Pure & Appl. Chem., Vol. 69, No. 8, pp. 1829–1837, 1997.
Printed in Great Britain.
© 1997 IUPAC

INTERNATIONAL UNION OF PURE
AND APPLIED CHEMISTRY

ENVIRONMENTAL CHEMISTRY DIVISION
COMMISSION ON OILS, FATS AND DERIVATIVES*

THE DETERMINATION OF *TRANS* UNSATURATED FATTY ACIDS IN EDIBLE OILS AND FATS BY CAPILLARY GAS–LIQUID CHROMATOGRAPHY

(Technical Report)

Results of collaborative studies and the standardised method

Prepared for publication by

A. DIEFFENBACHER AND P. DYSSELER

*Membership of the Commission during the period the report was prepared (1992–93) was as follows:

Chairman: A. Dieffenbacher (Switzerland); *Vice-Chairman:* P. Dysseler (Belgium); *Secretary:* P. W. Hendrikse (Netherlands); *Titular Members:* Gunhild Hölmer (Denmark); A. Karleskind (France); F. Mordret (France); S. Watanabe (Japan); G. Zwerenz (Austria); *Associate Members:* A. Bonekamp (FRG); T. Hashimoto (Japan); M. Jeránek (Hungary); J. Holm (Denmark); M. Pike (UK); K. Stig-Andersen (Denmark); A. Visipää (Finland); *National Representatives* (varying periods): R. Brenner (Argentina); J. Sabine (Australia); R. Lago (Brazil); N. Yanishlieva (Bulgaria); P. Kolodziejcyk (Canada); Y. Yang (China); J. Pokorny (Czech Republik); M. Nega (Ethiopia); D. Boskou (Greece); H. A. Bhakare (India); B. Connolly (Ireland); A. Dolev (Israel); M. Ramalho (Portugal); S. Goh (Malaysia); A. Krab (Netherlands); D. Woollard (New Zealand); Carmen Dobarganes Garcia (Spain); T. Tuglular (Turkey); D. Berner (USA).

Republication or reproduction of this report or its storage and/or dissemination by electronic means is permitted without the need for formal IUPAC permission on condition that an acknowledgement, with full reference to the source along with use of the copyright symbol ©, the name IUPAC and the year of publication are prominently visible. Publication of a translation into another language is subject to the additional condition of prior approval from the relevant IUPAC National Adhering Organization.

The determination of *trans* unsaturated fatty acids in edible oils and fats by capillary gas–liquid chromatography (Technical Report)

Abstract: Collaborative studies on the application of capillary gas-liquid chromatography for the determination of *trans* unsaturated fatty acids are described. The method investigated involves preparation of methyl esters with methanolic potassium hydroxide followed by analysis of fatty acid methyl esters by gas-liquid chromatography on polar capillary columns. Care has to be taken in selecting working conditions to obtain the best obtainable resolution of signals necessary for reliable identification and determination of *trans* unsaturated fatty acids.

INTRODUCTION

trans Unsaturated fatty acids are mainly found in processed oils and fats and only rarely in nature. These fatty acids are not considered as essential fatty acids and their biological effects are questioned. Therefor nutritionists are interested in knowing the content of these fatty acids in different foods. To determine the various unsaturated isomers encountered a suitable capillary column has to be selected together with an optimal choice of gas-liquid chromatographic working conditions. Special care must be taken in the interpretation of the chromatograms.

1ST (PRELIMINARY) COLLABORATIVE STUDY

Based on collaborative studies for the determination of *n*-3 and *n*-6 unsaturated fatty acids [1] the Commission decided to study the application of the standardised gas-liquid chromatographic method capillary column to hydrogenated vegetable oils. In a preliminary study in 1986/87 four samples of soybean oil with different degrees of hydrogenation, including a sample in duplicate, were sent for evaluation. Participants were asked to apply their usual working conditions and report results for the main fatty acids, as well as for the sum all unsaturated fatty acids containing 18 C-atoms. Additionally, it was appreciated when participants could identify some of the unsaturated fatty acids.

Eleven laboratories sent results for evaluation. Almost all of them identified the sample sent in duplicate. Interpretation of the chromatograms sent by participants revealed that signal evaluation should be more standardised. The Commission considered results columns of this preliminary study as promising. It was decided to continue the study during the following year with application of the method to animal oils and fats.

2ND COLLABORATIVE STUDY AND RESULTS

For the second collaborative study in 1987/88 samples of beef tallow and lard as well as a blend of 90 per cent of beef tallow with 10 per cent of lard were provided together with samples of fish oil, hydrogenated fish oil and a one-to-one blend of these two oils. Samples were sent in blind duplicates and participants were again asked to apply the preparation of methyl esters according to method 2.301 [2] and their usual working conditions to achieve maximal resolution of signals on their equipment.

Ten laboratories sent their results and the working conditions applied together with copies of chromatograms. Most capillary columns used were 50 m in length and coated with cyanopropylene-polysiloxane. Furthermore, most participants used split injection and temperature programming. Almost all participants could identify the pairs of samples, but had problems in identifying fatty acid signals. Statistical evaluation of some results is given in Table 1, where the number of results considered for a statistical evaluation are those who correctly recognised the signals of the different *trans* fatty acid isomers. The number of results eliminated are those considered as outliers by Cochrans' or Dixons' outlier tests. The remaining results after deduction of outliers are used for calculation of repeatability and reproducibility [3]. Values for repeatability and reproducibility were similar to those reported in the ring tests of the determination of *n*-3 and *n*-6 unsaturated fatty acids, but for isolated signals only e.g. for those of C-14:0,

© 1997 IUPAC

C-16:0 or C-18:0 [1]. The same is true for repeatability values for C-18:1 and its isomers, but not for reproducibility values (see Table 2). One reason for these phenomena was that not all participants could identify all fatty acid signals and some had chromatograms with poorly separated peaks.

However, those who could correctly interpret their data reported results suitable for a statistical evaluation of straight oils, as well as of blended samples.

Therefore the overall opinion of the Commission was that the analytical method tested gave satisfactory results for well-separated signals. Repeatability and reproducibility could, however, be improved in applying a more strict protocol particularly for evaluation of partially overlapping signals.

3RD COLLABORATIVE STUDY

For the 1988/89 study samples of pure and hydrogenated soybean oil and a one-to-one blend of these oils in duplicate were distributed. The codes were as follows, soybean oil; sample A; hydrogenated soybean oil; sample B; the one-to-one blend of pure soybean oil with hydrogenated soybean oil sent in duplicate ; sample C and D (see Tables 2 and 3). In the protocol emphasis was given to an uniform integration of the various *trans* fatty acid signals eluting in the region of C-18:1 isomers I to III. Participants were requested as in the previous studies, to apply the quick method for the preparation of fatty acid methyl esters according to method 2.301 [2] and to optimise their working conditions to obtain the requested resolution of signals.

Sixteen laboratories sent results together with their working conditions and copies of their chromatograms. As in the previous study most participants used capillary columns of 50 to 60 m in length and 0.25 - 0.32 mm internal diameter with a cyanopropylene-polysiloxane based coating. Again most participants applied split injection and only a few used on column injection. Helium was preferred to hydrogen as carrier gas and some participants even used nitrogen. Most laboratories applied temperature programming. Results for isomers I to III are given in Table 2. Statistical evaluation of results for fatty acids signals assigned to C-18:1 isomers I to III is given in Table 3. Once again results for repeatabilty r and reproducibiliy R were found to be acceptable for isolated signals. However, for overlapping peaks, such as those for *trans* fatty acids isomers were far from satisfactory. The commission agreed that the general method 2.304 for fatty acid determination by capillary gas-liquid chromatography as tested could also be adopted for hydrogenated oils and fats. It was, therefore, decided to repeat once more the collaborative study with hydrogenated vegetable oils. For this purpose the use of a work station was considered essential to ensure accuracy of integration where peaks could not be fully resolved.

Pure (SBO) and hydrogenated (SBO hydr) and a one-to-one blend of pure and hydrogenated soybean oil (SBO/SBO hydr 50/50) were distributed together with a sample of hydrogenated rape seed oil (RSO hydr, and a sample a one-to-one blend of pure and hydrogenated rape seed oil (RSO/RSO hydr 50/50). Samples were distributed again as differently coded blind duplicates. As in the previous studies participants were asked to apply the cold methylation method 2.301 [2] for the preparation of the fatty acid methyl esters.

Ten laboratories sent 12 sets of results. Their working conditions and the capillary columns used are summarised in Table 4. Results confirmed in more conclusively way previous findings. Identification of signals was not evident for all participants as not all of them could achieve the resolution necessary for a complete identification and determination of the *trans* fatty acids.

Those who could identify the various fatty acids reported results suitable for statistical evaluation as shown by the examples in Table 5.

Even the most polar capillary column can not resolve all ***cis*** and *trans* isomers of C-18 unsaturated fatty acids. Thus signals are partially overlapping and a correct integration of peaks is often impossible. Not all laboratories have work station with a computer program allowing integration of overlapping signals as proposed in the study. Not all laboratories could achieve the necessary chromatographic resolution too. Also there is a lack of reference standards for *trans* isomers of fatty acids which could help to ensure a correct and unambiguous identification of the signals. Results of those participants who correctly identified fatty acids showed acceptable values for repeatability $\boldsymbol{r}$ and reproducibility $\boldsymbol{R}$. For well resolved signals $\boldsymbol{r}$ and $\boldsymbol{R}$ were also good.

CONCLUSIONS

The Commission considers the general method 2.304 of the 1st supplement of its standardised method gas-liquid chromatography on capillary column as applicable for the determination of *trans* fatty acids in hydrogenated fats. For an unambiguous identification of the different types of fatty acid gas-liquid conditions have to be adapted accordingly. In case of a lack of reference standards the use of mass spectrometry is suggested.

Therefore the commission considers the task of the Working group on the determination of *trans* fatty acids in edible oils as completed.

ACKNOWLEDGEMENTS

The Commission wishes to express its thanks to collaborators in Belgium, Brazil, Canada, Czechoslovakia, Denmark, Finland, France, Germany, Hungary, Japan, Netherlands, Spain, Switzerland, UK and USA and to Mrs. Elizabeth Prior for her assistance in preparing this paper publication.

REFERENCES

1 Bear- Rogers, J.L. and Dieffenbacher, A., *Pure & Appl. Chem.* 1990, **62**(4), 795-802.

2 *IUPAC Standard Methods for the Analysis of Oils, Fats and Derivatives*, Blackwell Scientific Publications, 7th Edition, 1987.

3 Pocklington, W.D., *Guidelines for the development of standard methods by Collaborative Study.* Laboratory of the Government Chemist, 1986. LGC Publication No. 1986 OP 001.

APPENDIX. TABLES 1–5

© 1997 IUPAC, *Pure and Applied Chemistry* **69**, 1829–1837

Table 1:

	Fatty acid											
	C - 14 : 0						C - 16: 0					
	Beef tallow	Lard	Beef /Lard /90/10	Fish oil	Fish oil hydr	Fish /Fish hydr / 50/50	Beef tallow	Lard	Beef /Lard /90/10	Fish oil	Fish oil hydr	Fish /Fish hydr / 50/50
Number of results	11	11	11	11	11	10	11	11	11	11	11	10
Number of results eliminated*)	1	1				1	1	2		1	1	1
Number of results retained after elimination of outliers	10	10	11	11	11	9	10	9	11	10	10	9
Mean value (%) (surface/surface)	2.97	1.7	2.84	6.51	7.43	7.04	25.86	27.1	25.87	12.41	16.22	13.97
Repeatability												
Standard deviation(S_r)	0.1	0.04	0.7	0.19	0.52	0.32	0.42	0.29	0.32	0.24	0.18	0.43
Relative standard deviation	3.5	2.3	2.6	2.9	7	4.6	1.6	1.1	1.2	1.9	1.1	3.1
Repeatability value (r)(2.83 x S_r)	0.29	0.11	0.21	0.54	1.46	0.91	1.19	0.82	0.91	0.67	0.49	1.22
Reproducibility												
Standard deviation (S_R)	0.17	0.07	0.16	0.56	0.93	0.73	0.79	0.4	0.78	0.77	1.74	0.93
Relative standard deviation	5.6	4.1	5.7	8.6	12.4	10.3	3	1.5	3	1.9	10.7	6.7
Reproducibility value (R)(2.83 x S_R)	0.47	0.2	0.47	1.59	2.62	2.05	2.23	1.12	2.21	2.17	4.93	2.64

*) Elimination on the basis of Cochran and Dixon test

Table 2 :

Lab.	C - 18:1 isomers I SAMPLE A		B		C		D		C - 18:1 isomers II SAMPLE A		B		C		D	
	1st	*2nd*	*1st*	*2nd*	*1st*	*2nd*	*1st*	*2nd*	*1st*	*2nd*	*1st*	*2nd*	*1st*	*2nd*	*1st*	*2nd*
1	1.8	1.8	3.2	3.3	2.6	2.5	2.6	2.6	-	-	4.9	4.8	2.4	2.4	2.4	2.4
2	-	-	18	17.7	6.6	7	6.9	7	-	-	2.1	2.2	3.2	3.3	3.3	3.3
3	-	-	15	14.7	7.4	7.4	7.3	7.3	-	-	1.5	1.4	0.8	0.7	0.8	0.7
4	-	-	5.1	5.1	2.4	2.4	2.5	2.5	-	-	1.2	1.2	0.6	0.6	0.6	0.6
5	1.3	1.2	3.3	4.1	1.6	2.3	2.6	1.5	-	-	5.9	5.4	2.5	2.4	2.5	2.9
6	-	-	0.9	1	0.5	0.5	0.5	0.5	-	-	0.8	0.8	0.4	0.4	0.4	0.4
7	1.7	1.7	4	3.9	2.8	2.8	2.7	2.9	-	-	4.9	5	2.5	2.5	2.4	2.6
8	1.4	1.4	3.7	3.7	2.5	2.5	2.6	2.6	-	-	4.7	4.8	2.3	2.3	2.4	2.4
9	1.3	1.1	8	8	4.2	3.9	4	4.3	-	-	1.5	1.5	0.8	0.8	0.8	0.8
10	0.7	0	1.7	1.7	0.9	0.9	0.9	0.9	0	0	0.7	0.7	0.3	0.3	0.3	0.3
11	1.4	1.4	2.8°°)	2.8**)	2.1**)	2.1**)	2.1**)	2.1**)	-	-	4.8	4.8	2.3	2.3	2.3	2.3
12	0.4	0.6	21.2	21.1	0.7	0.7	8.2	8.3	0	0	10.4	12.1	2.6	2.9	3.5	3.4
13	1.3	1.3	2.6	2.5	1.7	1.9	1.9	1.9	-	-	4.7	4.7	2.3	2.3	2.3	2.3
14	1.3	1.3	2.8	2.8	2.1	2.1	2.1	2.1	-	-	4.7	4.7	1.9	1,7	2.3	2.3
15	1.4	1.3	2.4	2.6	2	2	2	2	-	-	4.4	4.5	2.2	2.1	2.2	2.1
16	1.6	1.5	3.1	4.2	2.2	2.2	2.2	2.2	0.3	0	4.8	5.4	2.4	2.4	2.4	2.3

°°) Reported as C-18:1, 11 c

**) Sum of 18:1 c isomers

© 1997 IUPAC, *Pure and Applied Chemistry* **69**, 1829–1837

© 1997 IUPAC, *Pure and Applied Chemistry* **69**, 1829–1837

Table 3 :

	C -18:1 isom. I				C -18:1 isom. II				C -18:1 isom. III			
	SAMPLE				SAMPLE				SAMPLE			
	A	B	C	D	A	B	C	D	A	B	C	D
Number of results	12	13	16	13	-	16	16	16	-	15	14	14
Number of results eliminated*)	2	3	2	1		3	1	1		2	2	2
Number of results retained after elimination of outliers	10	10	14	12		13	15	15		13	12	12
Mean value(%)(surface/surface)	1.43	2.93	2.04	2.2	-	3.15	1.78	1.89	-	1.34	0.7	0.83
Repeatability												
Standard deviation(S_r)	0.06	0.16	0.15	0.07	-	0.05	0.05	0.05	-	0.07	0.15	0.07
Relative standard deviation	3.8	2.2	7.4	3.4		1.5	2.7	2.7		4.9	22.1	7.9
Repeatability value (r)(2.83 x S_r)	0.16	0.18	0.42	0.21		0.14	0.14	0.15		0.18	0.44	0.18
Reproducibility												
Standard deviation (S_R)	1.4	1.17	0.92	0.92	-	1.82	0.93	1.4	-	0.65	0.35	0.51
Relative standard deviation	0.6	39.8	45	42		57.8	52.6	54.9		49	50	61.8
Reproducibility value (R)(2.83 x S_R)	1.3	3.3	2.6	2.61		5.16	2.65	2.74		1.85	0.99	1.45

***) Elimination on the basis of Cochran and Dixon test**

Table 4: Gas–liquid chromatographic conditions used

Lab.	Type of column	length (m)	internal diameter (µm)	Film Thickness (µm)	Injection mode	Carrier gas	Temperature program and remarks to the method
1	CP-Sil-88 (WCOT)	50	0.25	0.2	Split 1/100	Helium	not given
2	CP-Sil-88	50	0.25	0.2	Split 1/ 50	Helium	not given
3	CP-Sil-88	50	0.25	0.2	Split 1/100	not given	as proposed
4	CP-Sil-88	50	0.25	0.2	Split	not given	not given
5	Supelco SP-2330	50	0.25	0.2	Splitless	not given	Own methylation method
6	BPX70	50	0.22	0.25	Split 1/ 80	Hydrogen	not given
7	CP-Sil-88 (WCOT)	50	0.25	0.2	On column	Helium	temp.program according IUPAC
7A	-dito-	50	0.25	0.2	On column	Helium	Temp. program with longer heating at 200 and 220 °C
8	Supelco SP-2560	100	0.25	0.2	Split 1/100	Hydrogen	not given
9	Supelco SP-2560	100	0.25	0.2	Split	not given	not given
10A	ULTRA 2	25	0.20	0.33	Split 1/ 30	Nitrogen	not given
10B	FFAP	25	0.20	0.3	Split 1/ 30	Nitrogen	not given

© 1997 IUPAC, *Pure and Applied Chemistry* **69**, 1829–1837

© 1997 IUPAC, *Pure and Applied Chemistry* **69**, 1829–1837

Table 5 :

	Fatty acid													
	C - 18:1 cis					**C - 18:2 trans**					**C - 18:2 cis**			
	SBO3°)	**SBO hydr**	**SBO/SBO hydr/50/50**	**RSO hydr°°)**	**RSO/RSO hydr/50/50**	**SBO3°)**	**SBO hydr**	**SBO/SBO hydr/50/50**	**RSO hydr°°)**	**RSO/RSO hydr/50/50**	**SBO3°)**	**SBO hydr**	**SBO/SBO hydr/50/50**	**RSO hydr°°)**
Number of results	12	10	11	11	10	10	11	11	11	10	11	10	11	11
Number of results eliminated*)	1			1	1		1				2	1	1	2
Number of results retained after elimination of outliers	11	10	11	10	9	10	10	11	11	10	9	9	10	9
Mean value(%)(surface/surface)	22.8	40.4	31.7	34.35	46.46	1.13	1.65	1.22	0.91	0.33	52.56	35.46	26.82	1.89
Repeatability														
Standard deviation(S_r)	0.2	0.57	0.41	0.49	0.24	0.09	0.07	0.16	0.22	0.1	0.16	0.53	0.23	0.09
Relative standard deviation	0.94	1.4	1.3	1.4	0.5	7.7	4.1	12.7	24.6	30.3	0.3	1.5	0.9	4.8
Repeatability value (r)(2.83 x S_r)	0.61	1.62	1.16	1.39	0.68	0.25	0.19	0.44	0.63	0.28	0.46	1.5	0.44	0.26
Reproducibility														
Standard deviation (S_R)	0.4	2.61	0.89	1.53	1.55	0.23	0.78	0.49	0.55	0.23	0.51	1.41	0.49	0.18
Relative standard deviation	1.73	6.5	2.8	3.3	3.3	20.2	47.4	4.03	61.3	69.7	1	4	3.2	9.7
Reproducibility value (R)(2.83 x S_R)	1.12	7.38	2.53	4.38	4.38	0.64	2.2	1.39	1.57	0.65	1.45	4	2.42	0.52

***) Elimination on the basis of Cochran and Dixon test**

°) SBO means soyabean oil

°°) RPO means rapeseed oil