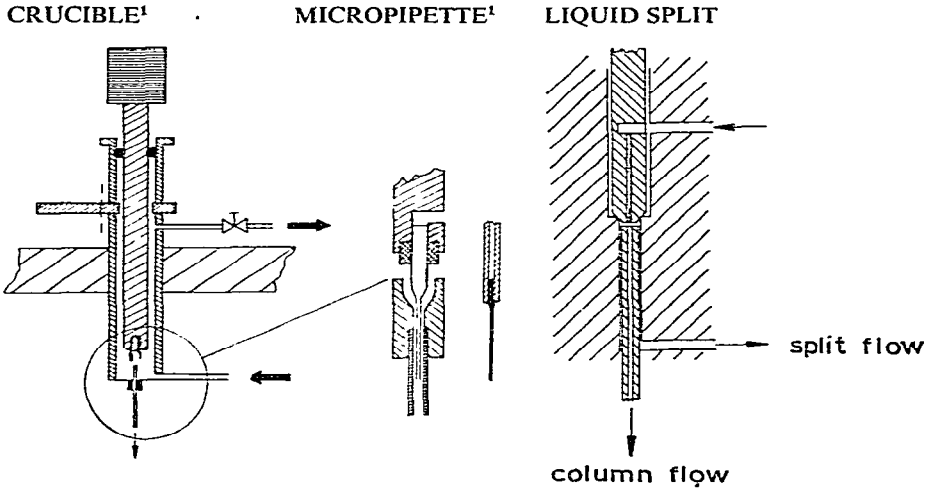

Fig. 5. Three versions of on-column sampling techniques. Liquid split: variable splitting of the liquid sample at the column inlet is used to minimize column load. The splitting ratio is varied with the carrier gas split flow. Discrimination is as low as with other on-column sampling devices.

The column load can be varied by changing the ratio of "split" flow and "column" flow of the carrier gas. Discrimination by volatility is as low as with other constructions of on-column sampling, as could be shown at the separation of various types of samples including the aforementioned discrimination test mixture containing the C_{18}–C_{36} alkanes.

Details of the construction and experimental results for practical applications will be published in the near future.

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