SALTS of FATTY ACIDS

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SYNONYMS INS No. 470

DEFINITION These products consist of calcium, potassium or sodium salts of commercial myristic, oleic, palmitic, stearic, acids or mixtures of these acids from edible fats and oils. The article of commerce can be further specified by:
- saponification value,
- solidification point for the fatty acids obtained from the salts,
- iodine value,
- residue on ignition including assay of the cation, and
- moisture content

Assay: Not less than 95% total fatty acid salts, dry weight basis

DESCRIPTION Hard, white or faintly yellowish, somewhat glossy and crystalline solids or semi-solids or white or yellowish-white powder

FUNCTIONAL USES Anticaking agent, emulsifier

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4): Potassium and sodium salts are soluble in water and ethanol; calcium salts are insoluble in water, ethanol and ether

Test for cations: Heat 1 g of the sample with a mixture of 25 ml of water and 5 ml of hydrochloric acid. Fatty acids are liberated, floating as a solid or oil layer on the surface which is soluble in hexane. After cooling, aqueous layer is decanted and evaporated to dryness. Dissolve the residue in water and *test for the appropriate cation*.

Fatty acid composition: Using the Method of Assay, identify the individual fatty sample. The fatty acid(s) in primary abundance should conform to those declared on the label of the product

PURITY

Free fatty acids: Not more than 3%
Measure free fatty acids as directed in the method *Free Fatty Acids.* Compute free fatty acid content using an equivalence factor (e) equal to 1/10th the molecular weight of the salt.

Unsaponifiable matter: Not more than 2%
See description under TESTS

Lead (Vol. 4): Not more than 2 mg/kg
Determine using an atomic absorption technique appropriate to the

specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

TESTS

PURITY TESTS

Unsaponifiable matter

Unsaponifiable matter is the whole of the products present in a fatty substance which, after saponification thereof with an alkaline hydroxide and extraction by a specified solvent, remains non-volatile under defined conditions of the test.

It includes lipids of natural origin such as sterols, higher aliphatic alcohols, pigments and hydrocarbons as well as any foreign matter non-volatile at 103° which may be present.

Weigh to the nearest 0.01 g about 5 g of the well-mixed sample into a 250 ml round-bottomed flask. Add 50 ml of approximately 0.5N potassium hydroxide solution and some pumice, attach a reflux condenser, and boil gently for 1 h. Stop heating. Add 100 ml of distilled water through the top of the condenser and swirl.

After cooling, transfer the solution to a separatory funnel. Rinse the flask and the pumice several times with diethyl ether (100 ml in all) and pour this into the separatory funnel. Stopper and shake vigorously for 1 min, periodically releasing pressure by inverting the separating funnel and opening the stopcock.

Allow to stand until there is complete separation of the two phases. Then draw off the soap solution as completely as possible into a second separating funnel.

Extract the aqueous ethanolic soap solution twice more, each time in the same way with 100 ml of diethyl ether. Combine the three ether extracts in one separating funnel containing 40 ml of water.

Gently rotate the separating funnel containing the combined extracts and the 40 ml water. Violent agitation at this stage may result in troublesome emulsions. Allow the layers to separate completely and draw off the lower aqueous layer. Wash the ethereal solution twice more with 40 ml portions of water, shaking vigorously each time and discarding the lower aqueous layers after separation. Draw off each washing solution up to 2 ml, then rotate the separating funnel around its axis, wait some min to give the last remainders the opportunity for collection and draw off the collected remainders, close stopcock when ether starts to pass the bore of the stopcock.
Wash the ethereal solution successively with 40 ml of 0.5 N potassium hydroxide solution, 40 ml of water, and again with 40 ml of potassium hydroxide solution, then at least twice more with 40 ml of water. Continue to wash with water until the wash-water no longer gives a pink colour on the addition of a drop of phenolphthalein solution.

Transfer the ethereal solution quantitatively a little at a time through the top of the separating funnel into a flask previously dried and weighed to the nearest 0.0001 g.

Evaporate the solvent by distillation on a boiling-water bath.
Add 5 ml of acetone and remove the volatile solvent completely in a gentle current of air, holding the flask obliquely while turning it in a boiling-water bath.

Dry the residue at 103±2° for 15 min, placing the flask in an almost horizontal position. Cool in a desiccator and weigh to the nearest 0.0001 g. Repeat the drying for successive 15 min periods until the loss of weight between two successive weighings is less than 0.0015 g.
Note: If constant mass is not obtained after three periods of drying, the unsaponifiable matter is probably contaminated.

After weighing the residue dissolve it in 4 ml of diethyl ether and then add 20 ml of ethanol previously neutralized to a faint pink colour, using phenolphthalein TS as indicator. Titrate with accurately standardized 0.1N ethanolic potassium hydroxide solution (prepare an approx. 1 N ethanolic solution by dissolving 60 g of potassium hydroxide in 50 ml of water and making up to 1 liter with ethanol; dilute this solution 1:10 with ethanol) to the same final colour.

Correct the weight of the residue for the free acidity content of the blank. Calculate the unsponifiable matter, in % (m/m) from:

$$\frac{100 \times (m_1 - 0.281 \times T \times V)}{m}$$

where
m = the mass, in g, of the test portion
m_1 = the mass, in g, of the residue
V = the number of ml of the standardized potassium hydroxide solution used
T = the exact normality of the potassium hydroxide solution used

METHOD OF ASSAY

Principle:
Saponification of the salts and esterification by methanol of the fatty acids in the presence of boron trifluoride, alkaline methanol. Gas liquid chromatography of the fatty acid methyl esters.

Part A - Preparation of the fatty acid methyl esters

Apparatus
- 50 and 100 ml ground-necked round-bottom flasks.
- Reflux condenser, 20 to 30 cm effective length, with ground joint appropriate to the flask.
- 250 ml separating funnels.
- Inlet tube for passing nitrogen.
- Test tubes with ground glass stoppers.
- Graduated pipette, capacity at least 10 ml, fitted with a rubber bulb or automatic pipette.

- Boiling chips (fat-free).

Reagents
- Heptane, chromatographic quality (Notes 2 and 4)
- Redistilled light petroleum (b.p. 40-60°), bromine value less than 1, residue free, or hexane (Note 2)
- Sodium sulfate, anhydrous
- Sodium hydroxide, appropriately 0.5 N methanolic solution: Dissolve 2 g of sodium hydroxide in 100 ml methanol containing not more than 0.5% (m/m) of water. When the solution has to be stored for a considerable time, a small amount of white precipitate of sodium may be formed; this has no effect on the preparation of the methyl esters.
- Boron trifluoride, 12 to 25% (m/m) methanolic solution. 14 and 50% solutions are available commercially (Note 2).
Caution: Boron trifluoride is poisonous. For this reason it is not recommended that the analyst prepare the methanolic solution of boron trifluoride from methanol and boron trifluoride (Note 3).
- Sodium chloride, saturated aqueous solution
- Methyl red, 1 g/l solution in 60% (v/v) ethanol
- Nitrogen, containing less than 5 mg/kg of oxygen

Procedure
Because of the toxic character of boron trifluoride, the following operations are best performed under a ventilated hood. All glassware must be washed with water immediately after use
Dry the sample at 105° to constant weight, using 2 hour increments of heating. Accurately weigh about 350 mg of dried sample. Sample sizes larger or smaller than 350 mg may be taken, however, the size of flask and quantities of reagents should conform to the following table:

weight of sample (mg)	*Flask capacity (ml)*	*0.5N NaOH (ml)*	*Methanolic soln. of BF_3 (ml)*
100-250	50	4	5
250-500	50	6	7
500-700	100	8	9
750-1000	100	10	12

Place the desired amount of prepared fatty acids in the appropriate flask. Add the appropriate amount of methanolic boron trifluoride solution. Boil for 2 min.

Add 2 to 5 ml of heptane (Note 4) (the precise amount does not affect the reaction) to the boiling mixture through the top of the condenser and continue boiling for 1 minute.
Withdraw the source of heat, and then remove the condenser. Add a small portion of saturated sodium chloride solution and shake the flask gently by rotating it several times.

Add more saturated sodium chloride solution to the flask in order to bring the level of liquid into the neck of the flask. Allow to separate and transfer about 1 ml of the upper layer (heptane solution) into a ground-necked test tube and add a little anhydrous sodium sulfate to remove any trace of water. If the sample taken was 350 mg, this solution contains about 7-17 percent

of methyl esters and may be injected directly onto the column for gas-liquid chromatography. In the other cases dilute the heptane solution to obtain a 5-10% concentration of methyl esters (Note 6).

In order to recover the whole of the dry esters, transfer the saline solution and the heptane layer into a separating funnel. Separate the layers. Extract the saline solution twice with 50 ml portions of light petroleum. Combine the heptane solution and the two extracts, and wash them with 20-ml portions of water until free from acid (methyl red indicator). Dry over anhydrous sodium sulfate, filter and evaporate the solvent over a boiling-water bath in a current of nitrogen (Notes 6 and 7). For samples under 500 mg it is desirable to reduce proportionately the volumes of solvent and water used.
Alternative methods which do not involve the use of boron trifluoride are available. In the general method the methylation reagents, 0.5 N methanolic sodium hydroxide and 12-25 % methanolic boron trifluoride, may be substituted with:

- 1 N Methanolic potassium hydroxide, (reacted with fatty substance in the presence of excess low moisture content methanol);
- Sodium methylate solution (prepared by dissolving 1 g of sodium metal in 100 ml of low-moisture content methanol).

Notes
1. If the unsaponifiable matter interferes, dilute the saponified solution with water and eliminate the unsaponifiable matter by extraction with diethyl ether or hexane. Acidify the aqueous soap solution and separate the fatty acids. Prepare the methyl esters from these as described.

2. In the course of the gas-liquid chromatography of the methyl esters, certain reagents, particularly the methanolic boron trifluoride solution may produce adventitious peaks on the graph (in the region of C_{20}-C_{22} esters in the case of methanolic boron trifluoride solutions). Consequently any new batch of reagent should be checked by preparing the methyl esters of pure oleic acid, and chromatographing them; if an extraneous peak appears, the reagent should be rejected. The various reagents must not give peaks interfering with those of methyl esters of fatty acids during the gas-liquid chromatography.
The methanolic solutions of boron trifluoride must be stored in a refrigerator.

3. If it is absolutely unavoidable to prepare a solution of boron trifluoride from gaseous boron trifluoride, the recommended method is:
Weigh a 2 l flask containing 1 l of methanol. Cool in an ice bath, and with the flask still in bath, bubble BF_3 from a cylinder through a glass tube into the methanol until 125 g BF_3 is absorbed. Perform the operation in a fume-cupboard. BF_3 must be flowing through the glass tube before it is placed in and until it is removed from methanol to prevent liquid from being drawn into the gas cylinder valve system. Gas should not flow so fast that white fumes emerge from flask.
This reagent is stable for 2 years.

4. If fatty acids containing twenty or more carbon atoms are absent, hexane may be substituted for heptane (mixture of pure C_7 isomers tested by gas-liquid chromatography).

5. If the suggested amount of sample is not available, 10 mg, or even less, may be used, provided that the amounts of reagents and the size of the containers are reduced proportionally.

6. Preferably, the solutions of methyl esters should be analysed as soon as possible. If necessary, the heptane solution containing the methyl esters may be stored under an inert gas in a refrigerator. In the case of prolonged storage, it is desirable to protect the methyl esters from autoxidation by adding to the solution an antioxidant in such a concentration as will not interfere with the subsequent analysis, e.g. 0.05 g/l of EHT (2,6-di-tertbutyl 4-methyl phenol).
If necessary, the dry and solvent-free methyl esters may be stored 24 h under inert gas in a refrigerator, or longer in sealed tube under vacuum in a deep-freeze.

7. There is some risk of losing part of the most volatile methyl esters if the evaporation of the solvent is prolonged, or if the current of nitrogen is too vigorous.
For infrared spectroscopy, elimination of the solvent must be as complete as possible.
For gas liquid chromatography remove solvent.

Part B - Gas-liquid chromatography of fatty acid methyl esters

Apparatus
The instruction given relate to the ordinary equipment used for gas-liquid chromatography, employing a packed column and a flame-ionization detector (Note 1). Any apparatus giving efficiency and resolution for the specific fatty substance is suitable.

Gas liquid chromatography
Injection system: The injection system should have the least dead space possible. If possible, it should be heated to a temperature 20 to 50° higher than that of the column.
Oven: The oven should be capable of heating the column to at least 220° and of maintaining the desired temperature to within 1°.
If programmed heating is to be employed, an apparatus with a twin column is recommended.
Packed column:
- Column: The column must be constructed of a material inert to the substances to be analysed: glass or, failing this, stainless steel (Note 2); Length: 1 to 3 m, a relative short column should be used when long-chain acids (C_{20+}) are present. For the determination of C_4 and C_6 fatty acids, a 2-m column is recommended; Internal diameter: 2 to 4 mm.

Packing
- Support: Acid-washed and silanized diatomaceous earth, or other suitable inert support with a narrow range (25 µm) of grain size between the limits 125-200 µm, the average grain size being related to the internal diameter and length of the column.
- Stationary phase: Polyester type of polar liquid (e.g. diethylene glycol polysuccinate, butanediol polysuccinate, ethylene glycol polyadipate ...) or

any other liquid (e.g. cyanosilicones ...) meeting the requirements below. The stationary phase should amount to 5 to 20% of the packing. A non-polar stationary phase can be used for certain separations

- Conditioning the newly prepared column: The column being disconnected from the detector, if possible, heat the oven gradually to 185° and pass a current of inert gas through the freshly prepared column at a rate of 20 - 60 ml/min for at least 16 h at this temperature, and for 2 h more at 195°.

Detector : The manipulations described below relate to the use of a flame ionization detector (Note 1).

Syringe: Syringe, maximum capacity 10 µl, graduated in 0.1 µl.

Recorder

If the recorder curve is to be used to calculate the composition of the mixture analysed, an electronic recorder of high precision is required. It should be compatible with the apparatus used. The characteristics of the recorder should be:

- Rate of response below 1.5 sec, preferentially below 1 sec (the rate of response is the time taken for the recording pen to pass from 0 to 90% following the momentary introduction of a 100% signal)
- Breadth of the paper: 25 cm minimum
- Paper speed: 25-100 cm/h

Integrator or Calculator (Optional)

Rapid and accurate calculation can be performed with the help of an electronic integrator or calculator. This must give a linear response with adequate sensitivity, and the correction for deviation of the base-line must be satisfactory.

Reagents

- Carrier gas: Inert gas (nitrogen, helium, argon ...) thoroughly dried and containing less than 10 mg/kg of oxygen.
- Auxiliary gases: Hydrogen (99.9% min.) free from organic impurities, air or oxygen.
- Reference standards: A mixture of methyl esters, or the methyl esters of an oil, of known composition, preferably similar to that of the fatty matter to be analysed.

Procedure

Conditions of Test

Determining optimal operating conditions

As a rule, the figures shown in Table 1 and 2 below, will lead to the desired results.

Table 1

Internal diameter of column	Carrier gas supply
2 mm	15-25 ml/min
3 mm	20-40 ml/min
4 mm	40-60 ml/min

Table 2

Concentration of stationary phase	Temperature

5 %	175°
10 %	180°
15 %	180°
20 %	185°

When the apparatus allows it, the injector should be at a temperature of about 200°C and the detector at a temperature equal to, or higher than, that of the column.

The flow of hydrogen to the flame-ionization detector is, as a rule, about half that of the carrier gas, and the flow of oxygen about 5 to 10 times that of the hydrogen.

Determining the efficiency and the resolution (Optional)

Carry out the analysis of a standard of methyl stearate. Choose the size of the sample, the temperature of the column and the carrier gas flow so that the maximum of the methyl stearate peak is recorded about 15 min after the solvent peak, and rises to three-quarters of the full scale

- Analysis

The sample for examination shall be 0.1 to 2 µl of the heptane solution of methyl esters obtained according to Part A. In the case of esters not in solution, prepare an approximate 10% solution in heptane and inject 0.1 to 1 µl of this.

As a rule, the operating conditions will be those defined above.

Nevertheless, it is possible to work with a lower column temperature where the determination of acids below C_{12} is required, or at higher temperature when determining fatty acids above $C_{20.}$

On occasion, it is possible to employ temperature programming in both the previous cases. If the sample contains the methyl esters of fatty acids below C_{12}, it is necessary to inject the sample at 100° (or at 50-60° if butyric acid is present) and immediately to raise the temperature at a rate of 4-8°/min to the optimum. In some cases the two procedures can be combined: after the programmed heating, continue the elution at a constant temperature until all the components have been eluted. If the instrument does not operate with programme heating, work at two fixed temperatures between 100° and 195°.

Expression of the results

Qualitative Analysis

Analyse the reference standard mixture of known composition in the same operating conditions as those employed for the sample, and measure the retention distances (or retention times) for the constituent fatty esters. Using a semi-logarithmic paper, construct the graphs showing the logarithm of the retention distance (or retention time) as a function of the number of carbon atoms of the acids; in isothermal conditions the graphs for straight chain esters of the same degree of unsaturation should be straight lines. These straight lines are approximately parallel.

Identify the peaks for the sample from these graphs, if necessary by interpolation. It is necessary to avoid conditions such that "masked peaks" exist, i.e. where the resolution is insufficient to separate two components.

Quantitative Analysis

Determination of the composition
Apart from exceptional cases, use the method of area normalization, i.e. assume that the whole of the components of the sample are represented on the chromatogram, so that the total of the areas under the peaks represents 100 percent of the constituents (total elution).
By appropriate standardization procedures (using a reference standard mixture or an internal standard), determine the total weight of fatty acids in the dried sample. Calculate the content of Fatty Acid Salts for the specific cation(s) in the sample. the content of total Fatty Acid Salts shall be not less than 95% of the dried sample. In addition if there are specifications on the label of the Fatty Acid Salt for content of individual fatty acids, the sample must comply with these specifications.

Notes
1. A gas-liquid chromatograph employing a catharometer (working on thermal conductivity changes) may be used. Operating conditions must then be modified as follows:

Column
- length: 2 to 4 m
- internal diameter: 4 mm
- support: grain size between 160 and 200 µm
- stationary phase: 15 to 25%

Carrier gas: helium, or failing this, hydrogen, with a content of oxygen as low as possible. No auxiliary gases.
Flow rate: usually between 60 and 80 ml/min
Temperatures
- Injector: 40° to 60° above that of the column
- Column: 180° to 200°

Quantitative analysis: correction of factors derived from the analysis of a reference mixture of esters of known composition, determined under operating conditions identical with those used for the sample, must be used.

2. If polyunsaturated components with more than three double bonds are present, they may decompose in a stainless-steel column.