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Short Communication

Use of a packed programmed-temperature vaporizer injector in the solvent elimination mode for the determination of fatty acid methyl esters by gas chromatography

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ABSTRACT

It is shown that when using a programmed-temperature vaporizer as the injection device for the gas chromatographic determination of fatty acid methyl esters in the solvent elimination mode, losses of medium-chain solutes during the solvent elimination step can be avoided by packing the glass liner of the injector with materials providing adequate retention characteristics. Silanized glass-wool proved to be a good material for this purpose. Other parameters such as solvent elimination temperature, nature of the solvent and solute vaporization temperature were also studied in order to achieve optimum injection conditions.

INTRODUCTION

Sample injection is an important step in high-resolution gas chromatography (GC), which becomes critical when quantitative analysis of complex mixtures (mixtures of compounds covering a wide range of polarities, concentrations or volatilities) is required. One of the most important problems in injection can be the discrimination of one compound over another in their transfer from the syringe (in the liquid state) to the column (in the vapour state) [1]. Such can be the case in analyses for fatty acid methyl esters (FAMEs): whereas some studies have shown that severe discrimination between acids with low and high boiling points can arise when using classical split injection techniques [2], other have shown that using the same technique and with

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strict control of the injection parameters, excellent results can be achieved [3].

Several solutions have been proposed to overcome the problem of sampling discrimination if it is caused by selective vaporization from the syringe needle when it is placed in a hot injector block, such as the so-called "cooled needle technique", which allows excellent results to be obtained in either the split or splitless sampling mode [4], and the cold on-column or the programmed-temperature vaporizer (PTV) injection techniques [5]. The PTV is an injection device which is cold when receiving the sample and subsequently, after sample release and syringe withdrawal, is rapidly heated so that the sample enters the column as vapour (in contrast to the on-column procedure).

The PTV injection technique is very versatile and allows sample vapour transfer to the column in three operational modes: split injection, splitless injection and solvent elimination [6]. In the solvent elimination mode, a two-step vaporization is used: first, in a low-temperature vaporization step, most of the solvent is vented from the system, then the split is closed and the injector temperature is increased so that the sample is transferred to the column. This technique allows the injection of large volumes without column flooding by the sample solvent (as can happen in the on-column and splitless injection modes) [4], and it is very convenient in the analysis of very dilute samples for which a preconcentration step is required. This can occur with FAME samples obtained from marine animal larvae in which the available biological material is always very scarce.

However, from an exhaustive study on PTV injection conditions for FAMEs, Eder *et al.* [7] concluded that when PTV injection is used in the solvent elimination mode, the recovery of low and medium boiling point FAMEs is far below 100% (*ca.* 80% for 14:0), because they are lost together with the solvent through the split exit. As a consequence, they recommended that the use of this injection mode be restricted to the determination of fatty acids containing more than sixteen carbon atoms.

However, solutes in the samples can be temporarily fixed during the solvent elimination step by using suitable inserts in the glass liner of the injector. The use of packed inserts in PTV injectors has been exhaustively studied by Herraiz *et al.* [8] and Loyola *et al.* [9]. These inserts improve sample evaporation, prevent droplets of liquid forming near the column entrance and adsorb solutes to prevent them from being lost while the solvent is being vented out.

In this paper, we show how, using a silanized glass-wool insert and appropriate injection conditions, FAMEs including the esters of $C_{14:0}$ to $C_{18:0}$ acids can be accurately determined using a PTV in the solvent elimination mode as the injection device.

EXPERIMENTAL

A Perkin-Elmer PTV cold injector coupled to a Perkin-Elmer Model 8500 gas chromatograph was used. The column was a 30 m × 0.25 mm I.D. fused-silica capillary coated with a 0.20-μm layer of SP-2330 (Supelco, Bellefonte, PA, USA). Nitrogen at 10 p.s.i.g. (1 p.s.i. = 6894.76 Pa) as the carrier gas and a flame ionization detector at 250°C were always used. The column temperature was raised from 140 to 205°C at a rate of 1°C/min. The vaporization insert was packed with a 2-cm plug of silanized glass-wool (Perkin-Elmer) or Tenax GC (0.15–0.18 mm) (Alltech).

The samples were injected at low temperature (solvent elimination temperature) while the split vent was open (splitting ratio = 140:1, with a septum purge of 4 ml/min). After a certain time (splitting time), the split vent was closed and the injector heated (15°C/s) to a certain temperature (sample vaporization temperature) which was kept constant for a further 6 min. Five solvent elimination temperatures (45, 50, 60, 70 and 80°C), three splitting times (30, 60 and 120 s) and four sample vaporization temperatures (250, 300, 350 and 400°C) were tried.

A quantitative FAME standard (MO-81; Larodan, Malmo, Sweden) containing acids of different boiling points and degrees of unsaturation ($C_{14:0}$, 5%; $C_{16:0}$, 10%; $C_{16:1}$, 5%; $C_{18:0}$, 6%; $C_{18:1}$, 25%; $C_{18.2}$, 15%; $C_{18:3}$, 17%; $C_{20:1}$, 7%; $C_{20:4}$, 5% and $C_{22:6}$, 5%) was used. To test for the effect of the injection solvent, one

TABLE I

INFLUENCE OF THE SOLVENT AND THE SOLVENT EVAPORATION TEMPERATURE OF THE INJECTOR ON THE RELATIVE RESPONSE FACTORS (RELATIVE TO C18:0) OF FATTY ACID METHYL ESTERS

Mean value from five measurements of relative response factor for each FAME with relative standard deviation (R.S.D.).

Solvent	FAME	Theoretical value	45°C		50°C		60°C		70°C		80°C	
			Mean	R.S.D. (%)	Mean	R.S.D. (%)	Mean	R.S.D. (%)	Mean	R.S.D. (%)	Mean	R.S.D. (%)
Hexane	14:0	1.04	0.88	1.68	0.83	1.32	0.57	2.77	0.29	3.75	0.16	16.56
	16:0	1.02	1.05	0.43	1.04	0.53	0.96	1.75	0.75	0.94	0.55	7.82
	16:1	1.01	0.94	0.48	0.92	0.91	0.82	1.84	0.61	1.89	0.42	8.91
	18:0	1.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00
	18:1	0.99	1.02	0.88	1.02	0.54	1.01	0.83	0.98	0.56	0.93	1.40
	18:2	0.99	0.98	1.55	0.97	0.46	0.96	0.57	0.93	0.59	0.88	1.87
	18:3	0.98	0.96	2.73	0.96	0.47	0.95	0.88	0.93	0.59	0.89	1.24
	20:1	0.98	0.97	1.70	0.97	0.56	0.99	0.55	1.04	1.25	1.14	1.83
	20:4	0.90[a]	0.90	3.97	0.90	1.36	0.91	1.21	0.95	1.20	1.01	1.34
	22:6	0.70[a]	0.69	4.92	0.70	1.19	0.75	2.19	0.82	1.33	0.91	3.27
Toluene	14:0	1.04	1.01	2.86	0.91	2.77	0.58	3.45	0.37	3.54	0.21	8.49
	16:0	1.02	1.06	0.94	1.06	0.79	0.97	1.35	0.82	0.67	0.58	5.03
	16:1	1.01	0.98	1.16	0.95	0.58	0.84	1.60	0.67	1.69	0.46	5.86
	18:0	1.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00
	18:1	0.99	1.03	1.19	1.02	0.82	1.01	0.54	0.99	0.45	0.94	1.62
	18:2	0.99	0.98	1.12	0.98	1.17	0.97	0.73	0.95	0.95	0.89	1.29
	18:3	0.98	0.97	1.35	0.96	1.20	0.96	0.87	0.94	1.17	0.90	1.27
	20:1	0.98	0.98	0.56	0.97	1.13	1.00	0.55	1.02	0.54	1.11	2.06
	20:4	0.90[a]	0.90	0.79	0.89	1.95	0.91	1.85	0.93	1.32	1.00	1.96
	22:6	0.72[a]	0.72	1.59	0.70	2.13	0.72	3.31	0.79	1.55	0.88	2.95

[a] Relative response factors calculated for Perkin-Elmer flame ionization detector.

aliquots of the text mixture was dissolved in hexane and another in toluene to a final concentration of 1 mg/ml. These are two solvents typically employed for FAMEs [10,11].

For statistical reasons, each injection for a given set of parameters was repeated five times and the relative standard deviation (R.S.D.) was calculated in each instance.

Experimental response factors for each FAME were determined. Good agreement with theoretical response factors was generally achieved [11], except for 20:4 and 22:6, whose response factors under our operating conditions were smaller (0.90 and 0.72, respectively). Such a deviation from the theoretical values for polyunsaturated fatty acid methyl esters in a Perkin-Elmer flame ionization detector has been reported previously [12]. To test for discrimination effects, experimental response factors for each FAME were determined and compared with the respective theoretical values under the different PTV injection conditions.

RESULTS AND DISCUSSION

Although Tenax GC was found to provide excellent results for PTV injection in the solvent elimination mode of volatile solutes [8], it did not allow the quantitative recovery of medium boiling point FAMEs and irreversibly adsorbed the long-chain FAMEs. Attempts to desorb these compounds using higher injector temperatures were unsuccessful, probably owing to this thermal decomposition.

We therefore studied the behaviour of the PTV when the insert was packed with silanized glass-wool as described by Herraiz *et al.* [8]. A splitting time of 60 s was chosen for all the subsequent experiments, as it allows an adequate solvent elimination with no solute discrimination. Higher splitting times led to partial losses of the most volatile FAMEs.

To assess whether, when packed with glass-wool, the injector allowed the retention of FAMEs during the solvent elimination step and their subsequent desorption, standard mixtures in hexane and toluene were injected several times using PTV injection with an initial temperature of 45°C; 45°C was the lowest temperature studied as it was the minimum temperature setting that the injection device could achieve while keeping the oven temperature above 100°C.

Table I gives the mean values and R.S.D.s of

TABLE II

INFLUENCE OF THE SAMPLE EVAPORATION TEMPERATURE ON THE RELATIVE RESPONSE FACTOR OF FATTY ACID METHYL ESTERS

Mean value from five measurements of relative response factor for each FAME with relative standard deviation (R.S.D.). Solvent: hexane.

FAME	Theoretical value	250°C		300°C		350°C		400°C	
		Mean	R.S.D. (%)	Mean	R.S.D. (%)	Mean	R.S.D. (%)	Mean	R.S.D. (%)
14:0	1.04	1.00	2.31	1.01	2.86	1.00	2.09	1.03	2.14
16:0	1.02	1.08	1.65	1.06	0.94	1.07	0.51	1.08	1.40
16:1	1.01	0.98	1.91	0.98	1.16	0.97	0.73	0.98	1.68
18:0	1.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00
18:1	0.99	1.01	0.88	1.03	1.19	1.02	0.54	1.01	0.54
18:2	0.99	0.96	1.36	0.98	1.12	0.97	0.57	0.96	0.94
18:3	0.98	0.92	2.74	0.97	1.35	0.94	1.22	0.91	1.96
20:1	0.98	0.77	11.17	0.98	0.56	0.95	1.57	0.93	2.78
20:4	0.90[a]	0.70	10.87	0.90	0.79	0.82	3.59	0.77	7.29
22:6	0.72[a]	0.24	25.28	0.72	1.59	0.46	6.62	0.44	35.71

[a] Relative response factors calculated for the Perkin-Elmer flame ionization detector.

the results expressed as relative response factors obtained for each FAME at different solvent elimination temperatures and with hexane (boiling point 68.9°C) and toluene (boiling point 110.6°C) as injection solvents. As can be inferred from comparison with the theoretical relative response factors, only toluene as solvent and a temperature of 45°C were not discriminative towards the low-boiling point FAMEs. Samples with hexane as solvent showed a clear discrimination at 45°C even for 16:1.

Loyola *et al.* [9] showed that different end temperatures in the sampling device could produce different results with respect to both the accuracy and the precision. To test for this effect, four sample vaporization temperatures were studied and the results are given in Table II. As can be seen, temperatures lower than 300°C were not able to desorb the long-chain FAMEs and showed high R.S.D.s. Higher temperatures gave an important and variable decrease in the relative response factors of long carbon chains, probably owing to thermal decomposition. As a consequence, 300°C was adopted as the temperature of sample vaporization.

CONCLUSIONS

A PTV injector in the solvent elimination mode can be used for the accurate and precise determination of medium- and long-chain fatty acids as their methyl esters in very dilute toluene solutions if the glass insert is packed with a material giving a sufficient retention power to avoid losses during the solvent evaporation step. Silanized glass-wool proved to be a good material for this purpose if low solvent evaporation temperatures and short splitting times are used. The use of 45°C as the solvent elimination temperature during a splitting time of 60 s allows the determination of fatty acids with less than eighteen carbon atoms without discrimination and with accuracies and precisions comparable to those obtained with other injection techniques.

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