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COLD SAMPLE INJECTION WITH EITHER THE SPLIT OR SPLITLESS MODE OF TEMPERATURE-PROGRAMMED SAMPLE TRANSFER

COMPARISON TO COLD ON-COLUMN INJECTION WITH A COMMERCIAL DEVICE

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SUMMARY

High precision and accuracy of relative (normalized) and absolute peak areas (column loads) can be achieved with both the cold (temperature programmed, TP) splitless and the cold (on-column) modes of "cold" injection techniques if applied to mixtures ranging widely in volatility. With the cold (TP) split mode technique the performance of quantitative analyses is also improved but a certain discrimination by volatility and a slightly increased standard deviation for the peak area data still cannot be avoided. Negligible discrimination by volatility and highly reproducible column loads are the typical features of both cold "splitless" techniques. With the cold (TP) splitless technique, no special syringes are needed and the glass insert can easily be exchanged and cleaned for the removal of non-volatiles.

INTRODUCTION

Samples characterized by a high average volatility, owing to dilution of the compounds of interest in volatile solvents, and that also have a wide range of component volatilities, cannot be introduced into the vaporization chambers of the common sampling devices, operated in the split or the splitless mode, without causing various problems, particularly in quantitative analyses of high precision and accuracy.

Of course, the optimum separation efficiency or resolution that is obtainable with capillary columns should actually be attained by appropriate performance of the sampling procedure. This may be difficult with large sample volumes, which are necessary if high enough column loads (signal-to-noise ratios at detection) of components significant for the purpose of the analysis are to be achieved.

Excessive temperatures in the injectors, which are to ensure discrimination-free vaporization and transfer of the sample into the column, give rise to decomposition

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of labile sample constituents. High volatilities of the solvents serving as "transfer" matrices for trace components cause difficulties in attaining the optimal peak shapes, the necessary resolution and reliable quantitative data with high-temperature sampling.

If components present in low concentrations also exhibit a wide range of volatilities (*i.e.*, molecular size and polarity), the precise and accurate determination of their concentrations in the injected sample with very low detection limits becomes the most difficult task in the quantitative analysis of mixtures by GC.

The GC of traces of various types of compounds including also those with strongly differing polarities is not easy because of other instrumental difficulties, such as adsorption on the system surfaces and lack of sensitivity, specificity and linearity of detection. Sufficiently high signal-to-noise ratios at adequate resolution (separation efficiency) for compounds at low concentrations are also necessary in order to guarantee reliable detection with high precision and accuracy.

Therefore, sufficiently high column loads for those components which are present in the mixture in the lowest concentrations are also required and are achievable only with splitless sampling techniques, including cold on-column injection. If the concentrations of these components are very low, excessively large sampling volumes must be introduced into the chromatographic system.

A decrease in the separation efficiency, *i.e.*, resolution, in the important part of the chromatogram, discrimination of either low- or high-volatility components and irreproducible column loads (absolute peak areas), depending on the sampling volume, are the consequences. Another kind of problem arises with overloading of capillary columns by major components of a mixture, owing to the very low sample capacity of this type of column. Samples that either do not contain a very large number of other components or that are not highly diluted in a solvent matrix must be dosed in volumes that are smaller than those which can be reproducibly handled with the common syringes, holding a total volume of about 10 μl, in order to avoid column overloading. The reproducible sampling of volumes below 1 μl and even lower, down to a few nanolitres, may be of great importance for this type of mixture. The split sampling usually applied to less diluted samples is still the most often used technique but needs to be carefully optimized[1] in order to achieve high precision and accuracy of both the relative and may be also the absolute peak areas in quantitative analysis. Discrimination effects, especially those occurring with mixtures ranging widely in volatility, and high standard deviations cannot easily be avoided. Careful calibration measurements with one or more internal standards must then be carried out. With more concentrated samples, analytical data of very high precision and accuracy cannot easily be attained by split sampling, but by cold on-column sampling, as has been shown previously[2]. For the on-column technique, such samples must be diluted with very pure solvents of suitable volatility and retention in order to avoid column overloading. The aim of our previous work[2] was to demonstrate that very low standard deviations and high accuracy can be achieved with the sampling technique used (cold on-column injection), which is of great importance for quality and production control in industrial practice.

OBJECTIVES

In this work we aimed to investigate whether the cold temperature-programmed (TP) sampling technique[3,4] is also capable of achieving quantitative data of the same quality as with the cold on-column injection. Moreover, the cold (TP) sampling technique could be used with the split mode of sample transfer into the column, which is advantageous in comparison with cold (on-column) injection, because the dynamic range of column load adjustment of the latter technique is very limited and can only be increased by dilution with solvents. However, with split sampling, the column load can also be varied via the splitting ratio. In comparison with "hot" split sampling, selective vaporization from the syringe needle and explosive vaporization can be avoided with cold (TP) injection. We have also been very interested in achieving reproducible column loads with varying syringe volumes (0.5–2 μl) and splitting ratios. Test mixtures with a wide range of component volatilities and an industrial sample (Baycor) were selected for systematic measurements, which were to be carried out in parallel, in the chromatographic laboratories of the Max-Planck-Institut and of Bayer, Wuppertal, because of the practical importance of the results to be expected.

All separations were performed with temperature programming. Therefore, the column inlet was cold during the sample transfer, so that band focusing of most of the test compounds is achieved by trapping, except for the species of higher volatility, which are eluted early. Concerning the performance of the cold (TP) split injection, we hoped to be able to avoid those errors which arise with "hot" split injection and lead to poor precision and accuracy of both the absolute and the relative peak areas.

Concerning the comparison with cold on-column injection, we mainly expected to attain a similar precision and accuracy of absolute and relative peak areas when adopting the splitless mode of sample transfer. In case the results of the two approaches to the comparison of different sampling techniques should prove to favour the cold (TP) injection, this technique may be considered a universal one, which, moreover, has the following other practical advantages, as discussed in previous papers[1–3].

(a) The syringe needle does not need to be introduced into the narrow-bore capillary column itself. The usual standard syringes with an external needle diameter of *ca.* 0.45 mm cannot be applied in on-column sampling.

(b) Columns with an inner diameter of less than 0.25 mm cannot be used in the cold on-column technique.

(c) The low-volume vaporization insert, which can be easily removed for cleaning or exchange, may act as trap for non-volatile or less volatile contaminants of poorly prepared samples.

(d) By packing it with stationary-phase material, the insert may also be used for pre-separations, which should take place with a higher separation efficiency than a simple vaporization step, *e.g.*, to remove solvents from highly diluted samples. This prevents them from entering the main separation at high resolution, in the same manner as proposed by Van den Berg and Cox[5] (moving-needle solid injection) and by Vogt *et al.*[6] ("split–splitless injection"). When the vaporization insert of the cold (TP) injector is operated as a miniaturized packed pre-column, the arrangement can be considered to act as a typical "multidimensional" GC system.

INSTRUMENTAL

We used a commercial cold injector version, the DANI (temperature-programmed vaporizer, PTV) cold injector in both the split and splitless modes, and coupled to a DANI Type 3800 gas chromatograph. For comparison we used two different cold on-column injectors from Carlo Erba and Chrompack, coupled to a Carlo Erba Model 4160 chromatograph. Different laboratory-prepared alkylpolysiloxane columns with fused silica and pre-treated alkali glass as the tubing materials were used for the separations. For statistical reasons, all measurements were repeated 6–9 times.

RESULTS

Fig. 1. illustrates the geometry of the apparatus with the syringe introduced. The position of the glass-wool plug, its length inside the insert and the position of the needle tip at the extrusion of the liquid sample into the insert can also be seen.

In our earlier experiments problems already arose concerning the sealing of the glass insert. Defined split conditions can be established in the injector only if

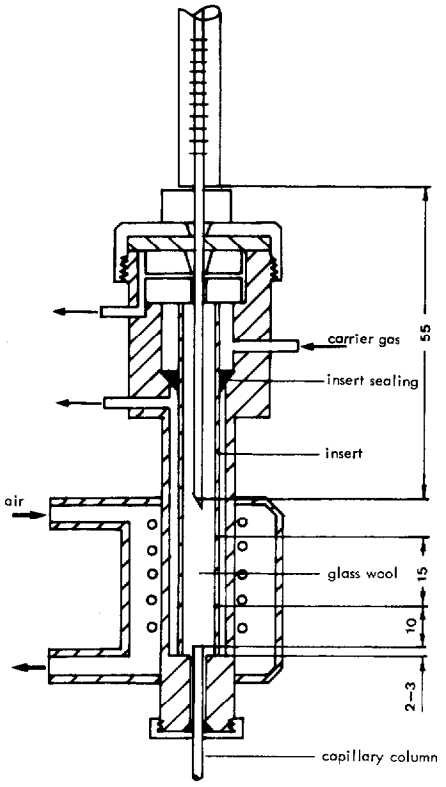

Fig. 1. Construction of cold (TP) injector of the DANI (TP) type. Penetration of syringe needle: (a) split mode, 35 mm; (b) splitless mode, 55 mm.

TABLE I

DISCRIMINATION TESTS USING ON-COLUMN AND COLD (TPV) INJECTION TECHNIQUES

Sample: C_{10}, C_{12}, C_{14}, C_{16} and C_{18} alkanes in *n*-heptane (15 μg/ml of each component in A, B and C). Column: (A) 25 m SE-54, fused silica; (B) 24 m OV-101, fused silica; (C) 38 m OV-101, alkali glass (all 0.25–0.30 mm I.D.). Temperatures: (A) 40–250°C; (B) 50–220°C; (C) 60–260°C. Carrier gas: hydrogen. No. of measurements: A and B, 6; C, 9.

System	*Parameter**	C_{10}	C_{12}	C_{14}	C_{16}	C_{18}	C_{18}/C_{10}
(A) Cold splitless (DANI)	$\bar{M}$	19.80	20.25	20.13	19.82	19.98	1.009
	s (%)	0.45	0.68	0.41	0.50	0.80	
(B) Cold on-column, secondary cooling (Carlo Erba)	$\bar{M}$	19.96	20.17	19.99	19.74	20.14	1.009
	s (%)	0.58	0.31	0.31	0.39	0.60	
(C) Cold on-column (Chrompack)	$\bar{M}$	19.81	20.22	19.95	19.77	20.25	1.022
	s (%)	0.46	0.26	0.20	0.61	0.47	

* $\bar{M}$ = average value; s = relative standard deviation.

column and split flow are independent of each other. If leaks of the insert seal occur, the splitting line and in the column is not proportional to the ratio of the amount of the injected sample and the column load, because a certain part of the split flow leaves the splitting outlet directly without entering the insert and the splitting region. Under these conditions, a larger proportion of the sample enters the column than calculated from the splitting ratio. With either graphite or Vespel a perfect seal of

TABLE II

DISCRIMINATION TESTS USING DIFFERENT SAMPLING TECHNIQUES WITH DANI COLD (PTV) INJECTOR

Split and splitless injection at constant or programmed sampling temperature. Sample: 0.4 μl of C_{10}, C_{12}, C_{14}, C_{16} and C_{18} alkanes in *n*-heptane (0.11% each component). Column: 25 m × 0.3 mm I.D. SE-54, fused silica. Temperatures: 50°C, 2 min isothermal, raised to 220°C at 15°C/min. Carrier gas: 0.25 bar hydrogen; splitting ratio, 1:50. Instrument: DANI 3800 with TPV.

*System**	*Parameter*	C_{10}	C_{12}	C_{14}	C_{16}	C_{18}	C_{18}/C_{10}
	Concentration (%)***	19.93	20.01	20.04	19.98	20.04	1.005
A	$\bar{M}$	20.78	20.65	19.92	19.18	19.43	0.935
	s (%)	1.16	0.59	1.07	0.61	0.70	
B	$\bar{M}$	20.01	20.87	20.67	19.57	18.90	0.945
	s (%)	3.57	2.03	1.90	2.50	3.60	
C**	$\bar{M}$	19.96	20.38	20.34	19.70	19.60	0.982
	s (%)	0.45	0.22	0.56	0.72	0.72	
D	$\bar{M}$	19.80	20.25	20.13	19.82	19.98	1.009
	s (%)	0.45	0.68	0.41	0.50	0.80	

* A, Split injection at a constant temperature of 150°C; B, split injection at a constant temperature of 250°C; C**, split injection with "cold" sample introduction (40°C) and subsequent ballistic heating to 250°C; D, splitless injection with "cold" sample introduction (40°C) and subsequent ballistic heating to 250°C, sample diluted 1:50.

** The data in line C were obtained under flow conditions that the splitting ratio does not correspond to the actual column load owing to imperfect sealing of the insert.

*** Known concentration of the test mixture which was made up by weight.

TABLE III

DISCRIMINATION TESTS USING THE COLD ON-COLUMN AND SPLITLESS COLD (TP) INJECTION TECHNIQUES

Determination of relative (normalized) peak areas of even-carbon C_{10}–C_{32} alkanes. Sample: (A) 0.2 μl of C_{10}–C_{32} alkanes in *n*-heptane, 0.002% of each component; (B) 0.4 μl of C_{10}–C_{32} alkanes in *n*-heptane, 0.002% of each component. Columns: 24-m OV-101. Temperatures: (A) 2 min isothermal, raised from 50 to 300°C at 15°C/min; (B) 1 min isothermal, raised from 50 to 300°C at 15°C/min. Injection temperature: 35–300°C. Carrier gas: (A) 0.45 bar hydrogen; (B) 0.58 bar helium. Instrument: (A) Carlo Erba 4160; (B) DANI 3800. No. of measurements: 7.

System	*Parameter*	C_{10}	C_{12}	C_{14}	C_{16}	C_{18}	C_{20}	C_{22}	C_{24}	C_{26}	C_{28}	C_{30}	C_{32}
(A) Cold (on-column)	Peak area (%)	8.18	8.26	8.21	8.29	8.44	8.53	8.31	8.24	8.26	8.42	8.42	8.42
(Carlo Erba)	S.d. (%)	0.38	0.34	0.44	0.55	0.66	0.88	0.29	0.52	0.55	0.72	0.69	0.71
(B) Cold TP (splitless)	Peak area (%)	8.26	8.37	8.27	8.23	8.26	8.22	8.52	8.39	8.62	8.39	8.29	8.16
(DANI TPV)	S.d. (%)	0.46	0.31	0.30	0.41	0.48	0.30	0.47	0.45	0.69	0.58	0.33	0.46

the insert is not without problems, because the packing is subjected to frequent, extreme changes of temperatures, ranging from 35 to 350°C.

The data in Table I were obtained with a hydrocarbon test mixture (C_{10}–C_{18}) with *n*-heptane (b.p. 100°C) as solvent. With all three series of measurements, performed with the cold splitless (TP) DANI device (A) and two different cold on-column injectors (Carlo Erba and Chrompack), average relative standard deviations of the relative peak areas of less than 0.5% were obtained. The discrimination measured by the C_{18}/C_{10} peak-area ratio was negligibly small with all three techniques. The theoretical value obtained by weighing was 1.005.

In Table II, the same, but less diluted, sample was first introduced with splitting at different, but constant, injector temperatures (A, B) and with cold (TP) injection (C). For comparison, the data obtained from splitless cold (TP) injection are also given in Table II (D). With the split mode and isothermal sample vaporization, the standard deviations of the relative (normalized) peak areas are between 1 and 4% and are higher at elevated sampling temperatures, whereas the discrimination (C_{18}/C_{10}) decreases only slightly at increased injector temperatures. However, the data of line C must be regarded with caution, as they were not reproducible in different series of repeated experiments, probably owing to imperfect sealing of the insert, as mentioned above. Under conditions of perfect sealing, a higher discrimination of the high-volatility (low carbon number) components was observed (see Table II).

For the data in Table III, a test mixture with a wider range of component volatilities (C_{10}–C_{32}) was used. The composition of the sample was well known from weighing but was controlled by the cold on-column (syringe) technique, which is known to deliver very reliable data for this type of sample. The relative standard deviations for the cold (TP) splitless mode injection and the cold on-column technique were found to be about the same and extremely low, *viz.*, 0.5% on average over the entire range of the hydrocarbons. In these analyses, the same diluted sample (containing 0.002% of each hydrocarbon in *n*-heptane) was applied. No appreciable

TABLE IV

REPEATABILITY OF ABSOLUTE PEAK AREAS USING A 10-μl SYRINGE AND A DOSAGE OF 1 μl OF SAMPLE WITH SPLIT AND SPLITLESS MODES OF COLD (TP) INJECTION (DANI)

Sample: (A) 1 μl of C_{10}–C_{18} alkanes in *n*-heptane, 0.002% of each component; (B) 1 μl of C_{10}–C_{18} alkanes in *n*-heptane, 0.1% of each component. Splitting ratio: 1:50. Column: 24-m OV-101. Temperatures: 1 min isothermal, raised from 50 to 240°C at 15°C/min. Injection temperature: 50–200°C. Carrier gas: 0.2 bar hydrogen. Instrument: DANI 3800 (TPV). No. of measurements: 5.

System	*Parameter*	C_{10}	C_{12}	C_{14}	C_{16}	C_{18}
(A) Splitless, cold (TP)	Absolute peak area	1,004,567	1,018,776	1,004,168	987,364	969,898
	S.D. (%)	2.14	1.77	1.88	1.95	2.11
	Relative peak area	20.15	20.43	20.14	19.81	19.45
	S.D. (%)	0.64	0.44	0.24	0.45	0.85
(B) Split, cold (TP)	Absolute peak area	937,538	1,030,816	1,126,470	1,175,726	1,187,941
	S.D. (%)	2.03	1.73	0.99	0.97	1.41
	Relative peak area	17.18	18.88	20.64	21.54	21.76
	S.D. (%)	1.07	0.65	0.37	0.56	0.67

discrimination can be recognized. With the split mode of cold (TP) injection, we observed relative peak areas that did not indicate any severe discrimination according to volatility, but increased standard deviations, primarily for the lower and the higher carbon numbers. Such data are in agreement with data published by Poy[7] who used the same device for his measurements. We decided not to include such data in Table III because we were unable to reproduce them under the same conditions in other series of experiments after previously resetting the column connection and the insert sealing.

We then started the tests for repeatability of the absolute peak areas (column load per component), using both the splitless and the split mode. These data are given in Table IV. Using the splitless mode for the sample of higher dilution we observed no discrimination, a low average relative standard deviation of the relative peak areas (0.5%) and an average relative standard deviation of the absolute peak areas of about 2%. Using the split mode for the undiluted sample, but with a splitting ratio of 1:50, we obtained data indicating a discrimination of the high-volatility (low carbon number) components, but the relative standard deviations of the relative and the absolute peak areas were as low as with the splitless mode, *i.e.*, less than 1% and 1.5–2%, respectively. These measurements were carried out in order to investigate whether the column loads obtained with split sampling actually correspond to the splitting ratio. Therefore, the flow system of the injector was properly adjusted by perfect sealing of the insert. This could be proved by the measurements. Indeed, with both series of experiments, the same absolute peak area values of about $1.00 \cdot 10^6$ on average were recorded, indicating that splitting in the ratio 1:50 and a dilution of 1:50 of the same sample led to the same column loads. This is not the case with the data of Poy[7] (see his Table 1).

Regarding the splitless mode of cold (TP) sampling, in the Bayer laboratories the same excellent data of discrimination and relative standard deviations of relative peak areas were obtained for the wide-range (C_{10}–C_{32}) mixture, whereas with the split mode unreliable data were obtained, in particular, very high relative standard deviations of the relative (normalized) peak areas.

The cold (TP) sampling in the splitless mode was also applied to the practical

TABLE V

DETERMINATION OF RESPONSE FACTORS OF THE FUNGICIDE BAYCOR RELATIVE TO ALKYL PHTHALATE STANDARDS OF VARYING VOLATILITY USING SPLITLESS COLD (TP) INJECTION (DANI)

Data obtained by G. M. Teller and M. Bender, Bayer, Wuppertal, F.R.G. Sample: 0.4 μl of Baycor + internal standard, 0.1 g in 4 ml of acetone, diluted 1:40. Column: 20-m methylpolysiloxane SE-30. Temperatures: 1 min isothermal, raised from 80 to 280°C at 15°C/min. Injection temperature: 40–280°C. Carrier gas: 0.5 bar helium. Instrument: DANI 3800 (PTV).

Parameter	*Phthalate*			
	Dimethyl	*Diethyl*	*Dibutyl*	*Diisooctyl*
Boiling point (°C)	284	298	340	384
Response factor	0.776	0.849	0.971	1.114
S.D. (%)	0.46	0.61	0.31	0.12

analysis of a fungicide (Baycor), as was done in our previous work on cold (on-column) sampling[2]. For these measurements, dialkyl phthalate standards of varying volatility were used. A negligible influence of the volatility of the standards on the precision (low standard deviation of response factors, relative peak areas) was noted. This observation indicates that the contribution of discrimination to the accuracy of relative peak areas may to be also neglected (see the data in Table V).

CONCLUSION

The cold injection of samples with a wide range of component volatilities, which may also be diluted in volatile solvents, is capable of improving the performance of quantitative analysis with capillary columns. The relative standard deviations of both types of data, *i.e.*, absolute and relative (normalized) peak areas, are decreased considerably. The repeatability of the absolute peak areas was surprisingly good (1–2% relative standard deviation) when a 10-μl standard syringe and an adjusted sampling volume of 1 μl were used. The relative standard deviation of the relative peak areas can be decreased by cold injection to even less than 0.5%, similar to the values obtained by cold (on-column) injection. The advantages in the practical use of the cold injection mentioned in the Introduction suggest that this technique may be used with advantage compared with cold (on-column) injection. These advantages are that the injection can be carried out with standard syringes into wide-bore glass inserts, which can be easily replaced and cleaned after contamination by non-volatiles deposited from poorly pre-treated samples. With split sampling, the same low relative standard deviations of absolute and relative (normalized) peak areas were achieved, but with discrimination of the high-volatility components of the test mixtures. Further systematic measurements on this subject are necessary, as well as measurements in which certain parameters that influence the performance of the cold (TP) injection are varied. These parameters include temperature at sample introduction, speed of injector heating and geometry of the insert.

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