Sampling in Capillary Gas Chromatography: A Comparison between the Split and the PTV-Split Procedures.

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Summary

Parallel studies on split-sampling and PTV-sampling procedures have been made to highlight the difficulties in quantitative analysis for capillary columns.

Parameters such as total sample amount, carrier gas and packing material used for the injector glass insert were studied.

The results clearly demonstrate the importance of optimizing all experimental parameters which give rise to discrimination effects during injection.

Introduction

One of the most important parameters in high resolution gas chromatography is the method of sample introduction; it is essential to select and optimize the sampling technique used with regard to the kind of sample to be analyzed.

The problem is especially difficult for the quantitative analyses of complex mixtures, i.e. mixtures of compounds having a wide range of polarities, concentrations and volatilities. This problem arises in the analysis of mixtures of biological origin such as volatile fractions of foods and beverages, crude oils, coal derived gasolines, tobacco extracts, environmental samples etc. In analyzing this type of sample, is often desirable to measure quantitatively with high accuracy and precision compounds which are present in very low concentrations. These compounds are often very influential factors in, for example, the aroma characteristics of foods or undesirable trace compounds in environmental samples.

A number of authors have studied different injection techniques that have been developed. The most important conclusions that they have drawn can be summarized as follows:

a) The split-sampling procedure is the simplest method of sample introduction in capillary column gas chromatography but quantitative results may be poor because of discrimination of one compound over another in their transfer from the syringe (in the liquid state) to the column (in the vapor state). Nevertheless it is possible to optimize the experimental parameters to avoid discrimination or at least to minimize it [1]. The main advantage of split-sampling is the ease of varying the column load by modifying not only the sample volume but also the split ratio. In spite of the disadvantages some authors consider that, for specific samples, it would be very difficult to replace the split sample procedure [2].

b) The splitless injection technique is considered to be one of the most suitable procedures for analyzing trace compounds in dilute solution. In this case the entire sample is transferred from the syringe to the capillary column and the solvent or thermal effects are used to achieve the necessary band focusing and thereby reduce peak broadening [3, 4]. The splitless technique requires optimization of all of the experimental parameters and above all, the difference between the initial oven temperature and the boiling point of the solvent selected is a particularly crucial parameter. However it can be difficult to obtain good quantitative results using the splitless procedure since selective vaporization remains a possible source of discrimination.

c) The use of the cold on-column technique gives good precision and accuracy [5, 6]. Since the liquid sample is introduced directly into the column, this procedure is recommended only for dilute samples in order to avoid column overloading. Moreover some problems in the first portion of the column which may produce distorted peaks have been described by Grob [7]. This type of peak distortion can be corrected by optimising the experimental parameters and by removing the stationary phase from the first few centimeters of the column [8].

d) The Programmed Temperature Vaporizer (PTV) is an injection device which is cold when receiving the sample from the syringe and is then rapidly heated so that the sample enters the column as vapor (as contrasted with the cold on-column procedure). The PTV procedure differs from the normal split or splitless technique in that the vaporization step occurs after the sample release. As a result

the problems due to selective vaporization from the syringe needle should be eliminated when the PTV is used. Subsequent temperature programmed transfer of the cold sample to the column can be carried out in either the split or the splitless mode [9, 10]. It has provided good results when used in the splitless mode [10–12]. On the other hand, there is disagreement about the precision and accuracy of the quantitative data when the split mode is used. Poy et al. have obtained excellent quantitative results [13, 14] but Schomburg et al. conclude that technical imperfections in a commercial PTV-design prevent a well defined split ratio and thereby cause discrimination [11]. Schomburg has suggested that the "cooled needle" technique is the most suitable option for split sampling by cold injection and subsequent vaporization [15, 16]. This procedure has also been shown to be suitable for splitless sampling onto capillary columns [17].

It is clear that in spite of the advances made during recent years, there is no universal injector and there probably never will be. Currently, techniques utilizing cold sample introduction appear to have a clear advantage, although further work is needed to determine optimal operating conditions. On the other hand, it must be recognized that in the selection of the proper injection mode, one of the factors to be considered (in fact the most limiting factor) is the availability of the injector system, and at present, the most common system is the standard split injector. Some interesting papers about the evaluation and optimization of the split injector [18, 19] have been published, and accurate quantitative results of specific types of samples using this injector [20, 21, 22] have been obtained.

Because of the split injector's availability and, particularly, because of its practical advantages, mentioned above, we decided to study the influence of some parameters on discrimination effects when the split mode is used.

In this paper we report the results from both cold and hot sample introduction using a commercial PTV operated in the split mode and a standard split injector. The use of glass wool for packing the glass insert can lead to difficulties and therefore Volaspher A-2*, which had not been studied previously, was tried. Our purpose was to study some difficulties encountered with both injection systems.

Experimental

The work was performed using a standard split injector of a Perkin-Elmer Sigma-3 Gas Chromatograph, equipped with flame ionization detector (FID), whose signals were processed by a Perkin-Elmer Sigma-15 Data Station. A second set of measurements was carried out using the Perkin-Elmer Programmed Temperature Vaporizer (PTV) cold injector, coupled to a Perkin-Elmer 8320 Gas Chromatograph, having essential software to integrate the peak areas obtained. This PTV design includes a graphite gasket that properly seals the injector at the bottom. The glass insert is held with a spring so as to compensate for differences in

thermal expansion. This configuration provides a constant pressure above the seal within the injector chamber which results in a well defined split ratio. The PTV injector was operated only in the split mode with the commercially provided glass insert containing a plug of silanized glass wool (Perkin Elmer).

The following instrumental conditions were used:

Column: 50m × 0.25mm i.d. glass capillary coated with a 0.2µm layer of cross-linked Carbowax 20M.

Column temperature programme: 80°C to 200°C at 15°C/min.

Detector temperature: 350°C.

Carrier gas flow in injector: 125ml/min.

Make-up gas flow: 30ml/min.

Split injector:

Temperature: 350°C

Split ratio: 1/80.

Length of the packing: 4 cm.

PTV injector:

Temperature Programme: 40°C to 350°C at 15°C/s.

Length of the packing: 4 cm.

In all cases a standard Hamilton syringe Model 701N with a 10µl capacity and a needle length of 5cm was used. Injections were made quickly in order to prevent selective vaporization from the syringe.

The test solution was a mixture of n-alkanes from C₁₁ to C₂₀ in hexane each in approximately 10% w/w concentration. The accurate weight percentages of these n-alkanes were (from C₁₁ to C₂₀ respectively): 9.96, 9.98, 9.91, 10.06, 9.94, 10.09, 10.07, 9.99 and 9.99. This test mixture was diluted 4 and 10 times with n-hexane to make suitable solutions for studying the influence of the amount sampled on the discrimination observed.

For statistical reasons, the reported values were calculated from a minimum of five replicate injections for each set of parameters.

Results and Discussion

The regression line from areas vs. sampled amount was calculated for all the n-alkanes. The calculated intercepts were subjected to the proper test for statistical significance [23] and all these values proved to be not significantly different from zero. New slopes were therefore calculated using the origin as an extra point. Since all of n-alkanes are present at about 10% w/w, their slopes should be about the same. However, discrimination effects caused differences in these slopes.

In order to facilitate the understanding of the results obtained Table I gives the percentage increase or decrease observed for each slope relative to C₁₄. This n-alkane was chosen as reference because it showed the least discrimination.

As can be seen from this Table, the standard split injector gives a continuous increase in the observed discrimination as the molecular weight of the n-alkane is increased. These results are in agreement with those previously reported [24]. The results with the PTV injector are roughly similar for all the carrier gases. The best results for the low volatility (high

* Volaspher A-2 is a siliceous synthetic silanized support for G.C. from Merck.

Table I. Deviations (%) of the area/concentration regression slopes of each n-alkane in relation to C₁₄, which shows minimal discrimination.

	C ₁₁	C ₁₂	C ₁₃	C ₁₄	C ₁₅	C ₁₆	C ₁₇	C ₁₈	C ₁₉	C ₂₀
Split	+ 11.5	+ 8.5	+ 3.9	—	− 7.7	− 14.9	− 19.7	− 24.1	− 27.5	− 29.3
PTV	+ 12.4	+ 6.3	+ 0.5	—	− 4.6	− 4.9	− 5.4	− 5.6	− 7.2	− 9.2

Table II. Regression line from area vs. sampled amount (160–1600ng) of n-alkanes from C₁₁ to C₂₀ using a standard split injector with the glass insert packed with different materials.

Glass insert packed with		C ₁₁	C ₁₂	C ₁₃	C ₁₄	C ₁₅	C ₁₆	C ₁₇	C ₁₈	C ₁₉	C ₂₀
Volaspher A-2	Slope	88.6	83.5	81.2	79.5	74.8	72.3	69.1	68.5	64.9	63.1
	r	0.99304	0.99672	0.99531	0.99702	0.99812	0.99799	0.99610	0.99859	0.99900	0.99433
Glass Wool	Slope	59.7	55.8	53.8	51.9	49.9	49.1	47.5	44.1	39.8	36.7
	r	0.99342	0.99277	0.99280	0.99243	0.99101	0.99027	0.99035	0.98308	0.98049	0.97375
None	Slope	85.3	83.0	79.5	76.5	70.6	65.1	61.4	58.1	55.5	54.1
	r	0.99992	0.99998	0.99996	0.99993	0.99996	0.99992	0.99660	0.99921	0.99888	0.99751

r: Regression coefficient

Sampled volume: 0.4 µl in all cases

Table III. Relative standard deviations (%) of the absolute peak areas obtained by using different packing materials for the glass insert and for several amounts of sample. Standard split injector.

Glass insert packed with	ng*	C ₁₁	C ₁₂	C ₁₃	C ₁₄	C ₁₅	C ₁₆	C ₁₇	C ₁₈	C ₁₉	C ₂₀
Volaspher A-2	160	4.4	3.4	3.4	3.3	2.5	2.6	5.2	5.3	4.8	7.3
	400	10.2	8.7	9.7	9.3	9.1	9.3	9.5	9.7	10.1	12.6
	1600	13.5	12.8	12.6	12.4	12.2	12.8	13.0	13.0	13.1	12.7
Glass Wool	160	14.9	14.9	14.3	14.4	14.0	6.7	10.0	9.9	4.2	2.5
	400	6.6	5.6	4.6	3.7	3.3	3.0	2.8	1.8	2.5	2.3
	1600	24.9	24.8	25.5	26.1	27.2	28.0	29.0	31.5	31.3	33.1
None	160	5.4	5.3	4.9	5.1	5.4	3.9	4.7	5.3	7.5	11.8
	400	1.7	5.0	4.1	4.4	4.0	3.8	3.4	3.2	4.7	5.8
	1600	7.9	9.3	11.1	14.5	16.0	19.8	22.7	24.5	25.4	26.2

* Sampled amount of each compound

Sampled volume: 0.4 µl in all cases

For the PTV injector the general discrimination trend is similar for all alkanes but the variation is not regular.

The importance of improving the vaporization of the sample and its homogenisation with the carrier gas is well known. However there is a lack of agreement about the most appropriate packing material for the glass insert of a split injector [25, 26]. We therefore carried out experiments using two types of packing for the glass insert: Volaspher A-2 (100–125 µm) and silanized glass wool. A third series of data were obtained from an unpacked glass insert.

Volaspher A-2 was chosen because of its unique characteristics [27] which suggested that the vaporization and homogenisation steps might be improved.

Tables II and III show the data from these experiments. Table II gives the slopes and regression coefficients of the straight lines obtained by plotting peak areas against the total sample amount for all the alkanes from C₁₁ to C₂₀. Data are given for the experiments in which the glass insert of the standard split injector is in each of the three packing conditions. (The data obtained from an unpacked glass insert are given here again for comparison.)

From Table II it is clear that Volaspher A-2 yields the highest values for the slopes and the lowest variation of the slope with the C-number of the alkane. This fact suggests that a better homogenisation is attained with Volaspher A-2 than with silanized glass wool or with no packing at all.

The relative standard deviations from the absolute peak areas obtained at the three sampling levels 160, 400 and 1600 ng are given in Table III.

The most important conclusion from the results in Table III is that, depending on the total amount of material to be introduced into the column and also on the volatility of the compound to be sampled, the most appropriate packing material must be carefully selected.

We then tested the repeatability of the absolute peak areas using either the standard split or the PTV-injectors with three different carrier gases: hydrogen, nitrogen and helium. The results from these experiments are given in Tables IV and V. With the standard split injector (Table IV) the use of hydrogen gave the highest standard deviations, while nitrogen gave the best results. These measurements are in agreement with the results previously reported [28]. Comparing these data to those in Table V it is noteworthy that

Table IV. Relative standard deviations (%) of absolute peak areas obtained with different carrier gases by using a standard split injector.

Carrier gas	C ₁₁	C ₁₂	C ₁₃	C ₁₄	C ₁₅	C ₁₆	C ₁₇	C ₁₈	C ₁₉	C ₂₀
Nitrogen	5.09	5.10	4.97	4.95	4.82	4.62	5.02	4.12	4.35	4.23
Helium	8.29	3.31	7.28	6.78	7.92	8.72	9.31	9.60	9.88	10.85
Hydrogen	29.38	29.22	25.80	29.78	30.11	30.96	31.82	33.80	31.73	35.65

Glass liner without packing

Sampled volume: 0.4 μ l in all cases**Table V.** Relative standard deviations (%) of absolute peak areas obtained with different carrier gases by using a PTV injector in the split mode.

Carrier gas	C ₁₁	C ₁₂	C ₁₃	C ₁₄	C ₁₅	C ₁₆	C ₁₇	C ₁₈	C ₁₉	C ₂₀
Nitrogen	4.25	2.00	4.69	1.46	5.51	2.92	3.55	3.50	2.16	1.09
Helium	3.33	2.72	3.57	4.79	1.21	2.05	3.61	1.50	2.94	0.91
Hydrogen	1.31	1.29	2.14	4.38	5.83	6.23	5.03	3.25	2.81	3.24

Glass liner with glass wool

Sampled volume: 0.4 μ l in all cases

carbon number) n-alkanes were obtained using higher viscosity carrier gases while the least viscous gas (hydrogen) gave the worst results with compounds of medium volatility.

In general, a comparison of Tables IV and V clearly shows the PTV injector to be superior in reducing discrimination than the split injector for all three carrier gases.

Conclusions

The conclusions of our work can be summarized as follows: It is necessary to re-emphasize again the impossibility of drawing generalized conclusions about the problems of sampling into capillary columns. For a given study the experimental parameters must be optimized to give the best possible sampling and separation results.

The choice of the carrier gas is especially critical when standard split injection is used but is less important from the point of view of discrimination with a PTV injector.

With regard to the packing for the glass insert, our experience has shown that careful selection of the packing material is important when a standard split injector is used. We have recently begun an extensive study on the influence of packing in the cold PTV split injection mode. Preliminary data indicate that in this case the packing of the glass insert is a particularly critical variable.

In relation to the amount sampled, the discrimination observed is less by us when a PTV injector is used than with the standard split injector.

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