Pure & Appl. Chem., Vol. 56, No. 2, pp. 301–304, 1984.
Printed in Great Britain.

0033-4545/84 $3.00+0.00
Pergamon Press Ltd.

INTERNATIONAL UNION OF PURE AND APPLIED CHEMISTRY

APPLIED CHEMISTRY DIVISION

COMMISSION ON OILS, FATS AND DERIVATIVES*

Results of a Collaborative Study on

DETERMINATION OF ERUCIC ACID

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RESULTS OF A COLLABORATIVE STUDY ON DETERMINATION OF ERUCIC ACID

Abstract - A TLC/GLC method for the determination of erucic acid (cis-13-docosenoic acid) in the presence of other docosenoic acid isomers studied collaboratively is described. It involves fractionation of the component fatty acid methyl esters by argentation-TLC at about minus 22°C. After addition of an internal standard the TLC fraction containing erucic acid and the fraction containing all of the other fatty acids are analysed by GLC. The percentage of erucic acid is calculated from the ratio of the internal standard in both fractions.

INTRODUCTION

Some countries restrict by legislation the proportion of docosenoic acids (C 22:1) in the total fatty acids of edible fat and oil products. A limit is also advocated by a Joint Expert Group of the Food and Agricultural Organisation and the World Health Organisation (ref. 1) for those populations in which fat constitutes a high portion of dietary energy.

The two basic sources of docosenoic acids in edible fats and oils are rapeseed oil and partially hydrogenated marine oil which are used to a large extent in the European fats and oils industry. Whereas in marine oils cetoleic acid (cis-11-docosenoic acid) predominates among the docosenoic acids, rapeseed oil contains erucic acid (cis-13-docosenoic acid) only. By the breeding of new rapeseed varieties the erucic acid content of rapeseed oil has been reduced from about 47 % to less than 5 %. In most European countries and in Canada only these new varieties are grown for the production of edible oils. By partial hydrogenation of both oils geometrical (trans)isomers as well as new positional isomers are formed. Most positional isomers are adjacent to the original cis ethylenic bond positions. In partially hydrogenated marine oils about 30 to 80 % of the docosenoic acid is of the trans configuration.

Total docosenoic acid can be determined using conventional GLC procedures for the separation of the fatty acid methyl esters. However an overlap of docosenoic acid with eicosatetraenoic acid (C 20:4) or eicosapentaenoic acid (C 20:5) is possible depending on the type of stationary phase, such polyunsaturated fatty acids being present in crude fish oils.A rapid method for the determination of total docosenoic acid using tetracosanoic acid (C 24) as internal standard has been described by H.B.S. Conacher (refs. 2,3).

In the European Economic Community (EEC) the amount of erucic acid in edible fats and oils and in compound foodstuffs to which fats and oils have been added has been limited to 5 %. From the corresponding erucic acid directive (ref. 4) specific analytical problems arise since it limits the amount of erucic acid but not of total docosenoic acid. Thus it is important for an analytical method to be available which distinguishes between erucic acid and other positional and geometrical docosenoic acid isomers that may be present. For the specific determination of erucic acid in the presence of other docosenoic acid isomers, procedures based on capillary GLC or on a combination of TLC and GLC have been developed.

A capillary GLC method using an internal standard has been studied collaboratively by the International Association of Fish Meal Manufacturers (ref. 5). A combined TLC/GLC procedure has been developed by an EEC working group in which the author participated. The method was adopted as the official EEC-method in 1980 (ref. 6).

COLLABORATIVE STUDY AND RESULTS

The EEC procedure was collaboratively studied by the IUPAC working group. The procedure provides first for a TLC separation of the methyl esters of the component fatty acids. Separation is carried out using argentation-TLC at low temperature (about minus 22°C). Under the conditions used erucic acid is

separated from cetoleic acid and from trans docosenoic acid isomers. After addition of an internal standard the fraction containing erucic acid and the fraction containing all of the other fatty acids are eluted and analysed by GLC. The percentage of erucic acid is calculated from the erucic acid content of the erucic acid fraction and from the ratio of the internal standard in both fractions. The method of separation and the mode of calculation preclude an overlap of erucic acid or of the internal standard with other fatty acids.

Before carrying out the collaborative study participants analysed one sample (no. 1) to get familiar with the method. The sample, composed of hydrogenated herring oil (erucic acid content 1.9 %), rapeseed oil (erucic acid content 36.2 %) and sunflowerseed oil, was analysed by 10 laboratories. The total docosenoic acid content of the sample was 18.4 % as determined by GLC, the erucic acid content was 14.2 % as calculated from the erucic acid content of the parent oils. The erucic acid content in the rapeseed oil was determined by GLC; in the hydrogenated herring oil by TLC/GLC. Results of this initial test are summarised in Table 1.

In the collaborative study four samples each containing less than 10 % erucic acid were analysed by 10 laboratories. Participants were requested to apply the method as strictly as possible.

Sample no. 2 was a mixture of hydrogenated capelin oil, rapeseed oil and sunflowerseed oil (35:20:45)
Sample no. 3 was a mixture of hydrogenated capelin oil, rapeseed oil and sunflowerseed oil (20:14:66)
Sample no. 4 was hydrogenated capelin oil
Sample no. 5 was identical to sample no. 3 in order to provide an unbiased determination of the repeatability of results.

The erucic acid content of the samples was calculated from the erucic acid content of the parent oils. Erucic acid content: rapeseed oil 36.4 % as

Table 1. TLC/GLC Determination of Erucic Acid - Initial Sample Test (Erucic Acid % m/m)

Lab.	Sample no. 1				Mean	Lab.	Sample no. 1				Mean
1	12.4	14.7	15.7	14.7	14.4	6	14.5	14.8	14.6	15.8	14.9
2	15.5	13.3			14.4	7	13.7	14.3			14.0
3	14.0	14.8			14.4	8					15.7
4	14.2	13.8	16.0	12.5	14.1	9[a]					14.5
5	14.1	14.1			14.1	10					16.5
Mean value [b]		14.5				Calculated value [c]		14.2			

a) By capillary GLC 15.1 % were found
b) Laboratory 10 not included
c) Calculated from the erucic acid content of the parent oils

Table 2. TLC/GLC Determination of Erucic Acid (Erucic Acid % m/m)

Lab.	Sample no 2			Sample no 3			Sample no 4			Sample no 5		
			Mean			Mean			Mean			Mean
1	7.8	7.6	7.7	4.2	4.3	4.3	1.4	1.6	1.5	5.0	4.7	4.9
2	8.8	7.2	8.0	4.5	6.5	5.5	10.1	8.0	9.1	4.2	6.1	5.2
3	7.7	9.7	8.7	7.0	5.9	6.5	1.6	2.2	1.9	5.9	5.3	5.6
4	8.7	8.5	8.6	6.2	6.3	6.2	1.1	1.1	1.1	5.8	6.0	5.9
5	7.8	7.4	7.6	3.2	2.8	3.0	1.5	1.9	1.7	3.7	4.2	4.0
6	9.0	9.2	9.1	5.1	5.4	5.3	1.0	1.2	1.1	7.3	7.4	7.3
7	8.1	8.4	8.3	5.8	5.7	5.7	1.7	1.8	1.7	6.2	5.9	6.0
8	9.9	9.7	9.8	6.1	6.1	6.1	4.7	4.5	4.6	4.9	5.0	5.0
9	7.6	7.9	7.8	5.6	5.5	5.6	2.4	1.7	2.1	5.4	5.6	5.5
10	8.4	8.3	8.3	6.3	5.5	5.9	-	-	-	4.4	5.7	5.1
11	7.8	8.4	8.1	4.8	4.4	4.6	1.6	1.0	1.3	5.0	5.6	5.3
Mean value			8.4			5.6[a]			1.6[b]			5.4
Calc. value [c]			7.8			5.4						5.4
12 Amb.temp. [d]			7.9			5.8			1.4			5.4
9 Capillary GLC			8.6			5.9			1.9			5.7

a) Laboratory no 5 not included
b) Laboratory no 2 and no 8 not included
c) Calculated from the erucic acid content of the parent oils
d) TLC separation at ambient temperature

Table 3. Statistical Evaluation of Results

		Sample no 2	3[a]	4[b]	5
Number of laboratories		11	10	8	11
Number of results		22	20	16	22
Mean	$\bar{\bar{x}}$	8.4	5.6	1.6	5.4
Calculated value [c]		7.8	5.4	-	5.4
Repeatability SD	s_r	0.58	0.55	0.30	0.54
Reproducibility SD	s_R	0.78	0.80	0.42	0.94
Repeatability	r (95)	1.64	1.56	0.85	1.54
Reproducibility	R (95)	2.20	2.27	1.18	2.65

a) Laboratory no 5 not included
b) Laboratory no 2 and no 8 not included
c) Calculated from the erucic acid content of the parent oils

determined by GLC; hydrogenated capelin oil 1.6 % as determined by TLC/GLC, mean value for sample no. 4. Results are summarised in Table 2.

Laboratory 12 carried out TLC separations at ambient temperature. Results were satisfactory as shown in Table 2, part 2. However since the quality and the reproducibility of the TLC separation is better at low temperatures, the method provides for separation at about minus 22 °C.

Laboratory 9 also analysed the samples by capillary GLC. Working conditions: 50 m WCOT/BMBT liquid crystal (N,N-bis(p-methoxy-benzylidene)-α,α-bi-p-toluidene) - carrier gas helium 1.6 ml/min - splitless injection - isothermal 181 °C. The results are in agreement with the results obtained by TLC/GLC (Table 2, part 2).

A statistical evaluation of results for laboratories 1 to 11, based on duplicate determinations is given in Table 3.

On the basis of the results received the Commission decided to adopt the method. The full text of the method 2.311 was published in 1982 (ref. 7).

ACKNOWLEDGEMENT

The Commission wishes to express its thanks to collaborators in Australia, Canada, Czechoslovakia, Denmark, France, FRG, Hungary, Sweden, Turkey, UK and USA for their participation and valuable cooperation and to Dr. W. D. Pocklington (UK) for his continued support.

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