

DETERMINATION OF METHYL ESTERS OF FATTY ACIDS BY GAS CHROMATOGRAPHIC METHOD

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INTRODUCTION

Methyl esters of fatty acids from fish and animal fats having 8-24 carbon atoms are separated and determined by gas chromatography. This method is not applicable for nonvolatile substances such as epoxy, oxidized, or polymerized fatty acids.

Esters appear in order of increasing number of C atoms and of increasing unsaturation for same number of C atoms. C₁₆ ester is ahead of the C₁₈ Me-esters which appear in order : stearate (18:0), oleate (18:1), linoleate (18:2), and linolenate (18:3). C₂₀ saturated ester (arachidic, 20:0) usually appears before 18:3 ester, but may be reversed on some columns, or positions may change with column used.

APPARATUS

1. Gas chromatograph (Shimadzu GC-9A)
2. Glass Spiral Columns
 - 2.1 For DEGS : length = 1.6 m, i.d. = 3 mm
 - 2.2 For Unisole 3000 : length = 2 m, i.d. = 3 mm
3. Packing material
 - 3.1 Diethylene glycol succinate (DEGS), 5 - 20% on support Shimalite W or Chromosorb W, (acid washed and silanized diatomaceous earth), mesh 60 - 80. Maximum aging temperature = 210°C.
 - 3.2 Unisole 3000 on support Uniport C, mesh 80/100. Maximum aging temperature = 250°C.
4. Microliter syringes
 - 4.1 Maximum volume 10 ul, graduated to 0.1 ul (Hamilton 701-N).
 - 4.2 Maximum volume 1 ul, graduated to 0.01 ul (Hamilton 7001 SN)
5. Recorder (Chromatopac C-R1B, Shimadzu)

REAGENTS

1. Carrier gas

Purified grade nitrogen gas with oxygen < 4.0 ppm, moisture < 2.5 ppm, hydrocarbons < 1.0 ppm.

2. Other gas

Purified grade air with oxygen = $21 \pm 1\%$, moisture < 3.0 ppm, hydrocarbons < 5.0 ppm.

Purified grade hydrogen with oxygen < 3 ppm, moisture and hydrocarbons < 1 ppm.

3. Reference standards

Known mixtures of methyl esters of fatty acids or methyl esters of oil of known composition, preferably similar to that of material to be analyzed.

OPERATING CONDITIONS

1. Column aging condition

1.1 DEGS column

Aging temperature	=	200°C
Carrier (N ₂) gas flow	=	60 ml/min
Duration	=	16 - 18 hours

1.2 Unisole 3000 column

Aging temperature	=	240°C
Carrier (N ₂) gas flow	=	70 ml/min
Duration	=	16 - 18 hours

1.3 Connect the long arm of column into the inlet or injection port of the gas chromatograph.

1.4 Do not connect the short arm to the detector. Leave it hanging in the oven.

- 1.5 Apply carrier gas flow at normal flow rate.
- 1.6 Then programme the oven temperature at 1°C/min rise to a temperature which is at least 10°C above that at which the column is operated.
- 1.7 Hold the final temperature for at least 2 hours and preferably for 16 hours.
- 1.8 Reduce temperature to the normal operating temperature and connect column to detector. The column is ready for use.

2. Isothermal programme for DEGS column

Column initial temperature	=	180°C
Column initial time	=	0.0 min
Column programme rate	=	0°C/min
Column final temperature	=	180°C
Column final time	=	100 min
Injection port temperature	=	200°C
FID detector temperature	=	200°C
Range	=	10 ²

3. Isothermal programme for Unisole 3000 column

Column initial temperature	=	220° or 230°C
Column initial time	=	0.0 min
Column programme rate	=	0°C/min
Column final time	=	100 min
Injection port temperature	=	270°C
FID detector temperature	=	260°C
Range	=	10 ²

4. Gas Flow rate and pressure

Carrier (N₂) gas = 30 - 40 ml/min

Hydrogen gas = 0.6 kg/cm²

Purified air = 0.5 kg/cm²

5. Recorder condition

Width = 5 sec

Slope = 100 uV/min

Drift = 0 uV/min

Min Area = 10 count

T-DBL = 0 min

Lock = 1.3 min

Stop time = 1000 min

Attenuation = 4 mV/full scale
(DEGS column)

= 3 mV/full scale
(Unisole 3000 column)

Speed = 10 mm/min

Method = 41

Sample weight = 100 (default value)

Internal standard weight = 1 (default value)

PROCEDURE

Sample Preparation

The fish oils used are first esterified by the boron trifluoride method. (See C-11)

Analytical Procedure

1. Open the carrier gas tank, set regulator outlet to 400 pKa, open the carrier valve on GC-9A and adjust carrier flow rate on GC-9A to that required.

2. Switch on GC-9A main switch, power and heater.
3. Select the File with the appropriate programme required and press ENTER. Then press START to start the programme. Press MONI COL to display the column initial temperature (CITP) on the display panel.
4. Wait for the temperature to reach that of the programmed temperature.
5. Open the purified air tank and set regulator outlet to 400 pKa.
6. Open the hydrogen gas tank and set regulator outlet to 400 pKa. Open the Hydrogen gas valve on GC-9A.
7. To ignite the FID detector, set the hydrogen gas to 0.9 kg/cm^2 and the purified air to 0.2 kg/cm^2 . Turn the ignitor to the detector till there is a "pop" sound. The detector is ignited and can be checked for vapour condensation on the flat surface of a metal spatula. Then set the hydrogen gas to 0.6 kg/cm^2 and the purified air to 0.5 kg/cm^2 . The GC-9A is ready for use.
8. Switch on the recorder and set the black key at open.
9. Press the PLOT key and adjust the baseline. Check SLOPE to be less than 300.
10. When the baseline is stable, inject 0.1 - 0.3 ul of 5 - 10% esterified sample in redistilled n-Hexane or chloroform by piercing the septum of the injection port and quickly discharging the sample.
11. Immediately press the START keys on GC-9A and the recorder.
12. Withdraw needle and note on chart the air or solvent peak as the start reference point.
13. If trace components are desired, the sample may be increased by < 10 times.
14. Adjust sample size so that major peaks is not attenuated > 8 times, preferably less (2-4).
15. Change setting of attenuator as necessary to keep peaks on chart paper. Mark attenuator setting on chart.
16. At end of run press the STOP key on recorder. The results will be printed out.

IDENTIFICATION

1. Analyze reference standard mixtures under same operating conditions as for sample.
2. Measure retention time (S) for known esters by measuring the distances from start point.
3. Plot log S as a function of number of C atoms of acids. Under isothermal conditions, graphs of straight chain esters of same degree of unsaturation should be straight lines, approximately parallel.
4. Identify peaks of sample from these graphs, interpolating if necessary.
5. Avoid conditions which permit "masked peaks" which are not sufficiently resolved.

CALCULATION

Method 41 of Chromatopac C-R1B is a normalization method. Use method of normalization, which assumes all components of sample are represented on chromatogram, so that sum of areas under peaks represent 100% of constituents (total elution). As the Chromatopack C-R1B is equipped with integrator, the figures shown can be used directly for calculation. Report results to following significant figures, with 1 figure beyond decimal point in all cases: 3 for > 10%, 2 for 1-10% and 1 for < %.

REFERENCES

Official methods of analysis of the Association of Official Analytical Chemists (13th Ed.), 1980 : 447-449.

Mirio SAITO et.al.(1985): Fatty acid composition of Fish Lipids, Jpn. J.Nutr., 43(6) 301-318.

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