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Variables affecting the introduction of large sample volumes in capillary gas chromatography using a programmed-temperature vaporizer

F.J. Señoráns and J. Tabera

Instituto de Fermentaciones Industriales, CSIC, Juan de la Cierva 3, 28006 Madrid (Spain)

J. Villén

Escuela Politécnica de Albacete, Universidad de Castilla-La Mancha, Cawetera de Peñas km 3.1, 02006 Albacete (Spain)

M. Herraiz* and G. Reglero

Instituto de Fermentaciones Industriales, CSIC, Juan de la Cierva 3, 28006 Madrid (Spain)

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ABSTRACT

The dependence of the programmed-temperature vaporizer (PTV) solvent split sampling technique on eight experimental variables affecting the introduction of large sample volumes in capillary GC was evaluated. The simplex method was used to improve the sensitivity of the analysis significantly by optimization of the operating conditions, namely sample volume, flow-rate during sampling, initial PTV temperature, length of the adsorbent bed, nature of the trapping material used in the glass liner, speed of sample introduction, elimination volume and solvent elimination temperature. With this method, reliable determinations of several volatile components at sub-ppb concentrations were obtained using a flame ionization detector, without the need for prior enrichment of the sample.

INTRODUCI'ION

Introduction of large volume samples in capillary GC is of special interest for the determination of trace compounds in strongly diluted or low-concentration samples. Also, injection of large amounts is usually required for on-line LC-GC coupling.

The determination of trace compounds by capillary GC has commonly been performed by using the so-called Grob-type splitless injection, which involves direct introduction into the chromatographic column of dilute solutions [1–3]. An evident disadvantage of this technique, however, is the high loading of the column with large amounts of solvents, as it may cause several problems mainly due to flooding sample liquid in the first portion of the capillary column, thus favouring phase stripping. The use of uncoated-column inlets with a reduced retention power (retention gaps) has proved suitable for reconcentrating the initial solute bands broadened in space. This reconcentration results from the accelerated movement of the solute materials through the uncoated inlet compared with the coated column [4]. Other approaches require the concentration of the sample prior to its intro-

* Corresponding author.

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duction into the injector [5–8], but all of them have several operational drawbacks and performance limitations.

Several years ago, an injection system allowing the introduction of up to 250 μl in a cold vaporizer was proposed for sampling large amounts of very dilute solutions [9,10]. Subsequently, a programmed-temperature vaporizer (PTV) was designed for a much wider field of application [11,12] and its usefulness for avoiding discrimination related to high-temperature sampling has been reported [13,15]. Moreover, the possibility of using the PTV injector as a pre-column enrichment device with a suitable adsorbent has also been emphasized [16–22].

The PTV vapour overflow technique is intended for introducing samples in large volumes of solvents by syringe injection. Evaporation and discharge of the solvent vapour occur by expansion, driven by the solvent vapour pressure. Subsequent heating of the vaporizing chamber brings about the transfer of the compounds into the column [23].

The use of a PTV operated in the solvent split (solvent elimination) mode [13,24], also allows the introduction of large volumes of solvents with very high diluted samples by means of a syringe injection. With this mode of operation, the sample is transferred to the column after the solvent has been eliminated at low temperature, whereas the analyte solutes are retained in a suitable trapping material. A few seconds after the injection, the split valve is closed, the vaporizer heated and the sample vapour transferred to the column.

Concerning the operation of a PTV in the solvent elimination mode for the determination of trace compounds, its use is only recommended if high-boiling solutes are to be measured, as the most volatile compounds are partly lost by evaporation together with the solvent. Therefore, further optimization of the experimental operation, especially regarding the determination of highly volatile materials, is necessary [25,26].

On the other hand, it is well known that in the development, improvement or adaptation of analytical methods, those variables affecting the experimentation should be carefully adjusted in order to establish the set of conditions that give the best results. This is why efficient and straightforward optimization procedures are strongly required, especially if the variables interact with each other so that each variable cannot be optimized independently of the others. In this respect, the sequential simplex method, first presented by Spendley et al. [27], has proved to be a useful optimization procedure [28–33].

With regard to the use of the PTV solvent split sampling technique, previous studies on the optimization of experimental variables by using the so-called response surface methodology suggested the usefulness of increasing the number of variables considered [34].

In this work, the optimization of the direct determination of trace compounds by using a PTV operated in the solvent elimination mode was studied. Eight variables were considered and the modified simplex method was used [35].

EXPERIMENTAL

Instrumentation

A gas chromatograph (Perkin-Elmer Model 8320) equipped with a flame ionization detector and a PTV injector was used. Data collection was done by using 2600 Chromatography Software (Perkin-Elmer Nelson Systems).

Sampling was accomplished by means of a simple, inexpensive, laboratory-made device designed to allow the introduction of large sample volumes by using a syringe (SGE, 500 μl). This device allows one to control effectively the speed of sample introduction (ranging between 0.083 and 1.47 μl/s) in order to adjust the injection speed to the rate of solvent evaporation.

Sample mixtures

A synthetic test mixture consisting of ethyl pentanoate, 1-butanol, ethyl hexanoate, 1-pentanol, ethyl heptanoate, 1-hexanol, ethyl octanoate, 1-heptanol and 1-octanol was used. The compounds were dissolved in ethanol-water (1:1, v/v) and their concentrations were about 0.1 mg/l per compound. The composition, concentrations and solvent of the test mixture were selected for subsequent real analyses of alcoholic beverages and foods.

Gas chromatographic analysis

Helium at 36 p.s.i.g. (1 p.s.i. = 6894.76 Pa) was used as the carrier gas and the detector was operated at 250°C. Gas chromatographic separations were carried out on a SGE (Ringwood, Australia) fused-silica column (50 m × 0.22 mm I.D.) coated with cross-linked BP-21 (film thickness 0.25 μm). The column temperature was held at 35°C for 8 min, then programmed to 60°C at 2°C/min and to 180°C at 4°C/min.

The initial PTV temperature was varied, as mentioned below, according to the optimization procedure. The final PTV temperature (250°C) was established taking into account the thermal stability of the trapping materials used and the need to achieve thermodesorption of the trapped solutes. After reaching the final temperature in the injector, it was maintained for 5 min and then cooled.

A silylated quartz insert (90 mm x 1 mm I.D. × 2 mm O.D.) from the PTV injector was packed with different lengths of packing material and plugged at both ends with silanized glass-wool. The packed liners were conditioned for 120 min at 255°C under a purge gas (helium) flow. According to our previous experience, the use of both Tenax TA and Gas Chrom 220 as alternative sorbents is of interest for trace enrichment because of their complementary characteristics. In order to evaluate the influence of the functionality of the trapping material (*i.e.*, the affinity of the sorbent for different organic compounds) on the performance of the analysis, a mixture of the two adsorbents mentioned above was finally considered to be one of the eight variables to be optimized. In all instances, the trapping material had been previously washed with acetone and subsequently dried and conditioned under a stream of nitrogen which was maintained either for 30 min at 90°C, 30 min at 180°C, 60 min at 300°C and 120 min at 350°C (for Tenax TA) or for 30 min at 90°C, 30 min at 180°C and 120 min at 250°C (for Gas Chrom 220).

Operating conditions

Table I shows the eight variables which were optimized for large sample introduction in the PTV: sample volume (V_{IO}), flow-rate during sampling (F), initial temperature of the PTV (T_i), length of the adsorbent in the glass liner (L), percentage of Gas Chrom 220 in the mixture of adsorbents used as a trapping material (%), speed of sample introduction (v_i), volume of gas that is purged during the splitting period (elimination volume, V_{OE}) and solvent elimination temperature (T_e). Prior to the sample introduction, the column end was withdrawn from the injector body so that solvent elimination was effectively performed through the injector bottom.

TABLE I

BASE LEVEL, STEP SIZE AND EXPERIMENTAL VARIABLES CONSIDERED FOR THE SIMPLEX OPTIMIZATION OF THE PTV SOLVENT SPLIT SAMPLING OF LARGE VOLUMES

Experimental variable	Base level	Step size
Sample volume, V_{IO} (μl)	300	150
Flow-rate during sampling, F (ml/min)	600	300
Initial PTV temperature, T_i (°C)	40	15
Length of adsorbent, L (cm)	2	2
Gas Chrom 220 in the adsorbent (%)	0	45
Speed of sample introduction, v_i (μl/s)	0.2	0.07
Elimination volume, V_{OE} (ml)	3000	3000
Solvent elimination temperature, T_e (°C)	20	15

The simplex optimization was initiated according to the matrix of mathematical coordinates proposed by Spendley et *al.* [27]. The following equation was applied to calculate the physical values of the experimental variables:

$$X_{phys} = X_0 + X_{math} S_z \quad (1)$$

where X_0 is the starting physical value of the variable X (base level), X_{phys} its actual physical value, X_{math} the corresponding mathematical coordinate in the Spendley matrix and S_z (the so-called step size) the physical value corresponding to a mathematical unit of the variable X.

Table I also includes the base level corresponding to the studied variables and the step size that was considered for each experimental variable.

RESULTS AND DISCUSSION

As previously described by several workers, the sequential simplex technique of Spendley *et al.* [27] suffers from several limitations, mainly because it does not have provision for acceleration and the possibility of attaining a false optimum. In order to overcome these drawbacks, a modification was proposed (modified simplex method) that has the advantage of acceleration and adaptation to fit the particular response being considered, as it allows operations of expansion and contraction in the searching progress [33,35].

Table II gives the experimental runs performed for optimizing large sample volume introduction in a PTV operated in the solvent split mode by applying the modified simplex method. Vertex 1 is defined by the base level of each variable and corresponds to the starting point of the experimental study. In order to investigate the influence of a modification of the variables affecting the process in the performance of the method, the sensitivity achievable from each run was evaluated. In this regard, the sum of the ratios of the measured peak areas (in area units) for each compound to the elution order of the corresponding solute was considered. It should be kept in mind that an interesting feature of the method investigated is the possibility of minimizing losses of the most volatile solutes. This is why the selected response included the elution order observed for each solute. Data obtained for the evaluated response throughout the optimization procedure are also given in Table II.

As a simplex is a geometric figure defined by a number of points equal to one more than that of the number of dimensions of the space, the

TABLE II

EXPERIMENTAL RUNS AND RESULTS FOR THE SIMPLEX OPTIMIZATION OF THE PTV SOLVENT SPLIT INJECTION

Vertex No.	Simplex No.	Factor level (physical values)								Response [b]
		V_{IO}	F	T_i	L	%	v_i	V_{OE}	T_e	
1	1	300	600	40	2.0	0.0	0.200	3000	20.0	3311
2	1	433	653	43	2.3	7.9	0.212	3530	22.6	3974
3	1	326	865	43	2.3	7.9	0.212	3530	22.6	3206
4	1	326	653	53	2.3	7.9	0.212	3530	22.6	3588
5	1	326	653	43	3.8	7.9	0.212	3530	22.6	6367
6	1	326	653	43	2.3	39.8	0.212	3530	22.6	7953
7	1	326	653	43	2.3	7.9	0.262	3530	22.6	2931
8	1	326	653	43	2.3	7.9	0.212	5651	22.6	3997
9	1	326	653	43	2.3	7.9	0.212	3530	33.2	2522
10	2	346	692	45	2.6	13.9	0.220	3928	11.4	5514
11	3	351	702	45	2.7	15.4	0.160	4027	19.0	5646
12	4	357	450	45	2.8	17.3	0.199	4151	18.3	5172
13	5	398	677	50	3.1	29.5	0.211	4970	20.5	6888
14	6	389	630	36	3.2	27.0	0.198	4799	17.3	9218
15'	7	421	619	27	3.6	36.5	0.191	5434	14.7	12 212
16[d]	8	453	608	18	4.0	46.0	0.184	6069	12.0	13 830
17'	9	485	597	9	4.5	55.5	0.177	6703	9.0	14 957
18	10	297	616	38	3.7	39.0	0.189	5593	14.0	9269
19	11	395	608	37	4.1	46.6	0.184	3457	11.8	10 493

[a] Units as in Table I.
[b] Sum of the ratios of the integration peak area (in area units) to the elution order of the corresponding solute (mean value of a minimum of two replicates).
[c] Obtained with expansion coefficient equal to 2.
[d] Obtained with expansion coefficient equal to 3.
[e] Obtained with expansion coefficient equal to 4.

initial simplex corresponding to the optimization of eight variables is formed by the first nine experimental runs (see Table II).

After having performed the initial simplex, the response values obtained were evaluated and the worst observation was rejected. Subsequently, a movement in the direction given by the rules of the simplex procedure was made. Experimentation was carried out until no further improvement of the response was observed. In all instances, a minimum of two replicates of each analysis were performed.

From Table II, the improvement observed in the sensitivity achievable with the analysis throughout the optimization is evident. As can be seen, experimental conditions defining vertex 17 (i.e., sample volume 485 μl, flow-rate during sampling 597 ml/min, initial temperature of the PTV 9°C, length of the adsorbent in the glass liner 4.5 cm, percentage of Gas Chrom 220 in the mixture of adsorbents 55.5, speed of sample introduction 0.18 μl/s, elimination volume 6703 ml and solvent elimination temperature 9°C) should be considered as the most suitable for the analysis, as they render the highest response.

Figs. 1 and 2 show respectively the initial (base level) and final chromatograms resulting from optimizing the PTV solvent split introduction of large sample volumes into the vaporizer of a gas chromatograph. It is evident that the optimization procedure brings about a significant improvement of the sensitivity attainable with the analysis, as under the selected conditions (vertex 17 in Table II) the response obtained is nearly five times higher than that corresponding to the initial experimental conditions (vertex 1 in Table II). It should be emphasized that a blank run performed on the trapping material by using GC-MS showed that the peaks assigned as adsorbent peaks are mainly due both to styrene (Fig. 2) and different isomers of dimethylstyrene (Figs. 1 and 2). In this respect, it is evident that the degradation of the adsorbent avoided the reliable determination of ethyl octanoate (see Fig. 2).

It is worth noting that according to the rules of the simplex procedure, vertices 15, 16 and 17 resulted from accelerations with expansion coefficients of 2, 3 and 4, respectively. After having performed nineteen experimental runs, the search was halted as the response values decreased from vertex 17 onwards, and the improvement achieved by the optimization procedure was satisfactory.

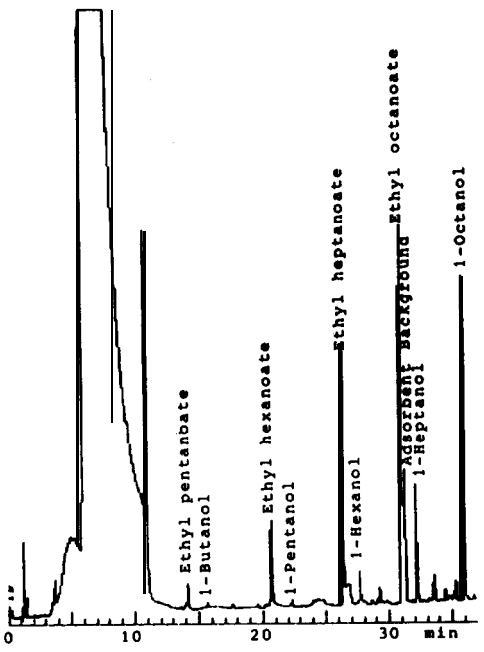

Fig. 1. Chromatogram resulting from the introduction of 300 μl of the test sample in a PTV operated in the solvent split mode under the initial experimental conditions of the optimization procedure (vertex 1 in Table II). The adsorbent background is due to different isomers of dimethylstyrene. See text for further details.

Table III gives the relative standard deviations obtained from the response values when a test mixture was analysed under the experimental conditions giving the highest response. As can be seen, values lower than 13% were generally obtained even though the analyses were performed with different glass liners in the PTV body. It is clear, however, that the use of only one glass liner for different experimental runs gives more precise analyses.

Table III also includes the recoveries obtained for the investigated compounds. These values were calculated from normalized peak areas (n = 3) using as a reference the normalized peak areas resulting from the cold splitless injection (n = 6) of a 2-μl volume of the standard solution, so that the same amount of each component was intro-

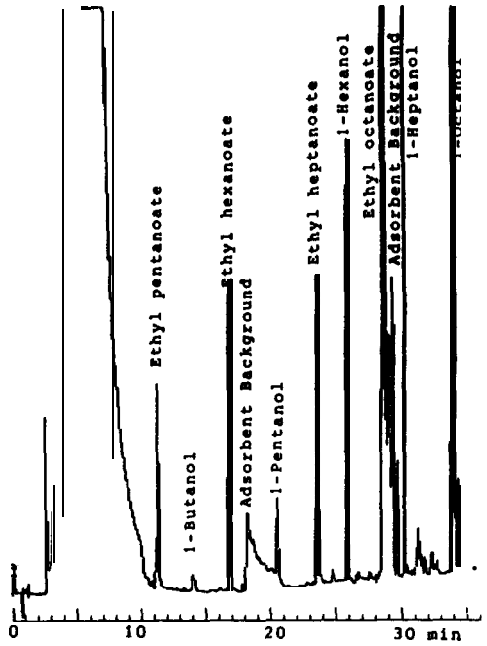

Fig. 2. Chromatogram following optimization of large volume sampling in the PTV solvent split mode of operation. Experimental conditions correspond to those of vertex 17 in Table II. The adsorbent background is mainly due to either (a) styrene or (b) different isomers of dimethylstyrene. See text for further details.

duced into the chromatographic column in both the cold splitless and the PTV solvent split modes of operation. Different procedures for the calculation of recoveries (including the consideration of both absolute peak areas and the ratio of the absolute peak area of each peak to that for 1-heptanol) provided similar results to those presented in Table III.

As expected, the highest recoveries were obtained for the less volatile compounds, but it is interesting that one of the most volatile components present in the sample (ethyl pentanoate) was more than 45% trapped. However, components with volatilities lower than or similar to that of ethyl pentanoate were largely lost. This was observed for the most volatile alcohols as the solvent mixture used for the standard dilution (ethanol-water) must promote the co-evaporation of the mentioned compounds during the solvent elimination period. Therefore, a reliable determination of 1-butanol is not possible using the proposed method, although the use of another solvent and subsequent optimization of the operating conditions would probably allow the determination of alcohols with higher recoveries and lower relative standard deviations.

Table IV gives the lowest concentrations that can be detected by using the described method, which involves (under the experimental conditions providing the best results) the injection of 485 μl, the speed of sample introduction being 0.18 μl/s. The mentioned detection limits are

TABLE III

RELATIVE STANDARD DEVIATIONS FOR THE RESPONSE VALUE (SUM OF THE RATIOS OF THE MEASURED PEAK AREAS FOR EACH COMPOUND TO THE ELUTION ORDER OF THE CORRESPONDING SOLUTE) FOR A TEST MIXTURE

Experimental conditions as given in Table II, vertex 17.

Compound	R.S.D. (%)	R.S.D. (%)[b]	Recovery (%)
Ethyl pentanoate	3.5	3.4	45.3
1-Butanol	11.6	25.6	2.6
Ethyl hexanoate	2.0	6.1	77.6
1-Pentanol	9.9	13.1	15.8
Ethyl heptanoate	3.2	8.3	87.4
1-Hexanol	5.5	12.0	56.5
1-Heptanol	7.6	10.8	95.2
1-Octanol	3.3	8.1	95.1

[a] Data obtained from five analyses performed with one glass liner.
[b] Data obtained from five different glass liners. In all instances, a minimum of two replicates were carried out ($n = 10$).

TABLE IV

DETECTION LIMITS WITH THE PROCEDURE INVOLVING THE PTV SOLVENT SPLIT INTRODUCTION OF LARGE SAMPLE VOLUMES

Experimental conditions as given in Table II, vertex 17.

Compound	Detection limit[a] (ng/l)
Ethyl pentanoate	378
1-Butanol	6557
Ethyl hexanoate	232
1-Pentanol	1227
Ethyl heptanoate	197
1-Hexanol	181
1-Heptanol	111
1-Octanol	99

[a] Corresponding to a signal which is equal to twice the baseline noise. The noise is determined from the width of the baseline.

taken as the concentration of solute giving a signal twice the background noise. In this respect, the noise is determined from the width of the baseline. From the data in Table IV, it is evident that the optimization of the PTV sampling procedure may allow the determination of less than 200 ng/l of some compounds by injection of large volumes without requiring a previous extraction and/or concentration of the sample.

After having performed the optimization procedure, it was intended to evaluate the influence of each variable on the obtained response. First the relationships between the response measured and the experimental values of each factor (variable) were studied by simple linear regression. The best correlations were achieved for the following variables: percentage of Gas Chrom 220 in the adsorbent, linear correlation coefficient r = 0.93; length of adsorbent, r = 0.88; and initial PTV temperature, r = -0.84. Therefore, a high positive correlation was observed between the response and both the percentage of Gas Chrom 220 in the adsorbent and the length of adsorbent, whereas a high negative correlation was obtained for the initial PTV temperature. It is clear that the higher the percentage of Gas Chrom 220 in the adsorbent, the higher is the response obtained, as this material exhibits a high retention power which lowers the losses of volatile materials. Also, an increase in the length of the adsorbent promotes the possibility of soaking up larger amounts of liquid samples, thus preventing the liquid from running out of the packing in the insert. With regard to the influence of the initial PTV temperature, its negative correlation with the observed response is evident as the more volatile compounds will be more easily retained if the insert is maintained at low temperature.

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