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BUREAU OF INDIAN STANDARDS

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भारतीय मानक मसौदा

पेट्रोलियम और उसके उत्पादों के लिए परीक्षण विधि (भाग -)
उच्च-प्रदर्शन तरल क्रोमैटोग्राफी द्वारा बायोडीजल मिश्रित ऑटोमोटिव डीजल/पैराफिनिक डीजल ईंधन में फैटी एसिड मिथाइल एस्टर का निर्धारण - अपवर्तक सूचकांक का पता लगाना

Draft Indian Standard

METHODS OF TEST FOR PETROLEUM AND ITS PRODUCTS (PART -)
DETERMINATION OF FATTY ACID METHYL ESTERS IN BIODIESEL BLENDED AUTOMOTIVE DIESEL/PARAFFINIC DIESEL FUELS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY— REFRACTIVE INDEX DETECTION

(ICS 75.080)

Methods of Sampling and Test for Petroleum and Related Products of Natural or Synthetic Origin (excluding bitumen) Sectional Committee, PCD 01	Last date for comment is 10 September 2024

FOREWORD

(*Formal clauses to be added later*)

India is the world's third-largest crude oil importer, with around 80 percent of its oil requirements met through imports. The transport sector comprises nearly 64 percent of the total crude oil consumption and is considered a significant source of energy-related CO_2 emissions. Given increasing automotive fuel consumption, India has planned to gradually reduce its crude oil import through technically feasible, indigenous, accessible, affordable, and environmentally benign alternative fuels. In the Indian National Policy on Biofuels backdrop, an indicative blending target of 20 percent ethanol in petrol and 5 percent biodiesel in automotive diesel is envisaged by 2030. In the compression-ignition engine, biodiesel or fatty acid methyl ester (FAME) is readily combustible alone or blends with diesel fuel. Biodiesel-blended automotive diesel significantly reduces greenhouse gas emissions, particulate matter, carbon monoxide, and polycyclic aromatic hydrocarbons. Infrared spectroscopy technology-based test methods, ASTM D 7371 and EN 14078 are referred in the automotive diesel fuel — specification (IS 1460:2017) and biodiesel fuel blend B8 to B20 — specification for determination of FAME content (IS 16531:2022). Test methods

ASTM D 7371 and EN 14078 are applicable for FAME concentrations from 1 percent by volume to 20 percent by volume as no precision data is available above 20 percent by volume to 50 percent by volume.

An indigenous test method for determining fatty acid methyl esters in biodiesel blended automotive diesel/paraffinic diesel fuels by high-performance liquid chromatography - refractive index detection has been developed by CSIR-Indian Institute of Petroleum, Dehradun, India. This method has established precision for diesel fuels and their blending components containing FAME content from 0.1 percent to 50 percent by volume. This method is simple, fast (run time 7 min), and requires no sample pre-treatment or back flushing.

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 standard prescribes the method of test for determination of fatty acid methyl esters (FAME) content in biodiesel blended automotive diesel/paraffinic diesel fuels. The method is applicable to the FAME concentrations ranging from 0.1 percent to 50 percent by volume.

2 REFERENCE

The following standards contain provisions, which through reference in this text, constitute the provisions of the standards. At the time of publication, the editions indicated were valid. All the standards are subject to revision, and parties to agreements based on this standard are encouraged to investigate the possibility of applying the most recent editions of the standard indicated below:

IS No.	*Title*
IS 1460 : 2017	Automotive diesel fuel — Specification (*sixth revision*)
IS 1447 (Part 1) : 2021	Methods of sampling of petroleum and its products Part 1 Manual sampling (*second revision*)
IS 15607 : 2022	Biodiesel (B-100) — Fatty acid methyl esters (FAME) — Specification (*second revision*)
IS 16531 : 2022	Biodiesel diesel fuel blend B8 to B20 — Specification (*first revision*)

3. TERMINOLOGY

3.1 Automotive Diesel — Also termed as high-speed diesel fuel, a petroleum-based middle distillate, a liquid fuel used in diesel engines.

3.2 Biodiesel — Also termed as fatty acid methyl esters (FAME), produced from the trans esterification of triglycerides or triacylglycerol's of vegetable oils or animal fats.

3.3 Paraffinic Diesel Fuels — Paraffinic diesel fuels are a class of liquid fuels similar to petroleum-derived diesel fuels that can be synthetically manufactured from feedstock's such as natural gas, vegetable oil, and biomass.

4 SUMMARY OF TEST METHOD

4.1 The test sample (diesel-biodiesel blend) is diluted 1:9 with *n*-hexane, and a fixed volume of this solution is injected into a high-performance liquid chromatography (HPLC) fitted with a strong polar column (polar stationary phase). This stationary phase has no affinity for the hydrocarbons (non-polar components) but exhibits a strong affinity for fatty acid methyl esters (polar components). As a result, the fatty acid methyl esters are separated from hydrocarbons. The method uses a silica stationary phase and an *n*-hexane mobile phase containing isopropanol as a modifier for optimum separation between hydrocarbons and FAME. The adsorption mechanism assists the separation.

4.2 A Refractive index (RI) detector detects the components as they elute from the column. A data processor continuously monitors the electronic signal from the detector. The amplitudes of the signals (peak areas) of FAME in the sample are compared with external calibration standards to calculate the percent *v/v* FAME in the sample.

5 APPARATUS

5.1 High-Performance Liquid Chromatography

Any HPLC capable of pumping the mobile phase at a flow rate of 1.0 ml /min, with a precision better than 0.5 percent RSD.

5.2 Sample Injection System

Capable of injecting 50 µl of sample solution with a repeatability of 1 percent RSD or better.

5.3 Column Oven

Any suitable HPLC column oven capable of maintaining a constant temperature (±1°C) within the range from 20°C to 40°C.

5.4 Column System

Any stainless steel HPLC column (s) packed with silica stationary phase (250 mm length, 4.6 mm i.d., and 5 µm particle size) is suitable.

5.5 Refractive Index Detector

Any refractive index detector may be used provided it is capable of being operated over the refractive index range from 1.3 to 1.6 or wider, meets the sensitivity and linearity of calibration requirement specified in the method, and has a suitable output signal for the data system.

5.6 Auto sampler Vials

5.7 Computer or Computing Integrator

Any data system can be used, provided it is compatible with the refractive index detector and is capable of peak area and retention time measurement. The data system shall have minimum capabilities for post-analysis data processing, such as automatic or manual baseline correction and reintegration.

5.8 Volumetric Flasks, Class A, 10 ml capacity

5.9 Pipettes

Class A, 1 ml and 0.1 ml capacity. Alternatively, calibrated micropipettes with 100 µl to 1000 µl and 10 µl to 200 µl capacity may be used.

6 REAGENTS AND MATERIALS

6.1 *n*-Hexane, HPLC grade. If necessary, dry the solvent with molecular sieves and then filter before use.

6.2 Isopropanol, HPLC grade. If necessary, dry the solvent with molecular sieves and then filter before use.

6.3 Methyl myristate, Purity 99 percent, *Min*. — Reference standard for FAME

6.4 *n*-Hexadecane, Purity 99 percent, *Min*. — Reference standard for hydrocarbons

7 PROCEDURE

7.1 Sampling

To obtain a representative sample of the base stock, follow the procedure as prescribed in IS 1447 (Part 1).

7.2 Apparatus Preparation

7.2.1 Set up the chromatograph, injection system, column, column oven, refractive index detector, and computing integrator according to the manufacturer's instructions before carrying out the measurement.

7.2.2 Maintain the sample injection valve at room temperature as the sample solution.

7.2.3 Install the HPLC column in the column oven and maintain the column oven temperature at 30 °C.

7.2.4 A mixture of *n*-hexane (99.4 percent, *v/v*) and isopropanol (0.6 percent, *v/v*) is to be used as the mobile phase.

7.2.5 Adjust the flow rate of the mobile phase to a constant 1.0 ml, and ensure the reference cell of the refractive index detector is full of the mobile phase. Allow the temperature of the column oven and refractive index detector, if equipped with temperature control, to stabilize.

7.3 Column Resolution

7.3.1 Prepare a system performance standard (SPS)-1 by measuring *n*-hexadecane (0.1 ml) and methyl myristate (0.1 ml) into a 10 ml volumetric flask and make up to the mark with *n*-hexane. Similarly, prepare a system performance standard (SPS)-2 by measuring *n*-hexadecane (0.2 ml) and methyl myristate (0.2 ml) into a 10 ml volumetric flask and make up to the mark with *n*-hexane.

> NOTE — Ensure that the *n*-hexadecane and methyl myristate is completely dissolved in the mixture; if not, an ultrasonic bath may be used.

7.3.2 When operating conditions are steady, as indicated by a stable horizontal baseline, inject 50 µl of the SPS-1 and SPS-2 separately and record the chromatograms using the data system. Chromatograms are illustrated in Fig. 1 and Fig. 2.

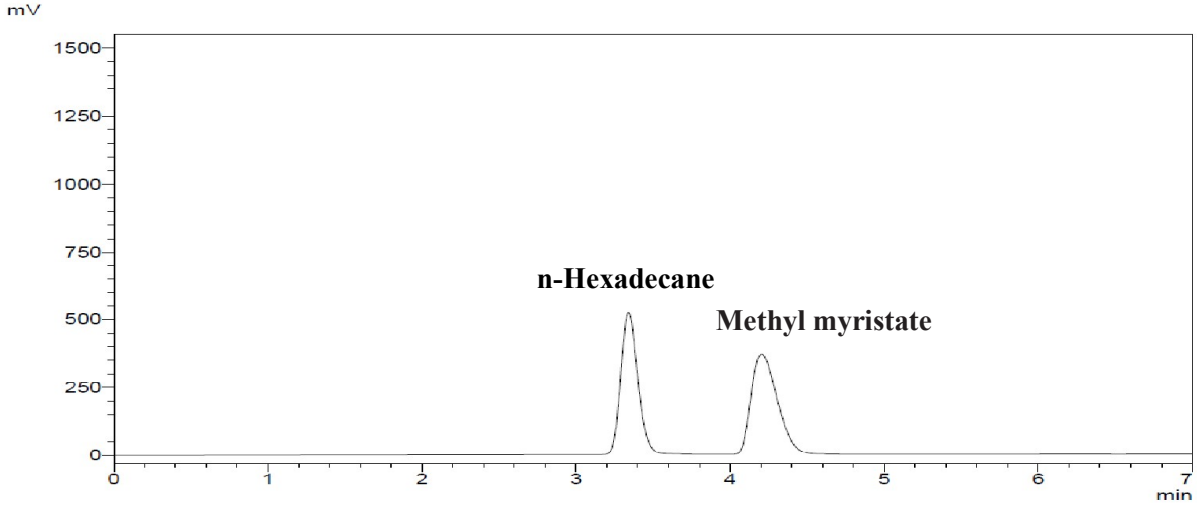

FIG. 1. CHROMATOGRAM DEPICTING ELUTION PROFILES OF REFERENCE STANDARDS PRESENT IN SYSTEM PERFORMANCE STANDARD-1.

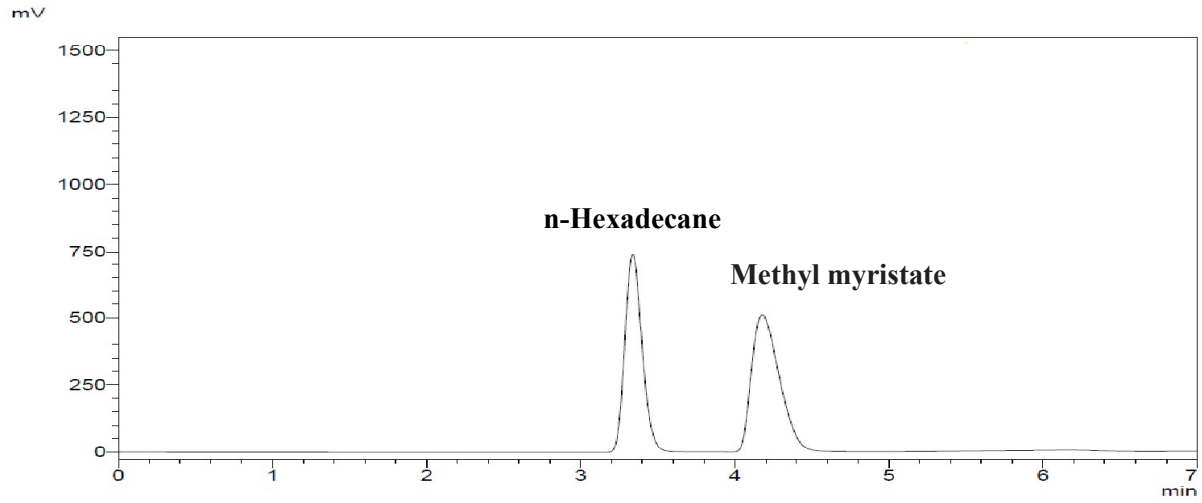

FIG. 2. CHROMATOGRAM DEPICTING ELUTION PROFILES OF REFERENCE STANDARDS PRESENT IN SYSTEM PERFORMANCE STANDARD-2.

7.3.3 Calculate the resolution between *n*-hexadecane and methyl myristate as follows:

$$R_s = \frac{2[RT_M - RT_H]}{W_H + W_M}$$

Where,

RT_H = retention time of *n*-hexadecane in min,

RT_M = retention time of methyl myristate in min,

W_H = width of the chromatographic peak of *n*-hexadecane in min, and

W_M = width of the chromatographic peak of methyl myristate in min.

NOTE — Resolution between *n*-hexadecane and methyl myristate shall not be less than 2.0.

7.4 Calibration

7.4.1 Prepare at least six calibration standard solutions (A, B, C, D, E, and F) at the concentrations given in Table 1. Take appropriate volumes of the methyl myristate (*see* **6.3**) and *n*-hexadecane (*see* **6.4**) into 10 ml volumetric flasks and make up to the mark with *n*-hexane (*see* **6.1**).

NOTE — During the preparation of calibration standard, corresponding weight may be taken instead of volume.

TABLE 1 Concentration of calibration standards
(*Clause* 7.4.1)

Sl. No.	Calibration standard (*v/v*)	Methyl myristate (*ml*)	*n*-Hexadecane (*ml*)
(1)	(2)	(3)	(4)
i)	A	0.5	0.5
ii)	B	0.3	0.7
iii)	C	0.1	0.9
iv)	D	0.05	0.95
v)	E	0.01	0.99
vi)	F	0.001	0.999

7.4.2 When operating conditions are steady, as indicated by a stable horizontal baseline, inject 50 µl of each calibration standard. Record the chromatogram, and measure the peak areas of methyl myristate. Chromatograms of all the six calibration standards are illustrated in Fig. 3 to Fig. 9.

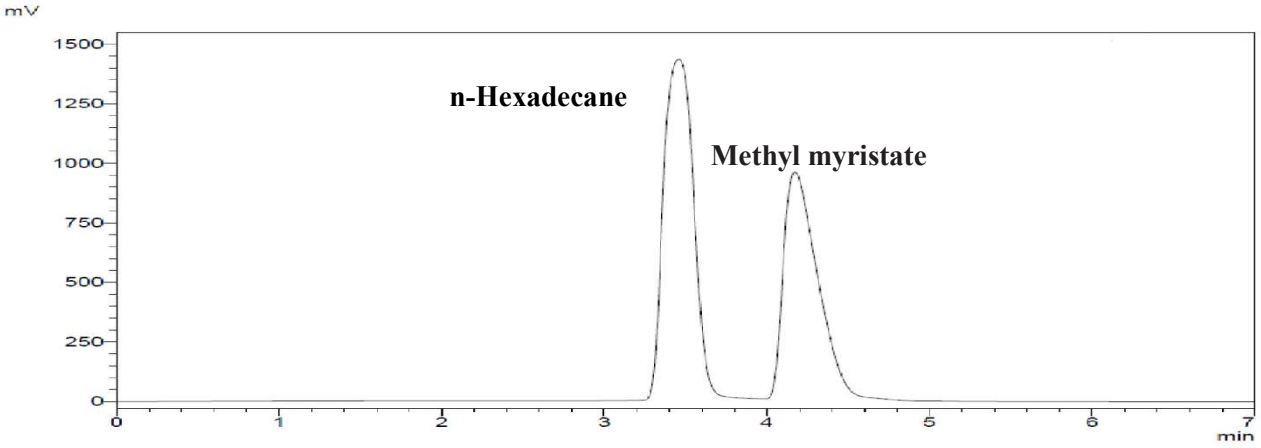

FIG. 3 CHROMATOGRAM OF CALIBRATION STANDARD A (*n*-HEXADECANE AND METHYL MYRISTATE: 50 PERCENT BY VOLUME EACH)

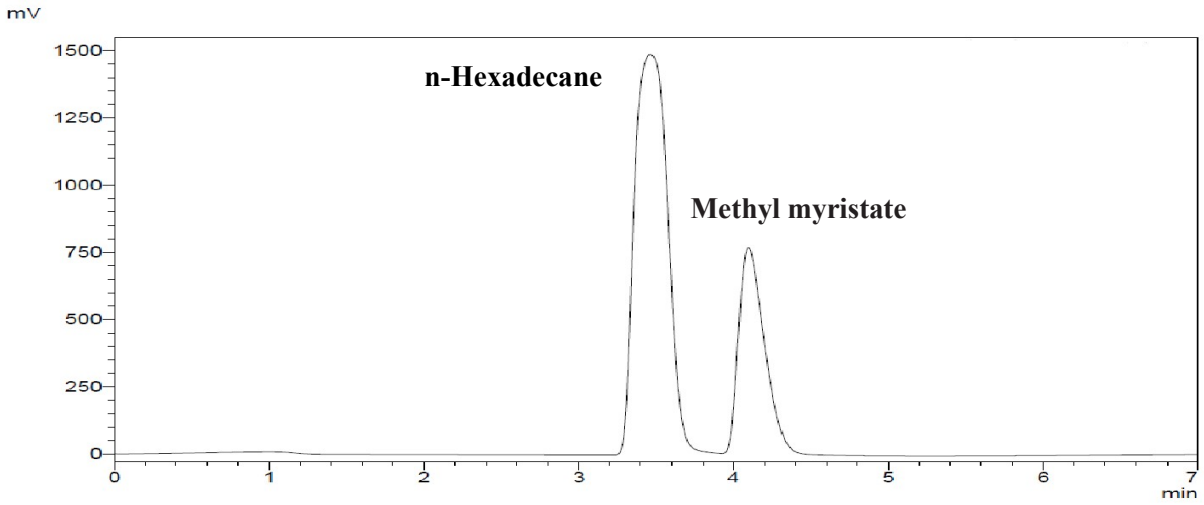

FIG. 4 CHROMATOGRAM OF CALIBRATION STANDARD B (*n*-HEXADECANE AND METHYL MYRISTATE: 70 PERCENT AND 30 PERCENT BY VOLUME, RESPECTIVELY)

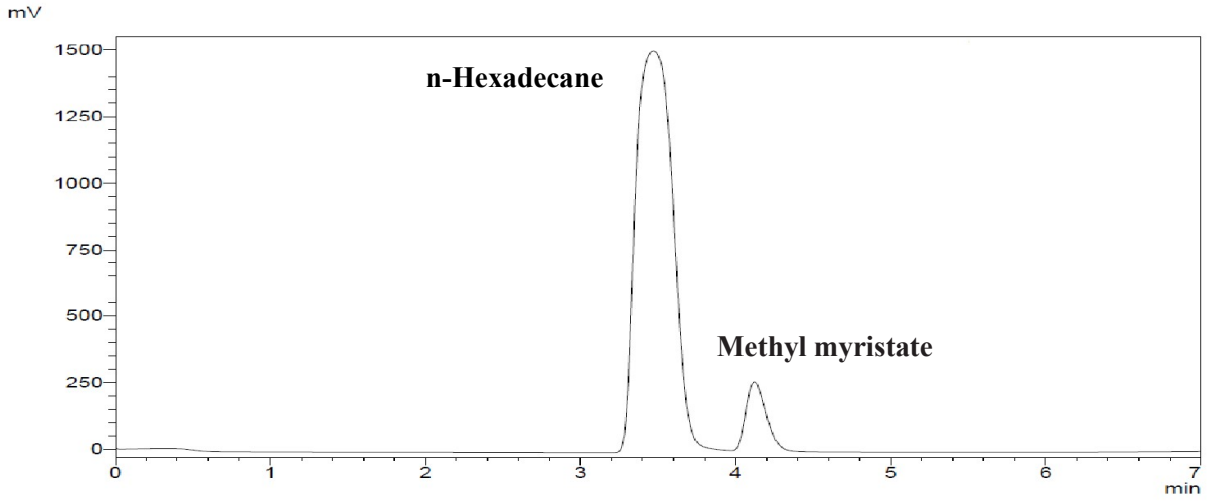

FIG. 5 CHROMATOGRAM OF CALIBRATION STANDARD C (*n*-HEXADECANE AND METHYL MYRISTATE: 90 PERCENT AND 10 PERCENT BY VOLUME, RESPECTIVELY)

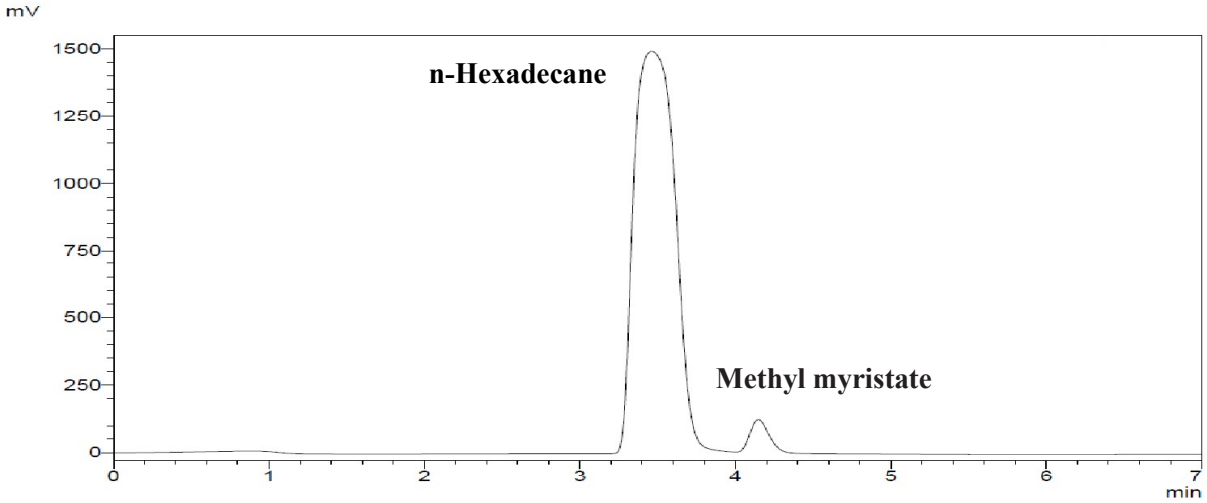

FIG. 6 CHROMATOGRAM OF CALIBRATION STANDARD D (*n*-HEXADECANE AND METHYL MYRISTATE: 95 PERCENT AND 5 PERCENT BY VOLUME, RESPECTIVELY)

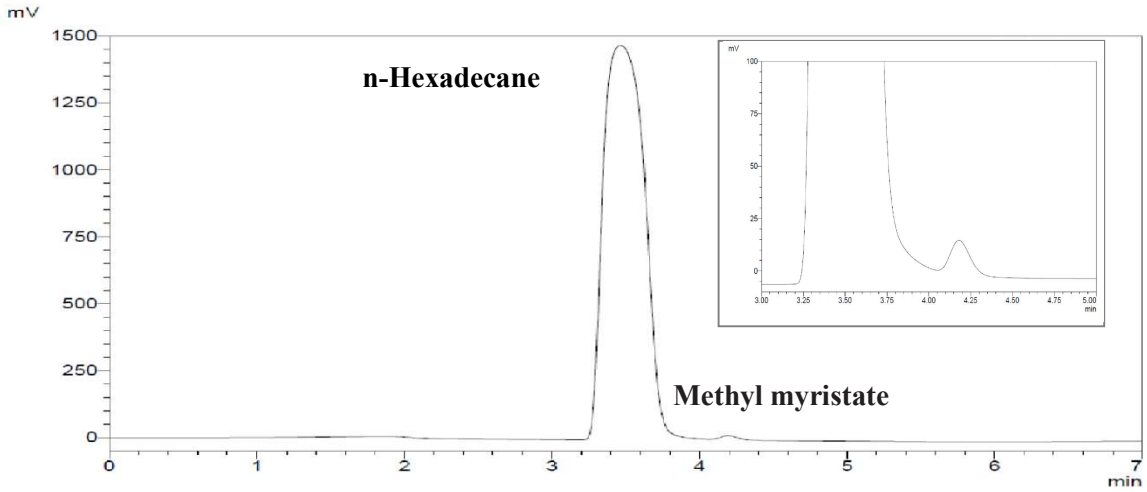

FIG. 7 CHROMATOGRAM OF CALIBRATION STANDARD E (ENLARGED PEAKS IN THE INSET; *n*-HEXADECANE AND METHYL MYRISTATE: 99 PERCENT AND 1 PERCENT BY VOLUME, RESPECTIVELY)

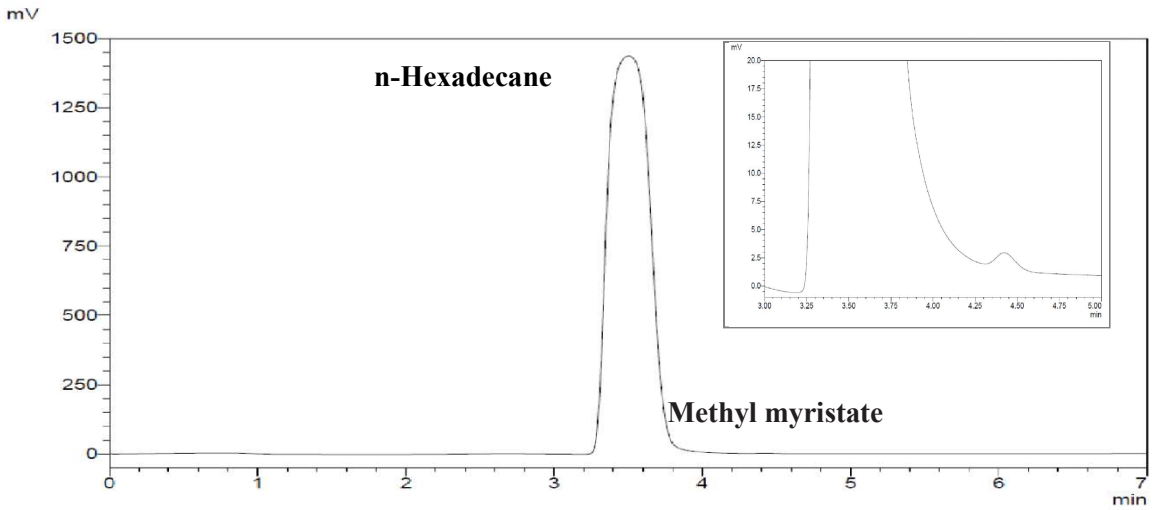

FIG. 8 CHROMATOGRAM OF CALIBRATION STANDARD F (ENLARGED PEAKS IN THE INSET; *n*-HEXADECANE AND METHYL MYRISTATE: 99.9 PERCENT AND 0.1 PERCENT BY VOLUME, RESPECTIVELY)

7.4.3 Plot concentration (percent, *v/v*) against area counts for each FAME standard (methyl myristate). Calibration plots shall be linear with a correlation coefficient greater than 0.99.

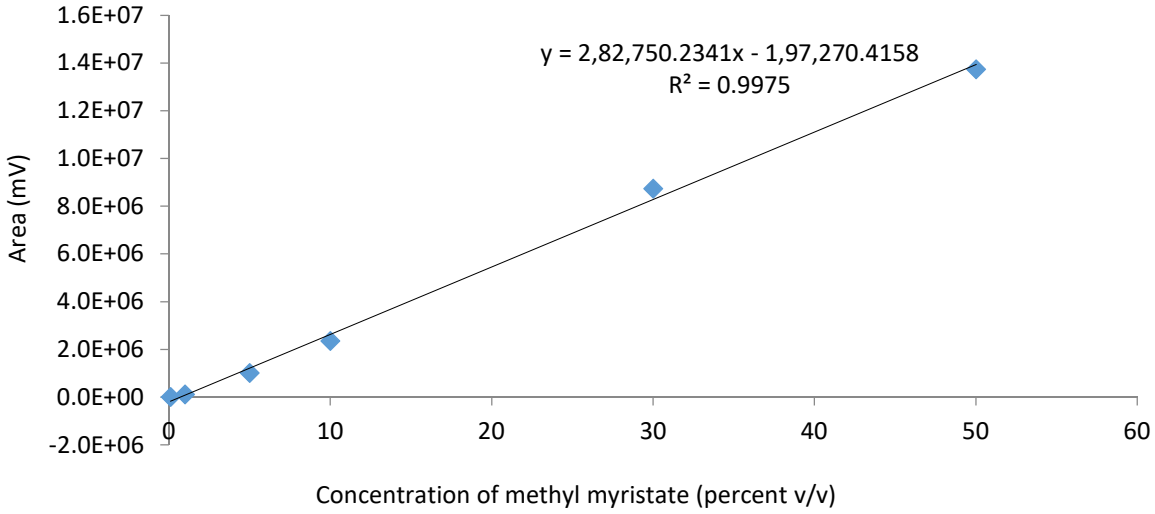

FIG. 9 CALIBRATION CURVE FOR METHYL MYRISTATE

7.5 Analysis of Samples

7.5.1 Take 1 ml of the sample using a pipette into a 10 ml volumetric flask and makeup to the mark with *n*-hexane. Shake thoroughly to mix, allow the solution to stand for 10 min. If required, filter the solution using a 0.45 µm or less porosity micro filter, which is chemically inert towards the mobile phase, to remove any insoluble material.

NOTE — During the preparation of sample, corresponding weight may be taken instead of volume. Refer IS 1460, IS 15607 and IS 16531 for density measurements.

7.5.2 When HPLC operating conditions are steady as indicated by a stable horizontal baseline and identical to those used for obtaining the calibration data, inject 50 µl of the sample solution. Record the chromatogram, and measure the peak areas for FAME. Chromatograms of automotive diesel – biodiesel blend fuels and paraffinic diesel – biodiesel blended fuels are illustrated in Fig. 10 to Fig. 14.

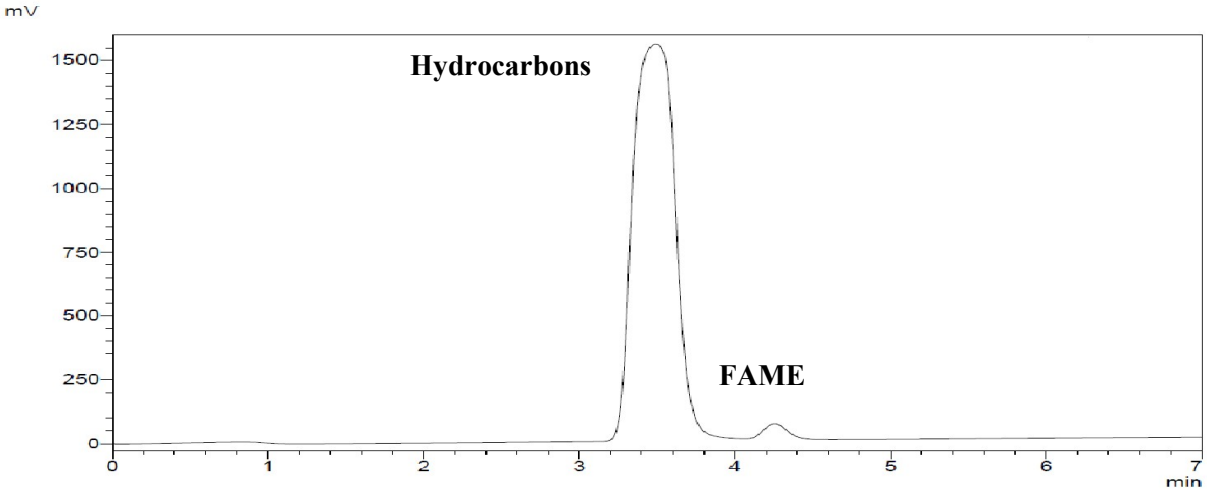

FIG. 10 CHROMATOGRAM OF AUTOMOTIVE DIESEL-BIODIESEL BLEND FUEL (BIODIESEL: 2 PERCENT BY VOLUME)

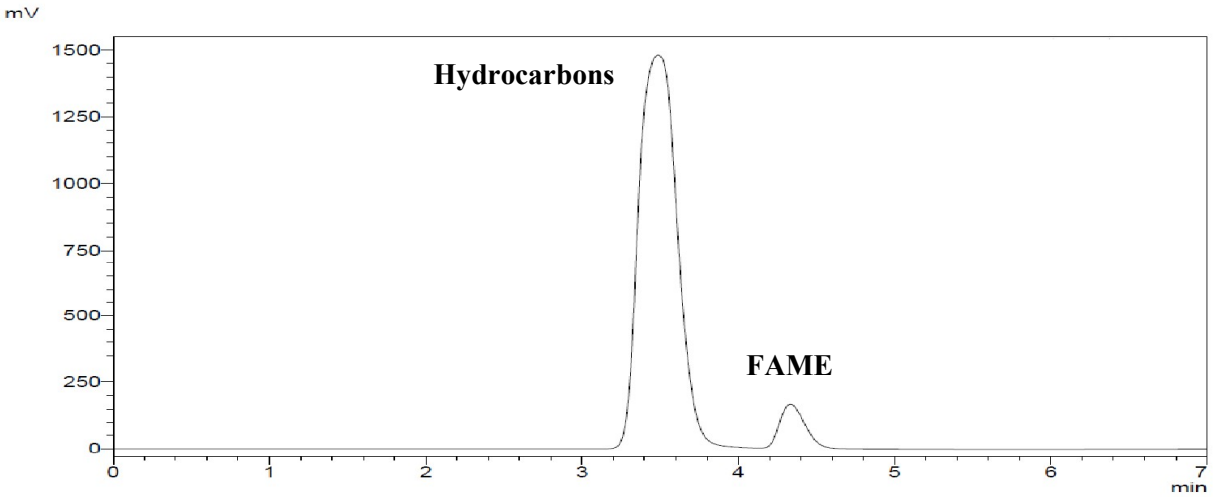

FIG. 11 CHROMATOGRAM OF AUTOMOTIVE DIESEL-BIODIESEL BLEND FUEL (BIODIESEL: 7 PERCENT BY VOLUME).

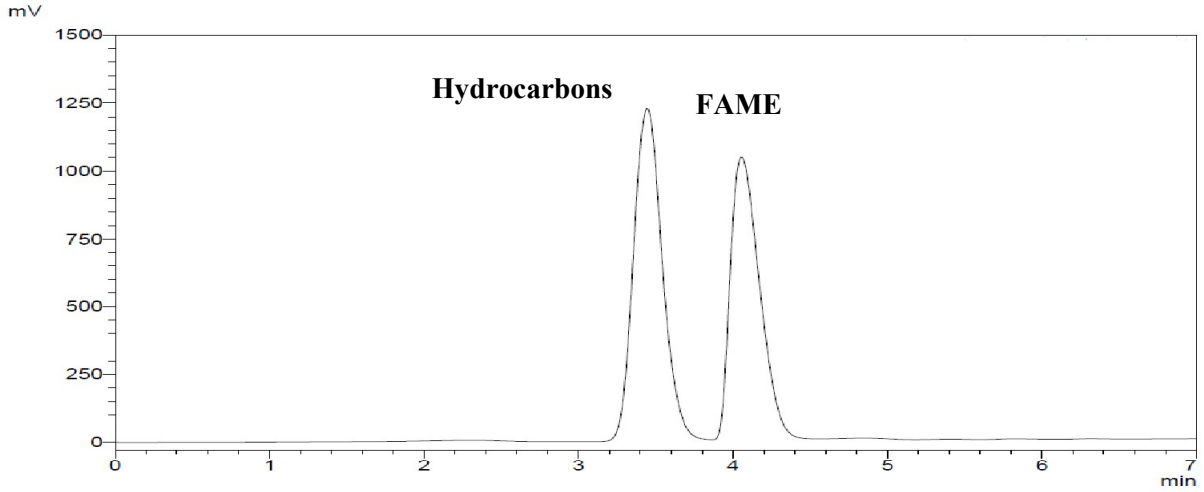

FIG. 12 CHROMATOGRAM OF AUTOMOTIVE DIESEL-BIODIESEL BLEND FUEL (BIODIESEL: 47 PERCENT BY VOLUME).

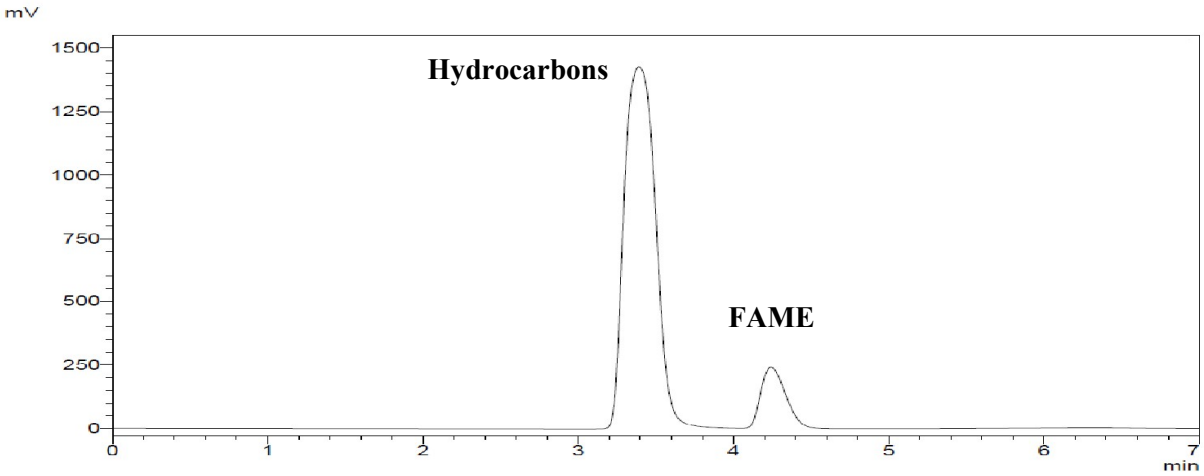

FIG.13 CHROMATOGRAM OF PARAFFINIC DIESEL-BIODIESEL BLEND FUEL (BIODIESEL: 10 PERCENT BY VOLUME).

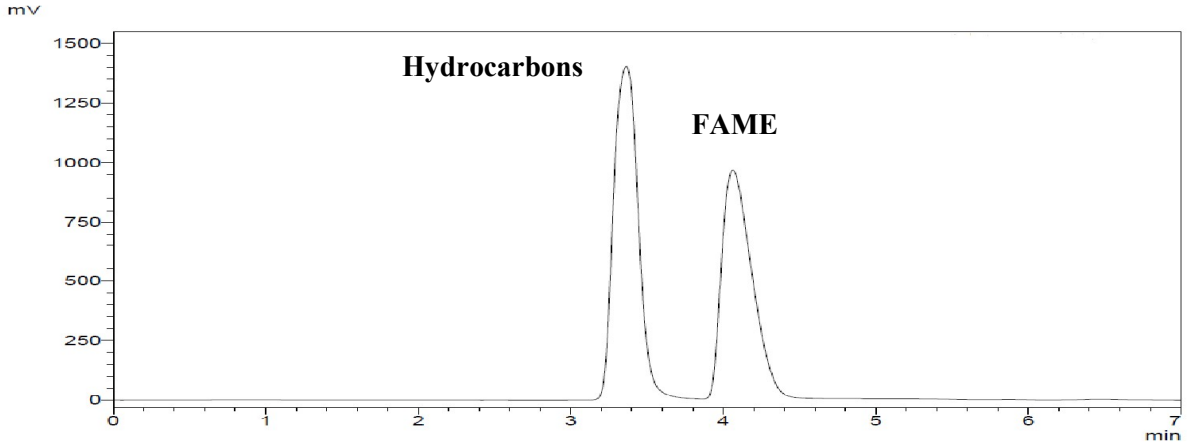

FIG. 14. CHROMATOGRAM OF PARAFFINIC DIESEL-BIODIESEL BLEND FUEL (BIODIESEL: 40 PERCENT BY VOLUME).

8 CALCULATION

8.1 Fatty Acid Methyl Esters (FAME) Content

$$x = \frac{[y - b]}{a}$$

Where,

x = concentration of FAME content (percent, *v/v*),

y = area of FAME peak,

a = slope calculated by linear equation, and

b = intercept calculated by linear equation.

9 REPORT

Report Fatty acid methyl esters (FAME) content and total hydrocarbon contents to the nearest 0.1 percent by volume.

10 PRECISION

10.1 Precision of this method has been established for diesel fuels and their blending components containing FAME content within 0.1 percent to 50 percent by volume.

10.2 Repeatability

The difference between two results obtained by the same operator on the same apparatus under constant operating conditions on identical test material would, in the long run, in the normal and correct operation of the test method, exceed the following values only in one case in twenty. Data are given in Table 2 and Table 3.

TABLE 2 Repeatability values of the method obtained with the actual blends of biodiesel in automotive diesel

(*Clause* 10.2)

Sl. No.	Blending of biodiesel in automotive diesel (percent *v/v*)	Calculated FAME concentration by the method[1] (percent *v/v*)	Relative standard deviation
(1)	(2)	(3)	(4)
i)	0.08	0.07	0.06
ii)	1	0.8	0.3
iii)	5	5.1	0.5
iv)	10	9.8	0.9
v)	20	19.7	0.6
vi)	30	30.0	0.9
vii)	40	39.8	1.7
viii)	50	49.8	1.1

[1]Calculated average value of 20 replications

TABLE 3 Repeatability values of the method obtained with the actual blends of biodiesel in paraffinic diesel

(*Clause* 10.2)

Sl. No.	Blending of biodiesel in Paraffinic diesel (percent *v/v*)	Calculated FAME concentration by the method[1] (percent *v/v*)	Relative standard deviation
(1)	(2)	(3)	(4)
i)	0.05	0.05	0.04

ii)	1	1.0	0.1
iii)	5	5.0	0.6
iv)	10	10.1	0.7
v)	21	21.1	0.9
vi)	30	30.2	0.7
vii)	40	40.1	1.0
viii)	50	49.3	1.4

[1]Calculated average value of 20 replications.

10.3 Reproducibility

The difference between two single and independent results obtained by different operators working in different laboratories on identical test materials would, in the long run, in the normal and correct operation of the test method, exceed the following values given in Table 4 only in one case in twenty.

TABLE 4 Reproducibility values of the method obtained with the actual blends of biodiesel in diesel fuel.

(*Clause* 10.3)

Laboratory code	Sample Id	Blending of biodiesel in diesel fuel[1] (percent *v/v*)	Calculated FAME concentration by the method[2] (percent *v/v*)	Relative standard deviation
(1)	(2)	(3)	(4)	(5)
i)	Sample-1	5.0	5.0	0.5
	Sample-2	20.0	20.4	0.9
	Sample-3	10.0	10.0	0.5
	Sample-4	30.0	30.6	1.3
ii)	Sample-1	5.0	5.0	0.8
	Sample-2	20.0	20.1	0.4
	Sample-3	10.0	10.0	0.6

	Sample-4	30.0	30.2	0.3
iii)	Sample-1	5.0	5.0	2.6
	Sample-2	20.0	20.1	1.7
	Sample-3	10.0	9.9	1.5
	Sample-4	30.0	30.8	1.6
iv)	Sample-1	5.0	5.0	2.4
	Sample-2	20.0	20.4	1.1
	Sample-3	10.0	10.0	1.1
	Sample-4	30.0	30.2	1.0
v)	Sample-1	5.0	5.6	4.5
	Sample-2	20.0	22.0	2.7
	Sample-3	10.0	11.1	3.6
	Sample-4	30.0	30.8	1.6
vi)	Sample-1	5.0	5.1	2.0
	Sample-2	20.0	20.2	0.7
	Sample-3	10.0	10.3	2.0
	Sample-4	30.0	30.1	0.5

[1]As per the certificate of analysis of Biodiesel / Diesel Fuel Blends (VHG, LGC Ltd.)

[2] Calculated average value of 20 replications.