

MAFF VALIDATED METHODS FOR THE ANALYSIS OF FOODSTUFFS

No. V3

ERUCIC ACID IN OILS AND FATS

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1. Scope and Field of Application

The method allows the determination of the erucic acid (*cis*-13-docosenoic acid) content of oils and fats, including: (i) oils and fats containing cetoleic acid (a *cis*-isomer of docosenoic acid, which occurs in fish oils), and (ii) hydrogenated oils and fats containing *trans*-isomers of docosenoic acid.

2. Definition

Erucic acid content: the content of erucic acid as determined by the method specified.

3. Principle

Separation of the methyl esters of the component fatty acids by low temperature argentation thin-layer chromatography and quantitative determination of the separated esters by gas-liquid chromatography.

4. Reagents

All solvents and chemicals should be of analytical reagent quality.

4.1 Diethyl ether, peroxide-free.

4.2 *n*-Hexane

4.3 Silica gel G, for thin-layer chromatography.

4.4 Silica gel, for column chromatography.

4.5 Silver nitrate solution, 200 g/l: dissolve 24 g of silver nitrate in water and dilute to 120 ml with water.

4.6 Methyl erucate solution, 5 mg/ml: dissolve 50 mg of methyl erucate in light petroleum (b.p.40 - 60°C) and dilute to 10 ml.

4.7 Methyl tetracosanoate (internal standard solution), 0.25 mg/ml: dissolve 25 mg of methyl tetracosanoate in light petroleum (as 4.6) and dilute to 100 ml.

4.8 Development solvent, toluene and hexane diluted 1 litre + 1 Litre.

4.9 2,7-Dichlorofluorescein solution, 0.5 g/l: dissolve by warming and stirring 50 mg of 2,7-dichlorofluorescein in 100 ml of 50%(V/V) aqueous methanol.

5. Apparatus

5.1 Thin-layer chromatography

The apparatus required for thin-layer chromatography to include, in particular, the following:

- 5.1.1 Deep-freeze unit, capable of maintaining the development tank and contents at a temperature of between -20°C and -25°C .
- 5.1.2 Glass plates, 200 mm x 200 mm.
- 5.1.3 Ultra-violet lamp
- 5.1.4 Column, glass, length about 200 mm, internal diameter about 10 mm, with sintered-glass filters.
- 5.1.5 Applicator, for depositing solutions in the form of a narrow band or streak on the plates.
- 5.2 Apparatus for gas-liquid chromatography, fitted with an electronic integrator.

6. Procedure

6.1 Preparation of fatty acid methyl esters

Take a representative sample of about 400 mg of the oil or fat and prepare a solution containing about 20-50 mg/ml of the fatty acid methyl esters in hexane.

6.2 Thin-layer chromatography

6.2.1 Preparation of plates

Place 60 g of silica gel in a 500 ml round-bottom flask with 120 ml of silver nitrate solution and shake for one minute to obtain a fully homogeneous slurry. Spread the slurry in the usual manner over the plates, adjusting the layer thickness to 0.5 mm. This quantity of slurry is sufficient for the preparation of five 200 x 200 mm plates. Allow the plates partially to air-dry (preferably in the dark for about 30 minutes) and then dry and activate them in an oven at 100°C for 2.5 hr. The plates should be used as soon as possible after activation, otherwise they should be stored carefully in a dark cabinet and activated before use. (Note: activation at 110°C for 1 hr. may be found satisfactory provided the plates are not darkened.) Score lines through the coating 10 mm from the sides and the top of each plate before use to reduce edge effects during the development.

6.2.2 Application of methyl esters

Using the applicator, deposit 50 μl of the prepared sample methyl esters solution in a narrow streak about 50 mm long, at least 40 mm from the side of the plate and 10 mm from the bottom. Apply in a similar way 100 μl of a solution containing equal volumes of the prepared solution of methyl esters (6.1) and the methyl erucate solution (4.6). Particular care is necessary during the application of solutions because of the fragile nature of the coating. (Note: if desired, 50 μl of the methyl erucate solution may be applied to the plate as above to assist in identification of the methyl erucate band after development.) After the application of the methyl esters the bottom edge of the plate may be stood in diethyl ether until the ether ascends to about 5 mm above the area of sample application; this will concentrate the methyl esters into a narrow band.

6.2.3 Development of plates

Pour sufficient development solvent into the tank to give a depth of about 5 mm and place the tank, complete with lid, in a deep freeze cabinet at -25°C , or as near to this temperature as possible. (Lining the tank with paper may in some cases be advantageous.) After two hr. place the plate carefully in the tank and allow the solvent to ascend to about one-half to two-thirds of the height of the plate. Remove the plate and gently evaporate off the solvent from the plate with a stream of nitrogen. Replace the plate in the tank and allow the solvent to ascend to the top of the plate. Remove the plate and dry with a stream of nitrogen as before, then spray with 2,7-dichlorofluorescein solution. When viewed under ultra-violet light, the methyl erucate in the sample can be located by reference to the intensified band in the sample to which methyl erucate has been added.

6.2.4 Separation of the methyl ester fractions

Scrape off quantitatively the methyl erucate band derived from the sample into a 50 ml beaker. Scrape off quantitatively the silica gel above and below the methyl erucate band, which contains all the other fatty acid methyl ester fractions, into another 50 ml beaker. To each beaker add 1.0 ml of the methyl tetracosanoate standard solution and 10 ml of diethyl ether. Stir and transfer separately the contents of the beakers to the column or filters containing about 1 g of silica gel, and extract the esters using three or four 10 ml portions of diethyl ether. Collect the filtrates in small flasks. Evaporate the filtrates to small volumes under a gentle stream of nitrogen and transfer them to small glass tubes with pointed bottoms. Remove all solvent by evaporation under nitrogen in such a way that the methyl esters are concentrated at the bottom of the tubes. Dissolve the methyl esters in about 25-50 μl of hexane.

6.3 Gas-liquid chromatography

Inject 1-2 μl of the solutions of methyl esters obtained from (i) the fraction containing methyl erucate and (ii) the fractions containing the remainder of the fatty acid methyl esters. From the electronic integrator obtain the following peak areas:

- (i) from the chromatogram of the fraction containing methyl erucate,
 - (a) methyl erucate $[E]$;
 - (b) internal standard $[L_1]$;
 - (c) total methyl ester peak areas excluding the internal standard $[EF]$;
- (ii) from the chromatogram of the fractions containing the remainder of the fatty acid methyl esters,
 - (a) total peak areas excluding the internal standard $[RF]$;
 - (b) internal standard $[L_2]$.

7. COSHH

Analysts are reminded that appropriate hazard and risk assessments required by the Control of Substances Hazardous to Health Regulations, 1988 (See "Control of Substances Hazardous to Health - Approved Code of Practice, Control of Substances Hazardous to Health Regulations, 1988") must be made before using this method.

8. Expression of Results

8.1 Formula and method of calculation

The erucic acid content of the sample, expressed as a percentage of the total fatty acids, is given by:

$$\% \text{ erucic acid} = \frac{E}{L_1 \{EF/L_1 + RF/L_2\}} \times 100$$

where E , EF , RF , L_1 and L_2 are the peak areas referred to in the previous section, corrected as necessary by the use of calibration factors. If peak areas are obtained in percentages, the values for EF and RF may be calculated as follows:

$$EF = 100 - L_1$$

$$RF = 100 - L_2$$

The method of calculation assumes that the level of tetracosanoic acid in the sample is negligible. If significant amounts of this acid are found to be present, the value for tetracosanoic acid (L_2) obtained from the chromatogram of the fractions containing the remainder of the fatty acid methyl esters must be reduced to L_2^* , where:

L_2^* is the peak area of tetracosanoic acid derived from the internal standard and is calculated using $L_2^* = L_2 - T_2$,

T_2 is the peak area of tetracosanoic acid derived from the sample and which forms part of the peak area attributed to the internal standard in the chromatogram of the remaining fraction of fatty acid methyl esters and is calculated using $T_2 = T_0 P_2 / P_0$,

P_2 is the peak area of palmitic acid obtained from the same chromatogram as T_2 ,

T_0 is the peak area of tetracosanoic acid obtained from the chromatogram of the total fatty acids,

P_0 is the peak area of palmitic acid obtained from the same chromatogram as T_0 .

8.2 Derivation of formula

The fatty acid content of the fraction containing methyl erucate, expressed as a percentage of the total fatty acids in the sample, is given by:

$$\% \text{ total fatty acids} = \frac{EF/L_1}{EF/L_1 + RF/L_2^*} \times 100$$

or

$$\% \text{ total fatty acids} = \frac{EF}{L_1 \{EF/L_1 + RF/L_2^*\}} \times 100$$

The erucic acid content of the fraction containing methyl erucate, expressed as a percentage of the fatty acids in the methyl erucate fraction, is given by:

$$\% \text{ erucic acid in methyl erucate fraction} = E/EF \times 100$$

Hence the erucic acid content of the sample, expressed as a percentage of the total fatty acids, is given by:

$$\% \text{ erucic acid content} = \frac{EF}{L_1\{EF/L_1 + RF/L_2^*\}} \times \frac{E}{EF} \times 100$$

or

$$\% \text{ erucic acid content} = \frac{E}{L_1\{EF/L_1 + RF/L_2^*\}} \times 100$$

9. References

- 9.1 RB Player and R Wood, J. Assoc. Publ. Analysts, 1980, **18**, 77-89
 9.2 RS Kirk, RE Mortlock, WD Pocklington and P Roper, J. Sci. Fd. Agric., 1978, **29**, 880.

APPENDIX 1

Analytical Quality Control

General principles of analytical quality control are outlined in protocol V0 of the series.

A1 Repeatability

The absolute difference between two test results obtained under repeatability conditions should not be greater than the repeatability, r , deduced from the collaborative trial data summarised in Table 1. At about 7% erucic acid, r may be taken as 1.3%; at 11% erucic acid r is about 1.9%. This precision corresponds to an overall relative standard deviation of repeatability (coefficient of variance of repeatability), RSD_r , of about 7%.

A2 Reproducibility

The absolute difference between two test results obtained under reproducibility conditions should not be greater than the reproducibility, R , deduced from the collaborative trial data summarised in Table 1. R may be taken as 4% at erucic acid levels of 7%; this precision corresponds to a relative standard deviation of reproducibility (coefficient of variance of reproducibility), RSD_R , of some 20%.

A3 Trueness (Bias)

The collaborative trial included the analysis of a sample of oil before (A) and after (D) spiking with 4.78% authentic methyl erucate. An overall mean difference of 4.37% erucic acid was found, suggesting a recovery of 95%; however there was no statistical evidence that this recovery was less than quantitative. The method is therefore unlikely to be systematically biased.

A4 Limit of Detection

This limit has not been established, but the collaborative trial data suggests an accuracy which, if maintained, corresponds to an extrapolated lower limit of roughly 1% erucic acid for a single determination.

A5 Statistical Data Derived from the Results of Interlaboratory Tests

Participants in the collaborative trial each analysed four samples (A-D) in duplicate. The samples were known mixtures of rapeseed oil, corn oil and/or partially hydrogenated marine oil; one of them (D) was sample A spiked with 5% methyl erucate. A summary of statistical data is tabulated below; the erucic acid levels were expressed as a percentage of total fatty acids.

TABLE 1
Statistical Analysis of the % Erucic Acid in Mixed Oil Samples

Sample	A	B	C	D
Number of Laboratories retained after eliminating outliers	18	18	18	17
Number of Laboratories eliminated as outliers	6	6	5	7
Number of results accepted after eliminating outliers	33	33	35	32
LEVEL OF ANALYTE				
Mean observed value $\bar{x}$	6.84	6.08	6.10	11.21
REPEATABILITY				
Standard Deviation S_r	0.41	0.47	0.46	0.69
Relative Standard Deviation RSD_r (%)	6.0	7.7	7.5	6.2
Repeatability r [$2.5 \times S_r$]	1.16	1.32	1.29	1.94
REPRODUCIBILITY				
Standard Deviation S_R	1.43	1.25	1.21	1.57
Relative Standard Deviation RSD_R (%)	20.9	20.6	19.8	14.0
Reproducibility R [$2.5 \times S_R$]	4.00	3.51	3.39	4.40

A6 Key to Table 1

Symbol	Definition
$\bar{x}$	Overall mean value
S_r	The standard deviation of repeatability
RSD_r	The relative standard deviation of repeatability, expressed as a percentage of the mean (coefficient of variance of repeatability CV_r)
r	Repeatability
S_R	The standard deviation of reproducibility
RSD_R	The relative standard deviation of reproducibility, expressed as a percentage of the mean (coefficient of variance of reproducibility CV_R)
R	Reproducibility