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 epoxy, oxidized, or polymerized fatty acids.

I SAMPLE PREPARATION

The fish oils used are first esterified by the boron triflouride method.

II APPARATUS

The following conditions are for use with flame ionization detector (FID).

a) Gas chromatograph (Shimadzu GC-9A).

With minimum dead space in injection system, which is maintained at 20-50°C higher than column temperature. The column temperature should be maintained within ±1°C to at least 220°C. If programmed heated, dual columns are used.

b) Columns

1.600 mm × 3 mm (i.d.) glass spiral columns.
Maximum aging temperature = 210°C.

c) Packing

Chromosorb W, (Acid-washed and silanized diatomaceous earth) mesh 60-80.
Coated with 5-20% diethylene glycol succinate (DEGS).

Condition column while disconnected from detector at 200°C with current of nitrogen gas at 60 ml/min for 16-18 hours.

d) Microliter syringes

Maximum volume 10 ul, graduated to 0.1 ul (Hamilton 701-N).

e) Recorder (Chromatopac C-RIB, Shimadzu)

Method of recording	:	Thermal printer-plotter
Chart width	:	21 cm
Chart speed	:	0-50 mm/min
Pen speed	:	0.89 sec/full scale; 57 characters/sec.
Span	:	1 mv (automatic attenuation by time programming). With attenuation switch to change range.
Integration sensitivity	:	1 uV. sec (= 1 digit)
Linearity	:	±0.1% or better.

III REAGENTS

a) Carrier gas

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

b) Other gas

Purified grade air with oxygen 21 ± 1%, moisture <3.0 ppm, hydrocarbons <5.0 ppm.

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

c) 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.

IV OPERATING CONDITIONS

a) Isothermal program

Column initial temperature = 180°C.

Column initial time = 0.0 min.

Column final temperature = 180°C.

Column final time = 500 mins.

Injection port temp. = 200°C.

Range = 10^2.

b) Gas flow rate and pressure

Hydrogen gas : 0.6 kg/cm^2

Purified air : 0.5 kg/cm^2

Nitrogen gas : 60 ml/min.

c) Recorder conditions

Width : 5 sec

Slope : 300 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

Speed : 5 mm/min

Method : 41

Sample weight : 100 (default value)

Internal standard weight : 1 (default value)

V PROCEDURE

1. With recorder showing stable baseline, inject 0.1-0.3 ul 5-10% n-hexane solution of methyl esters.

2. If trace components are desired, the sample may be increased by <10 times.

3. Pierce septum of inlet port and quickly discharge sample.

4. Withdraw needle and note on chart small peak due to air or solvent, marking start reference point.

5. Press 'Start' on both GC-9A and recorder.

6. Adjust sample size so that major peak is not attenuated >8 times, preferably less.

7. Change setting of attenuator as necessary to keep peaks on chart paper. Mark attenuator setting on chart.

VI 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 from sample from these graphs, interpolating if necessary.

5. Avoid conditions which permit "masked peaks" which are not sufficiently resolved.

N.B. Esters appear in order of increasing number of C atoms and of increasing unsaturation for same number of C atoms. C_{16} ester is ahead of the C_{18} ester and C_{18} Me esters appear in order: stearate (18:0), oleate (18:1), linoleate (18:2), and linolenate (18:3). C_{20} 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.

VII CALCULATIONS

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 Chromatopac 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 <1%.

REFERENCES

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

Through personal communication with Mr Kinumaki and Dr Tsukuda.