Determination of Tetrachloroethylene—Results of a Collaborative Study and the Standardized Method

W. Dennis Pocklington*
Laboratory of the Government Chemist, Teddington, United Kingdom

The development, by collaborative study, of a standardized method for the determination of tetrachloroethylene in olive oils is described. The results of the study, which was carried out in 1989 under the direction of the International Olive Oil Council (IOOC), are presented and show that tetrachloroethylene can be readily determined, to an acceptable degree of precision, by gas-liquid chromatography with electron capture detection. A limited number of results obtained for the determination of trichloroethylene and chloroform by the same method indicate that a similar precision could be obtained with the method when determining these solvents also.

KEY WORDS: Chloroform, collaborative study, electron capture detection, olive oil, solvent contamination, tetrachloroethylene, trichloroethylene.

In recent years, traces of the solvent tetrachloroethylene (which is commonly employed in automated systems for the determination of the oil content of crushed olives) have been detected in some batches of olive oil (usually virgin olive oil). The presence of this solvent in the oil may be attributed to the accidental contamination of the olive oil mill environment and the subsequent contamination of the stored olive oil. Similar problems have arisen with other chlorinated solvents such as trichloroethylene and chloroform.

In 1988, the Commission of the European Communities (EEC) introduced a regulation (1) prohibiting the sale, in the Community after 31 December 1988, of olive oils and olive-pomace oils with a tetrachloroethylene content of more than 0.1 mg per kg. Because different methods for the determination of this solvent were being used by Community laboratories, a further regulation (2) was issued in 1988, which described a method to be used for the enforcement of the regulation. The method, which is based on gas chromatography with a packed column, electron capture detection and headspace injection, had not been subjected to collaborative study, and it had been established that the precision of the procedure, as outlined in the regulation, needed to be improved. Therefore, the International Olive Oil Council (IOOC) arranged for the convening of a working group (mainly made up of chemists from olive oil producer countries) and assigned it the task of studying the procedure with a view to improving its precision characteristics.

1989 COLLABORATIVE STUDY

Four materials (spiked olive oils) were provided for the study, each material being provided in duplicate (blind-coded) giving a total of eight samples for analysis. Three of the sample materials were olive oils spiked with tetrachloroethylene only (in the range 0.02 to 0.3 mg/kg [20–300 ppb]), and one of the sample materials was spiked additionally with trichloroethylene and chloroform at a level of 0.3 mg/kg (both solvents). Each sample was required to be injected twice, under repeatability conditions, and participants were allowed a certain amount of freedom in choice of column type and injection method (*i.e.*, direct or headspace). Where headspace injection was used, duplicate analysis of the same prepared headspace was allowed. Participants were given more precise instructions than those appearing in the EEC regulation method regarding the calibration procedure to be followed and recommendations for the use of capillary columns (not required by the regulation method) in the analysis of the provided samples.

*Address correspondence at 55 South Lane, New Malden, Surrey KT3 5HX, England.

RESULTS

The individual results reported by each laboratory are reproduced in Table 1, which shows that the recovery of the spiked amounts of the added solvents was generally satisfactory. Table 2 shows that the values for the relative reproducibility standard deviation (RSD_R) at 9, 12, 17, and 26% are (except for the 43% value at the 0.02 mg/kg [20 ppb] level) considerably less than the 45% indicated by Horwitz (3) as having generally been experienced with methods determining analytes at the parts per billion level. The participants (representing laboratories in France, Italy, Portugal, Spain and the United Kingdom) were requested to report the gas-liquid chromatographic conditions employed for the analysis, and these conditions are given in Table 3. Figure 1 shows the typical gas chromatographic separation of chloroform, trichloro- and tetrachloroethylene from olive oil.

TABLE 1

Results Obtained from the Collaborative Study (raw data—results expressed as ppb [μg/kg])

Sample	Laboratory number						
	1	2	3	4	5	6[a]	7
A—olive oil spiked with 300 ppb tetrachloroethylene	340 325	300 300	420 380	381 354	180 350	245 255	328 317
B—olive oil spiked with 100 ppb tetrachloroethylene	110 120	100 100	800[a] 830[a]	129 139	70 70	100 105	70 100
C—olive oil spiked with 300 ppb tetrachloroethylene	280 260	300 300	800[a] 760[a]	329 296	— 300[a]	225 —	259 250
D—olive oil spiked with 20 ppb tetrachloroethylene	30 30	10 10	250[a] 250[a]	27 26	90[a] 70[a]	30 30	22 15
E—olive oil spiked with 300 ppb trichloroethylene	— —	300 300	240 230	335 324	— 	175 —	184[a] 196[a]
F—olive oil spiked with 300 ppb chloroform	— —	300 300	430 400	335 309	— 	265 —	315 272

[a]Results not included in statistical analysis.

TABLE 2

Summary of Statistical Analysis of Results

Sample	Mean value (ppb)	r^a	R^a	$RSD_R{}^a$ (%)	Number of accepted results	Total number of results
A—olive oil spiked with 300 ppb tetrachloroethylene	345	46	116	12	10	12
B—olive oil spiked with 100 ppb tetrachloroethylene	287	32	70	9	10	12
C—olive oil spiked with 300 ppb tetrachloroethylene	101	30	74	26	10	12
D—olive oil spiked with 20 ppb tetrachloroethylene	21	7	26	43	8	10
E—olive oil spiked with 300 ppb trichloroethylene	288	17	137	17	6	8
F—olive oil spiked with 300 ppb chloroform	333	59	164	17	8	8

$^a r$ = repeatability limit; R = reproducibility limit; RSD_R = relative reproducibility standard deviation.

TABLE 3

Gas Chromatographic Conditions Used by Participants

Laboratory number	Column Type[a]	Column Dimensions	Stationary type	Phase loading/ film thickness	Column temp. (°C)	Injection[b]	Carrier gas[c]
1	P	2 m × 4 mm	SE 30	10%	70	HE	N
2	P	2 m × 2 mm	Porapak Q	?	200	HE	A-M
3	C	30 m × 0.25 mm	SE 52/54	0.2 μm	35	HE	H
4	P	3 m × 2 mm	OV 101	10%	60	DI	N
5	C	50 m	HPS			HE	
6	P	2 m × 1/8″	OV 101	10%	up to 350	HE	N
7	C	50 m × 0.32 mm	CPSU 8	1.2 μm	70	HE	He

[a] C = capillary column; P = packed column.

[b] HE = headspace injection; DI = direct (on-column) injection.

[c] A-M = argon/methane; H = hydrogen; He = helium; N = nitrogen.

Although a more accurate assessment of the precision of the method would have been made possible by having more results available for statistical analysis, it was agreed that on the basis of the submitted results a standardized procedure could be drafted. The text of the standardized procedure, as adopted by the International Olive Oil Council, is given below.

STANDARDIZED PROCEDURE

TETRACHLOROETHYLENE DETERMINATION IN OLIVE OILS BY GAS-LIQUID CHROMATOGRAPHY

1.0 SCOPE AND FIELD OF APPLICATION

This Standard describes a method for determination of the tetrachloroethylene content of olive oils in a concentration range of 0.02–0.3 mg/kg. It may also be used for the determination of certain other chlorohydrocarbons (Note 8.1).

2.0 PRINCIPLE

Incubation of the sample in a closed vial and analysis of the headspace by gas-liquid chromatography using an electron capture detector. Quantitative estimation of the tetrachloroethylene following calibration with an external standard. Alternatively, analysis of the sample following direct injection onto the column.

3.0 APPARATUS

3.1 Vials, glass, 15–20 mL capacity, capable of being hermetically sealed with an aluminum cap containing a Teflon/rubber septum (Note 8.2).

3.2 Pipette, automatic, suitable for dispensing 40 μL volumes, preferably positive displacement type.

3.3 Gas syringe, 2500 μL (Note 8.3). Alternatively, for direct injection analysis, ordinary syringe, 10 μL.

3.4 Gas-liquid chromatograph equipped with an electron capture detector and integrator.

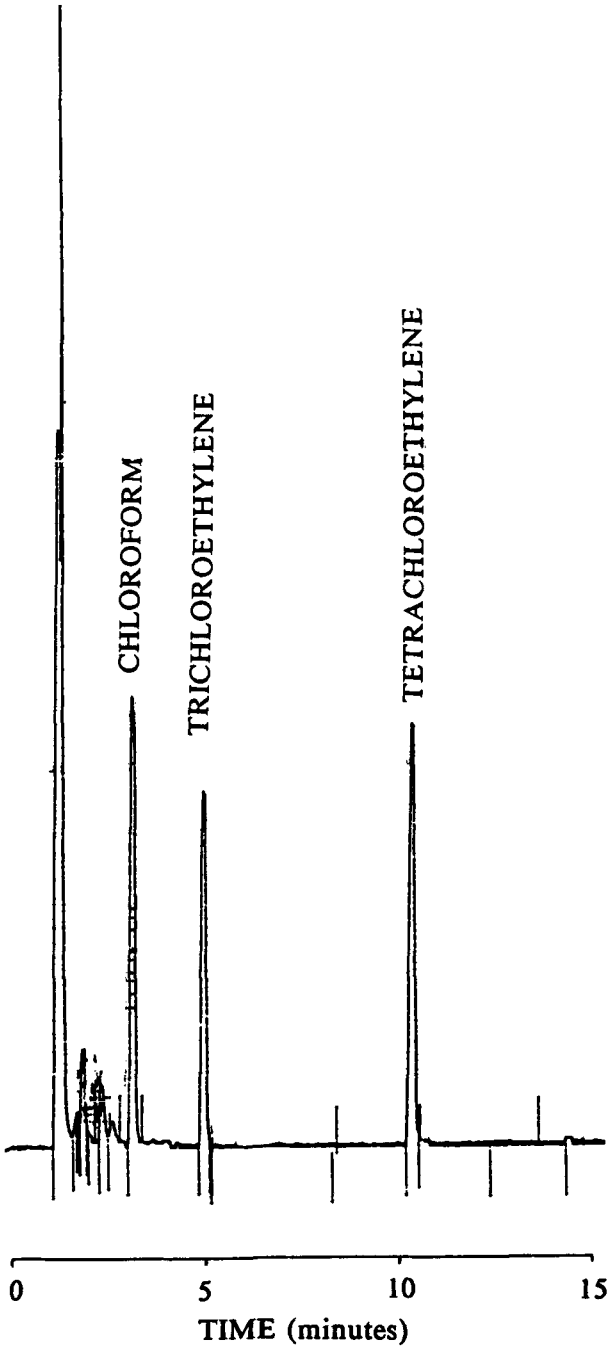

FIG. 1. Typical gas chromatographic separation of the three subject chlorinated solvents, added at levels of 0.1 mg/kg olive oil.

3.5 Column, capillary or packed (Note 8.4), to fit the chromatograph (3.4), with a stationary phase suitable for the separation of chlorohydrocarbons (Note 8.5). For use with direct injection of test sample a suitable pre-column should be fitted. Recommended operating conditions (Note 8.6): injector: 150°C; detector: 350°C; oven: 35–85°C.

3.6 Carrier and auxiliary gases: nitrogen, hydrogen, helium, or argon/methane (Note 8.7) for gas chromatography and suitable for use with electron capture detection.

4.0 REAGENTS

4.1 Tetrachloroethylene, chromatographic grade.

4.2 Dekalin (decahydronaphthalene), or dimethylformamide, or *N,N* dimethylacetamide, chromatographic grade (Note 8.8).

4.3 Tetrachloroethylene, solution in dekalin or other suitable solvent (4.2). Prepare as follows:

4.3.1 *Solution A* (concentration equivalent to 10 g/kg): Weigh 1.00 g tetrachloroethylene (4.1) into a 100-mL volumetric flask and dilute to volume with solvent (4.2).

4.3.2 *Solution B* (concentration equivalent to 100 mg/kg): Pipette 1 mL Solution A into a 100-mL volumetric flask and dilute to volume with solvent (4.2).

4.3.3 *Solution C* (concentration equivalent to 5 mg/kg): Pipette 5 mL Solution B into a 100-mL volumetric flask and dilute to volume with solvent (4.2).

4.3.4 *Solution D* (concentration equivalent to 10 mg/kg): Pipette 10 mL Solution B into a 100-mL volumetric flask and dilute to volume with solvent (4.2).

5.0 PROCEDURE

5.1 Weigh accurately (to the nearest 10 mg) about 2 g test sample into each of three vials (3.1) and add using the automatic pipette (4.3), to:
vial 1—40 μL of solvent (4.2)
vial 2—40 μL of Solution C (4.3.3)
vial 3—40 μL of Solution D (4.3.4)
[Note: The test portions in vials 2 and 3 will contain tetrachloroethylene concentrations equivalent, for practical purposes, to 0.1 and 0.2 mg/kg, respectively (Note 8.9)].

5.2 Seal the vials hermetically, mix (Note 8.10) the contents of each vial by gentle shaking of the vials several times and, if headspace analysis is to be used, incubate the vials at 70°C for 60 min.

5.3 **Chromatography:**

5.3.1 **Headspace analysis:** Using the gas syringe (3.4), inject about 250–2000 μL volumes of the headspace from each of the vials (5.1) which have been incubated for 60 min. Record the peak areas obtained for tetrachloroethylene (TCE).

5.3.2 **Direct injection analysis:** Using the ordinary syringe (3.4), inject, in turn, about 5–10 μL of the solutions in each of the vials. Record the peak areas obtained for tetrachloroethylene (TCE).

6.0 CALCULATION AND EXPRESSION OF RESULTS

6.1 Construct a calibration graph of the recorded peak areas (5.4) against the corresponding concentration of TCE added in mg/kg.

6.2 Extrapolate the straight line obtained and record the concentration of TCE in the sample

given by the value of the intercept of the abscissa.

6.3 Express the results obtained for tetrachloroethylene to the nearest mg/kg.

7.0 QUALITY ASSURANCE—See Annexe.

8.0 NOTES

8.1 The determination of trichloroethylene and chloroform at a concentration range equivalent to 0.3 mg/kg has been shown to be satisfactory when using the procedure described.

8.2 The vials are not to be used for more than one determination.

8.3 The syringe should be warmed to a temperature of about 85°C before use. Alternatively, an automatic headspace sampler, together with an integral thermostatically controlled incubator and temperature controlled heated gas syringe, may be used with advantage.

8.4 Suitable column dimensions are:
—*capillary column*: length 25–50 m and internal diameter 0.25–0.35 mm (a split ratio of 1:5 has been found suitable)
—*packed column*: length 2–3 m and internal diameter 2–4 mm i.d.

8.5 Suitable stationary phases are:
SE 30, SE 52/54, OV 17, OV 11, OV 101, HP 5, CPSil 8, CPSil 13, and DB 24.

8.6 An oven temperature programming of 65°C for 8 min, then to 85°C at 3°C/min has been found satisfactory.

8.7 A carrier gas consisting of a mixture of 10% argon in methane will be found to increase detector sensitivity.

8.8 These solvents have high lipid solubility, relatively low vapor pressure, and a density and viscosity similar to that of olive oil. Alternatively, pentane may be used but is not recommended.

8.9 For concentrations of tetrachloroethylene outside the range of 0.1–0.2 mg/kg, other calibration solutions with appropriate concentrations should be prepared.

8.10 Mixing of the vial contents by electro-mechanical means is preferred—mixing by inversion is not advised as this could result in oil being introduced into the syringe when it pierces the septum.

ANNEXE
Analytical Quality Control

1) Repeatability

When the mean value [m] of two single test results obtained under **repeatability conditions**# lies within a range of the values shown in the table below, the absolute difference between the two test results obtained should not be greater than the **repeatability limit (r)** deduced by linear interpolation from the data in the table.

#**repeatability conditions**: conditions where independent test results are obtained with the same method on identical test material in the same laboratory by the same operator using the same equipment within short intervals of time.

2) Reproducibility

When the values of two single test results obtained under **reproducibility conditions**# lie within the range of the values shown in the table below, the absolute difference between the two test results obtained should not be greater than the **reproducibility limit (r)** deduced by linear interpolation from the data in the table.

#**reproducibility conditions**: conditions where test results are obtained with the same method on identical test material in different laboratory with different operators using different equipment.

3) Trueness (bias)—the bias of the method was demonstrated in a collaborative study of the method (see table of statistical data below) not to be significant at levels of tetrachloroethylene in the range 0.02–0.3 mg/kg.

4) Limit of detection—below 0.02 mg/kg when capillary columns are used for the assay.

5) Statistical and other data derived from the results of an interlaboratory test

An interlaboratory test carried out in 1989 at the international level under the direction of the International Olive Oil Council and in which 7 laboratories participated, each obtaining two test results for each sample, gave the statistical results (evaluated in accordance with ISO 5725-1986) summarized in the following table:

Sample	A	B	C
Number of laboratories retained after eliminating outliers	5	6	5
Number of outliers (laboratories)	2	1	2
Number of accepted results	10	12	10
Mean value (mg/kg)	**0.02**	**0.1**	**0.3**
True, or accepted value (mg/kg)	0.02	0.1	0.3
Repeatability standard deviation (S_r)[a]	0.002	0.01	0.01
Repeatability relative standard deviation[b]	9.7%	9.6%	3.9%
Repeatability limit (r)[a] [2.8 × S_r]	**0.006**	**0.027**	**0.032**
Reproducibility standard deviation (S_R)[a]	0.009	0.023	0.025
Reproducibility relative standard deviation[b]	38.2%	23.2%	8.6%
Reproducibility limit (R)[a] [2.8 × S_R]	**0.025**	**0.066**	**0.070**

[a]Expressed as mg tetrachloroethylene/kg sample.

[b]Coefficient of variation.

ACKNOWLEDGMENTS

The International Olive Oil Council is indebted to the seven laboratories who submitted results, and to the Laboratory of the Government Chemist (UK) for its organization of the collaborative study. The author thanks Mr. Fausto Luchetti (IOOC Executive Director) for permission to publish this paper.

REFERENCES

1. *Off. J. European Communities,* Commission Regulation (EEC) No. 576/88. OJ No L 56, 2.3.88, Commission of the European Communities, Brussels, Belgium, 1988.
2. *Ibid.,* Commission Regulation (EEC) No. 1858/88. OJ No L 166, 1.7.88.
3. Horwitz, W., *Anal. Chem. 54*:67A-76A (1982).

[Received August 20, 1991; accepted March 30, 1992]