BUREAU OF INDIAN STANDARDS

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भारतीय मानक मसौदा

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Draft Indian Standard

DETERMINATION OF FATTY ACIDS IN NATURAL AND SYNTHETIC PERFUMERY MATERIALS BY GAS CHROMATOGRAPHY

(ICS 71.100.60)

Fragrance and Flavour Sectional Committee PCD 18	Last date for receipt of comment is **19 July 2024**

FOREWORD

(*Formal clauses shall be added later*)

This document supersedes earlier Wide Circulated Doc No. PCD 18 (14314). Following comments have been incorporated in the earlier Wide Circulated draft:

i) Need/importance of the standard incorporated in the foreword of the draft;
ii) Grade of Hexane (from Chromatographic quality to Chromatographic grade solvent), and carrier flow of Helium incorporated;
iii) Recording of the RT in clause 4.3.2 also modified; and
iv) Sentence "This method would be useful in conforming presence of adulteration like vegetable oil if any unusual peak is observed" included by BIS Secretariat in the foreword.

Essential oils are the most frequently adulterated commodity in modern times. Some of the most common adulterants are castor oil, liquid paraffin, vegetable oils, etc. There are instances of adulteration of essential oils by addition of vegetable oils. Routine chromatographic methods prescribed for quality evaluation of essential oils are not capable of detecting presence of vegetable oils, as they are composed of non-volatile fatty acids.

The purpose of this standard is to prescribe method for determination of vegetable oils in essential oils. The method involves the procedure for conversion of fatty acids into volatile methyl esters followed by gas chromatographic analysis.

This method would be useful in conforming presence of adulteration like vegetable oil if any unusual peak is observed.

In reporting the result of a test or analysis made in accordance with this standard, if the final value, observed or calculated, is to be rounded off, it shall be done in accordance with IS 2: 2022 ‘Rules for rounding off numerical values (*second revision*)’.

1 SCOPE

1.1 This Indian standard prescribes the method of test for determination of fatty acids in natural and synthetic perfumery materials by gas chromatography.

NOTE — This method is also suitable for detection of adulteration of essential oils by vegetable oils.

2 APPARATUS

2.1 Gas Chromatograph — equipped with split/split-less injector and Flame Ionization Detector (FID).

2.2 Capillary Column — suitable for Fatty Acid Methyl Ester (FAME) analysis.

3 REAGENTS

3.1 Hexane — chromatographic grade solvent.

3.2 Methanolic Potassium Hydroxide (0.2 N) — prepared by dissolving 11.2 g of KOH in 100 ml of HPLC grade methanol.

3.3 FAME Mix Standard — (C_4 – C_{24}).

4 PROCEDURE

4.1 Preparation of Sample Solution

Take 0.2 g sample in a test tube, add 2 ml hexane and shake well**.** Add 0.2 ml of methanolic potassium hydroxide solution (*see* **3.2**) and shake well for 1 min. Allow the two layers to separate. Upper hexane layer containing FAME is suitable for injection into gas chromatograph. Transfer 1 ml aliquot of upper layer into a 1.5 ml GC vial and preserve until GC analysis.

4.2 Preparation of FAME Standard Solution

Dissolve 0.1 ml of stock FAME mix standard solution in 1 ml hexane in a 1.5 ml GC vial.

4.3 Gas Chromatographic Analysis

4.3.1 Set up following GC operating conditions:

Column	Fused silica polar capillary column coated with (50 percent Cyanopropyl)-dimethylpolysiloxane, length 60 m, ID 0.25 mm, film thickness 0.25 μm
Injector Temperature	250 °C
Split Ratio	50 : 1
Carrier Gas	Helium
Carrier Flow	1 ml (constant flow)

Oven Temperature Program	50 °C (1 min) to 175 °C with ramp rate of 5 °C, to 240 °C (hold for 30 min) at ramp rate of 4 °C/min
Detector (FID) temperature	270 °C
Injection volume	1 µl prepared in hexane

4.3.2 Inject FAME standard solution; acquire chromatogram and record retention times (RTs) of each peaks corresponding to the fatty acid starting from C_4 onwards.

4.3.3 Inject the derivatized FAME sample solution, acquire chromatogram and identify peaks of fatty acids by comparing with Retention Time of peaks eluting in FAME standard solution.

4.3.4 Gas chromatographic conditions shall be same for both the sample injections.

5 CALCULATION

Calculate area percent of peak due to each fatty acid by following equation:

$$\text{Area percent of individual fatty acid} = \frac{Area\ of\ peak\ due\ to\ individual\ fatty\ acid}{Sum\ of\ area\ of\ all\ peaks\ due\ to\ fatty\ acids} \times 100$$