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BUREAU OF INDIAN STANDARDS

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भारतीय मानक मसौदा

गैस क्रोमेटोग्राफी द्वारा एसेंसियल ऑइल में चिराल कि शुद्धधता का निर्धारण

Draft *Indian Standard*

DETERMINATION OF CHIRAL PURITY OF ESSENTIAL OILS BY GAS CHROMATOGRAPHY

(ICS 71.100.60)

Fragrance and Flavour Sectional Committee, PCD 18	Last date for receipt of comment is 16 March 2025

FOREWORD

(Formal clauses shall be added later)

Some essential oils contain enantiomeric isomers having distribution ratio within a specified range. Any change in the distribution ratio indicates addition of synthetic compounds and naturalness of the oil becomes doubtful.

It is not possible to resolve these isomers using polar or non-polar column and routine chromatographic conditions and therefore specific chiral columns are used to properly resolve them. Also most of the standards do not prescribe chiral chromatographic methods to address this issue.

This draft standard specifies general guidance for chiral analysis of essential oils having enantiomeric isomers, which can be useful in establishing the naturalness as also in the detection of addition of synthetic molecules.

GC operating conditions may be modified and optimized, depending upon the nature of components, enantiomer distribution, polarity, boiling point etc., in order to achieve proper resolution and complete elution of all the components in the essential oils.

1 SCOPE

1.1 This draft standard specifies procedure for the determination of chiral purity of essential oils, by gas chromatographic technique using chiral gas chromatographic column.

1.2 This draft standard is applicable for essential oils containing enantiomers (optical isomers).

2 REAGENTS

2.1 Suitable solvents such as ethanol, acetone, hexane etc. as per the requirement.

2.2 Any solvent capable of dissolving the essential oils can be used, provided it does not co-elute with components of interest.

2.3 Gas

2.3.1 *Carrier gas* ─ Such as hydrogen, helium or nitrogen, or any other gas, depending on the type of detector used.

2.3.2 *Detector supply gas* ─ Air and hydrogen of high purity for a flame ionization detector, helium of high purity for a mass detector.

2.4 Reference substance ─ Corresponding to the pure and mixed enantiomers to be detected.

3 APPARATUS

3.1 Gas Chromatograph ─ Equipped with spilt/spiltless inlet, suitable capillary chiral column and flame ionization detector (FID) and also conforming to the requirements given under **3.1.1** or **3.1.2** as applicable.

3.1.1 GC Conditions for β – DEX 225 Chiral Column

Column	Fused silica capillary column coated with non-bonded, 25 percent Silyl[(6-O-*tert*-butyldimethyl)-2,3,-di-O-acetyl)-β-cyclodextrin (20 percent phenyl/80 percent dimethylpolysiloxane) / [β-DEX 225]
Film Thickness	0.25 µm
Column Dimension	30 m × 0.25 mm ID
Injector Temperature	230°C
Split Ratio	200 : 1
Sample Size	0.5 µl (2 percent solution in suitable solvent)
Carrier Gas & Flow	Helium, at constant pressure of 89.2 kPa
Column oven Temperature	60°C (10 min) to 120°C @ 1.0 °C/min then to 160°C @ 2.5°C/min, final hold time 40 min
Detector type	FID
Detector Temperature	240°C

3.1.2 GC Conditions for γ– DEX 225 Chiral Column

Column	Fused silica capillary column coated with Non-Bonded; 25percent 2,3-di- O-acetyl-6-O-TBDMS-γ-cyclodextrin in SPB-20 poly (20 percent phenyl/ 80 percent dimethylpolysiloxane) [γ-DEX 225]
Film Thickness	0.25 µm
Column Dimension	30 m × 0.25 mm ID
Injector Temperature	230°C
Split Ratio	200 : 1
Sample Size	0.5µl (2 percent solution in suitable solvent)
Carrier Gas & Flow	Helium, at constant pressure of 89.2 kPa

Column oven Temperature	60°C (10 min) to 120°C @ 1.0 °C/min then to 160°C @2.5°C/min , final hold time 40 min
Detector type	FID
Detector Temperature	240°C

4 PROCEDURE

4.1 Sample Preparation — Dissolve 200 mg of essential oil in ethanol or other suitable solvent and dilute to 10 ml.

4.2 Sample analysis — Analyze by GC using conditions listed in **3.1.1** or **3.1.2** as applicable.

5 CALCULATION

Calculate the percent of each isomer by

$$\text{Area percent of specified isomer} = \frac{\text{Peak area of the specified isomer}}{\text{Sum of areas of isomers in the chromatogram}} \times 100$$

Notes

1) The modern instruments are equipped with the software, which automatically calculates area percent of each peak and the values are presented in the peak table of chromoatogram.
2) The above method has been successfully applied for testing of lavender oil, coriander oil, peppermint oil, spearmint oil and tea tree oil.
3) GC operating conditions may be modified and optimized, depending upon the nature of components, enantiomer distribution, polarity, boiling point etc. in order to achieve proper resolution and complete elution of all the components in the sample.