Determination of Styrene in Olive Oil by Coevaporation, Cold Trap, and GC/MS/SIM

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A new procedure to determine styrene in olive oil, a simulant of fatty food, is presented. The method is based on the coevaporation of styrene with a solvent of low boiling point at 90 °C. The vapors obtained are condensed in a frozen syringe, and this liquid is injected in GC/MS/SIM mode. The method has been validated using deuterated styrene (d_8) and α-methylstyrene as internal standards. The detection limit is 10 μg/kg with a relative standard deviation (RSD) of 5% for 50 μg/kg styrene in olive oil.

INTRODUCTION

Styrene monomer can be in direct contact with food when polymers such as polystyrene, acrylenitrile–butadiene–styrene (ABS), are used as packaging material with foods. Several adverse effects to the health of human population have been detected (Bond, 1989), and styrene concentration in food has been limited in the European community countries.

A number of methods of analysis for residual styrene in food from packaging polystyrene have been previously reported. The analysis of aqueous liquids or, in general, hydrophilic foods with little fat content implies that liquid–liquid extraction and gas chromatography with FID detection can be used (Varner et al., 1981a,b). However, with fatty food a cleanup step to eliminate the fat matter, which interferes in the GC analysis, is necessary. This cleanup step can be carried out either by HPLC or by classical liquid chromatography. This process makes the time of analysis longer and also may introduce additional errors because of the sample handling. Also, the cost of the analysis can be higher if an automatic injector, fraction collectors, and automatic systems are used to reduce both the handling and the time. Another method of analysis is headspace gas chromatography (Gilbert et al., 1983). Manual and automatic headspace procedures (Jickells et al., 1993) have been developed for styrene, and they have several advantages compared to the other mentioned methods. First, headspace sampling does not need a cleanup step. Second, the number of interferences is less than with other methods, and finally, no extraction is required. Manual headspace procedures usually have problems of condensation in the syringe. Automatic headspace is expensive, and only one chromatographic injection of each sample can be done. This paper shows a modified manual procedure in which the styrene is coevaporated with an organic solvent, the vapors being condensed on a cold syringe. Several replicates of the liquid obtained, which contains the styrene, can be injected in the GC. The method has been developed for determining styrene in olive oil, and the quantification has been made by GC/MS. The detection limit is 10 μg/kg, which is lower than that corresponding to the automatic headspace.

EXPERIMENTAL PROCEDURES

Materials. Twenty-milliliter headspace vials, Teflon-coated silicone septa, and a 0.5-mL (0.2 cm i.d.) gastight syringe (Pressure Lok, Precision Sampling Corp.) were used.

The syringe was cleaned using, sequentially, acetone and hexane, and it was dried in at oven a 50 °C before a new use.

The glass vials and the septa were preheated a 180 °C for 12–16 h before use.

Styrene, styrene-d_8, and α-methylstyrene were obtained from Aldrich Chemical Co., Madrid.

The thermostated oven of a Perkin-Elmer 8010 gas chromatograph was used.

Methods. Five grams of olive oil was placed in a 20-mL headspace vial and 200 μL of n-hexane and 5 μL of a solution containing 42 ng/μL of styrene-d_8 and 41 ng/μL of α-methylstyrene were also added to the vial. The vial was sealed with a Teflon-coated silicone septum, and it was shaken for 2 min to achieve homogenization. The vial was introduced into a thermostated air oven at 95 °C. After an equilibrium period of 60 min, the syringe with the plunger inside previously frozen at −20 °C was inserted through the septum. The plunger of the syringe was taken out to facilitate the vapors coming out and condensing on the inner frozen walls of the syringe. When the vapor flow stops and both internal and external pressures are equilibrated, the liquid contained in the syringe (about 25 μL) is placed in a microvial, and 1 μL of this liquid was injected in GC/MS system.

To quantify the styrene, the standard addition method was use; different amounts of styrene were added to several aliquots of 5 g of the olive oil sample, and the recommended procedure was followed.

The reproducibility study was carried out with an olive oil sample that contained 50 μg/kg styrene-d_8 and 46 μg/kg of styrene. Seven aliquots of 5 g each were prepared, and the recommended procedure was followed.

Chromatographic Conditions. A Hewlett-Packard 5970 Series II gas chromatograph coupled to a Hewlett-Packard 5971 mass selective detector and the following conditions were used: GC column, 30 m × SPB1; carrier gas, He; flow, 1 mL/min; volume, 1 μL; Injector temperature, 250 °C; Detector temperature, 280 °C; oven temperature, 50 °C (initial time 1 min); rate, 5 °C/min; final temperature, 120 °C (final time 5 min); splitless time, 1 min; ion masses selected: 104 (styrene), 112 (styrene-d_8), 117 (α-methylstyrene).

RESULTS AND DISCUSSION

For the analysis of styrene in olive oil, headspace partition is extremely unfavorable and adequate limits of detection are not attainable by headspace GC/MS. However, a steam distillation approach followed by GC/MS using a stable isotope-labeled internal standard has been carried out. So, the combination of the headspace procedure with a coevaporation with low boiling point solvent can improve the partition for styrene and consequently its sensitivity.

Among the common solvents of low toxicity, hexane was selected to study the coevaporation effect. To be sure

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Table I. Influence of the Volume of Hexane Added to the Oil Sample for the Coevaporation of Styrene and Hexane

hexane added, μL	area styrene	area ratio styrene/styrene-d_8
100[a]	67.400	3.99
200	39.300	3.91
250	38.400	3.85
350	35.000	3.93

[a] The obtained volume of condensed vapor is very small, and it does not allow handling.

that the quantitative results obtained for the styrene were representative of each experiment, two compounds of similar chemical structure to styrene were used as internal standards: α-methylstyrene and deuterated styrene (styrene-d_8).

Table I shows the results obtained when different volumes of hexane were added to the oil sample for the coevaporation step. Several points can be emphasized from these data. First, independent of the volume of hexane added, the area ratio styrene/styrene-d_8 is constant. Consequently, the amount of hexane in the vial does not influence the quantification of styrene. However, the more the absolute response (area) for the styrene was obtained, the less concentration of styrene can be determined, which implies a lower detection limit for the method. If this situation were applied to Table I, a volume of 100 μL would be considered the optimum for use in the method. Nevertheless, when this volume is used, the volume of condensed vapor is too low to handle it and cannot be recommended as the optimum value. So, a volume of 200 μL of hexane was selected as the optimum value.

The syringe is also very important for several reasons, one being the internal diameter of the syringe. If it is lower than 0.2 cm, the vapor pressure pushes out the condensed liquid. With a syringe of 0.2 cm i.d., the vapor bubbles through the condensed liquid.

On the other hand, to facilitate the vapors coming out, the plunger of the syringe has to be taken out. However, if the plunger of the frozen syringe is taken out some time before the injection into the headspace vial, the moisture from the room could condense on the inner walls of the syringe. This situation would affect very much the condensation of the organic vapors from the sample in the vial. To avoid this problem, the plunger is kept inside the syringe during the frozen step, and it is taken out just after the liquid in the syringe is injected into the vial.

As the method implies the evaporation of several compounds, the temperature is a very important factor. To achieve accurate control of the temperature, a chromatographic oven was used in the work. The temperature should not be modified more than ±0.5 °C. With the method developed in this paper the sensitivity can be deduced from the slope of the straight line obtained when the response (area counts in this case) is plotted against the concentration of styrene, as shown in Figure 1 . As can be seen in Figure 1 , a difference of 500 area counts in the response gives a difference in the concentration value of 6 μg/kg, which is quite a high value. Consequently, good sensitivity can be attributed to this method.

Figure 2 shows the calibration plot obtained for styrene using both α-methylstyrene and styrene-d_8 as internal standards. A linear range was obtained up to 400 μg/kg styrene. It can be observed that the slope for α-methylstyrene is higher than that obtained for styrene-d_8. This is a result of both the different volatilities of styrene-d_8 and α-metylstyrene and the lower response in the GC/MS for α-methylstyrene than for styrene-d_8 for similar concentration values. Figure 3 shows the plots obtained when
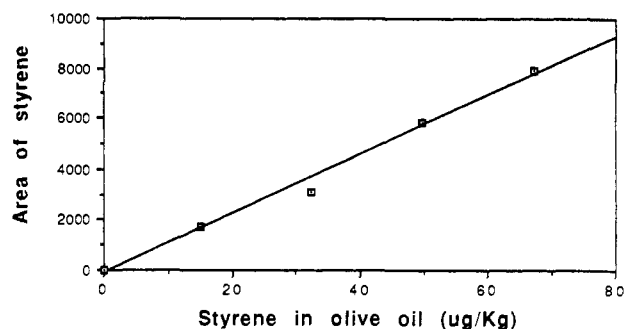

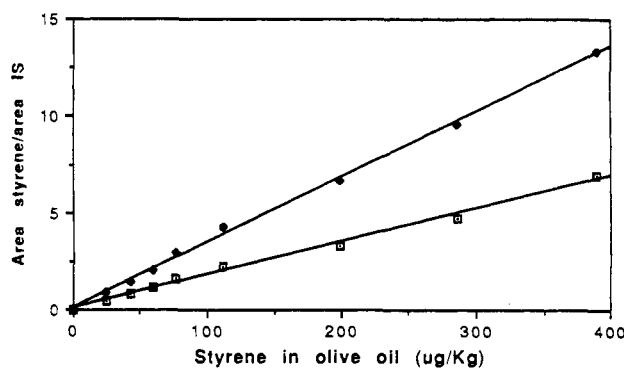**Figure 1.** MS response (area counts) for styrene monomer against the concentration of styrene in olive oil.

Figure 2. Calibration plot for styrene by GC/MS/SIM using two different internal standards: styrene-d_8 (□) and α-methylstyrene (■).

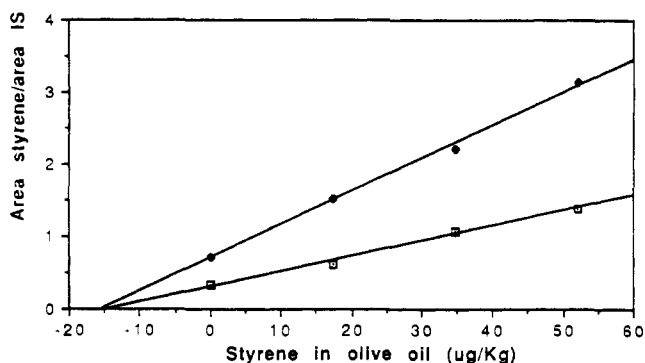**Figure 3.** Standard addition procedure for styrene using styrene-d_8 (□) and α-methylstyrene (■) as internal standards to quantify the styrene by GC/MS/SIM mode.

the standard addition method was applied to a sample of olive oil in which a low amount of styrene (15 μg/kg) had been added. In the quantification of styrene both styrene-d_8 and α-methylstyrene were used as internal standards. The results obtained show the accuracy of the method and also are important to point out that quantitative results can be achieved using a nondeuterated internal standard such as α-methylstyrene by the standard addition method. The result obtained with concentration values of styrene of 50 and 100 μg/kg are also very good.

CONCLUSIONS

The described procedure for determining styrene in olive oil, an established simulant of fat food, has several advantages:

(1) It is a direct method, which means no cleanup step is necessary and no interferences appear from other compounds or from the matrix of the sample.

(2) It is a very cheap procedure, and no automatic injection system or automatic headspace is necessary.

(3) The vapor obtained, once condensed, can be injected several times, providing replicates of chromatographic data of the same sample.

(4) The sensitivity is higher than that of other manual methods. It is possible to quantify 10 μg/kg styrene in olive oil.

(5) This method permits preparation and analysis of samples several hours after using a normal automatic injector for liquid samples.

(6) Both styrene-d_8 and α-methylstyrene used as internal standards give good results to quantify styrene; nondeuterated internal standard can be used in styrene cuantification.

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