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Method Validation of Fatty Acid Profile with Mustard Oil by Gas Chromatograph

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ABSTRACT

Mustard oil has fatty acids composition predominant with eicosanoic (C22:0), and erucic (C22:1) acids. The study had the aim of method validation of fatty acids with mustard oil by Gas Chromatograph with FID detector. The LOD, LOQ and precision studies were done with C22:0 and C22:1n9 fatty acids whish are naturally present in mustard oil. The LOD and LOQ from calibration curve were 1.14 and 3.46 µg/mL for C22:0 standard and 44.83 and 135.85 µg/mL for C22:1n9. The recovery study at Central Agmark Laboratoy, Nagpur was done with spiking of C11:0 standard in mustard oil. The recovery percentage were 83.13, 81.27, 95.76, 101.52,106.86 for solutions A,B,C, D and E respectively. The recovery percentage of spiked C11: 0 standard meets the acceptance criteria within 70-120% and ranged between 81.27 to 106.86% . The regression coefficient was 0.9999 for C22:0 and C22:1n9.

Keywords: Method Validation, Regression coefficient, Gas Chromatograph, mustard oil.

INTRODUCTION

The oils extracted from plant seeds are an essential part in nutrition in terms of fats. As an energy source, these compounds are essential for human health [1] . The vegetable oils extracted are the primary source of essential fatty acids and each vary with the composition [2].

The presence or absence of double bonds as saturated fatty acids (without double bonds), monounsaturated fatty acids (with one double bond), and polyunsaturated fatty acids (with more than two double bonds) in each fatty acids vary. The chain length and degree of unsaturation may have great influence on the chemical, biological properties of these compounds. In addition, genetic and environmental factors can determine the proportions of saturated and unsaturated fatty acids present in vegetable oils[3],[4].

Mustard oil used widely in India is extracted from genus *Brassica.*Mustard oil contains the major saturated fatty acids like palmitic and stearic acids alongwith mono and polyunsaturated fatty acids like oleic, eicosenoic, erucic, linoleic and linolenicacids. The fatty acid composition of the seed oil depends on the genetic, ecological, morphological, physiological and cultural factors. [5].

Mustard oil has fatty acids composition in higher genetic variations compared to those contained in other major vegetable oils. The seven major fatty acids were extracted from members of the genus *Brassica* are found to be palmitic (C16:0), stearic (C18:0), oleic (C18:1), linoleic (C18:2), linolenic (C18:3), eicosanoic (C22:0), and erucic (C22:1) acids. *Brassica* species seed oil is characterized by significant amount of long-chain monounsaturated fatty acids, mainly erucic acid (C22:1) absented in any other commercial plant oil[6].

Mustard oil is predominant in C22:0 and C22:1n9 fatty acids. The FSSAI standard for C22:0 and C22:1n9 in mustard oil are 0.2-2.5 and 40.0-58.0 % respectively. The objective of the study was to identify the recovery percentage of C11:0 spiked standard in mustard oil and also to identify the interference of the naturally present fatty acids like C22:0 and C22:1n9 in spike recovery of C11:0, which is not naturally present in mustard oil. With the above, objective, a study named Method validation of Fatty Acid profile with Mustard oil by Gas Chromatograph was conducted at Central Agamark Laboratory, Nagpur.

2. MATERIALS AND METHODS:

2.1APPARATUS AND REAGENTS:

24/29 joint 100ml conical flasks of adequate quantity, Air condensers, Centrifuge tubes of 50 ml capacity, water bath, weighing balance, centrifuge, vials, micropipette – 5ml capacity and Gas chromatograph- Agilent make- model-7890B. FAME 37 mix standard CRM, Methanolic HPLC grade, sodium hydroxide, methanolic boron Tri fluoride, Heptane.

2.2EXTRACTION OF METHYLATED ESTERS:

The analytes were weighed separately in the labelled 100ml conical flasks. 10 ml of 0.5 N Methonolic sodium hydroxide was added. Air condenser was fixed and in water bath for 45 minutes. The extracts are saponified, cooled and added 10mI of 14% methanolic BF3. Then each extracts were placed in fuming chamber for 30 minutes to volatile the solvent. Then the solution is transferred to the 50mL Centrifuge tube. The Conical flask was rinsed with 10-15 mL of Saturated NaCl Solution. 5ml of heptane was added in the centrifuge tube. The centrifuge tubes were vortexed and then Centrifuged at 5000 rpm for 5 minutes. Upper Hexane layer of the centrifuged analytes filtered through 0.45micron filter paper and transferred to the 1.5 mL vial. The labelled analytes filed vials kept on Auto sampler of GC. The individual fatty acids present in the analytes were read with peaks obtained in GC under the following instrumental conditions.

2.3.INSTRUMENT CONDITION:

The following conditions were adopted in GC during the method validation of Fatty acid profile in mustard oil

Table-1 INSTRUMENTAL CONDITIONS

Make	Agilent
Model	Agilent-7890B
Oven details	
Equilibration time	1 min
Maximum Temperature	240^0C
Initial	60^0C
Temperature:Hold Time	1 Minute
Ramp rate	4^0C /minute
Final Temperature	220^0C
Hold Time-	20 Minutes
Injection Volume	1µL
column	DB-225-30m x 250µm x 0.25 µm
Carrier Gas	Nitrogen
Column- flow rate	1 mL/Minute
Average Velocity	26.511 cm/Sec
Detector	FID
Make up gas	Nitrogen

Heater Temperature	300^0C
Hydrogen flow	40mL/Min
Air flow	400 mL/Min
Make Up flow	25mL/min
Flame	on
Inlet (Injector) details	
Injector Temperature	250^0C
Septum purge flow	3 mL/min
Total flow	34ml/min
Mode	split
Split flow	30mL/Minute
Liner	Agilent 5190-2293:900µL (Split less, single tapper, wool, ultra)

2.4 PREPARATIONS OF SOLUTIONS FOR METOD VALIDATION- LINEARITY AND ACCURACY :

The Stock Solution (Internal standard C11) was prepared with 50µL of C11 (ρ= 0.872g/mL at 25^0C) is transferred in to 25 mL volumetric flask and makeup by Hexane upto the mark which consititued to 1744 µg/mL of C11 standard as stock solution. The Working standard solution was prepared with 1 ml of stock solution transferred to 10mL volumetric flask and makeup by Hexane constituted with the Concentration of 174.4 µg/mL.

2.5 PREPARATIONS OF SOLUTIONS FOR METOD VALIDATION- RECOVERY AND LINEARITY AND RESPONSE FACTOR:

Solution A, B, C, D and E were prepared with the internal standards with the sample concentration of 10mg/mL, 1mg/mL, 0.5mg/mL, 0.25mg/mL, 0.125mg/mL respectively and with the internal standard(C11) concentration of 348.8 µg/mL, 34.88 µg/mL, 17.44 µg/mL, 8.72µg/mL, 4.36 µg/mL

Table-2 –SUMMARY TABLE FOR CONCENTRATION OF SOLUIONS

Sr. No	**Solution**	**C11 (internal standard)-(µg/mL)**	**Mustard oil concentration (mg/mL)**
1	A	348.8	10
2	B	34.88	1
3	C	17.44	0.5
4	D	8.72	0.25
5	E	4.36	0.125

2.7 Calculation of C22:0 and C22:1n9 µg/ML standard:

Area of C22:0 in sample × Conc.of Mix-Std (µg / mL) × Response factor

Area of C22:0 in FAME- Mix Std

The study was conducted with the standard C11 as the fatty acid is not present in the vegetable oils and Mustard oil sample is having more C22:0 and C22:1n9 µg/ML concentration than any other vegetable oils. C11 is not getting disintegrated during the analysis as spiked with mustard oil when compared to other vegetable oils.

2.8 CALCULATION FOR INDIVIDUAL FATTY ACIDS:

% of Fatty acid Component = $\sum A$ x 100

At = Area of desired fatty acid

A = Total area of Fatty acid Component

RESULTS AND DISCUSSION

The study was conducted with the standard C11 as the fatty acid is not present in the vegetable oils and Mustard oil sample is having more C22:0 and C22:1n9 µg/ML concentration than any other vegetable oils. C11 is not getting disintegrated during the analysis as spiked with mustard oil when compared to other vegetable oils. The methylation process was done to prior to GC analysis for all the spiked mustard oil samples involved with methonolic Boron trifluoride as mentioned in procedure for extraction of methylated

2.6 CALCULATIONS FOR STANDARDS AND RESPONSE FACTOR:

C11:0 Internal standard (µg/mL) is calculated as below

$$\text{C11:0 (µg/mL)} = \frac{\text{Area of C11:0 in sample}}{\text{Area of (C11:0) in FAME-Mix}} \text{ X Concentration of C11:0 (µg/mL) in FAME-Mix}$$

$$\text{Response factor} = \frac{\text{C11 :0 in sample (µg/mL)}}{\text{C11(Added)(µg/mL)}}$$

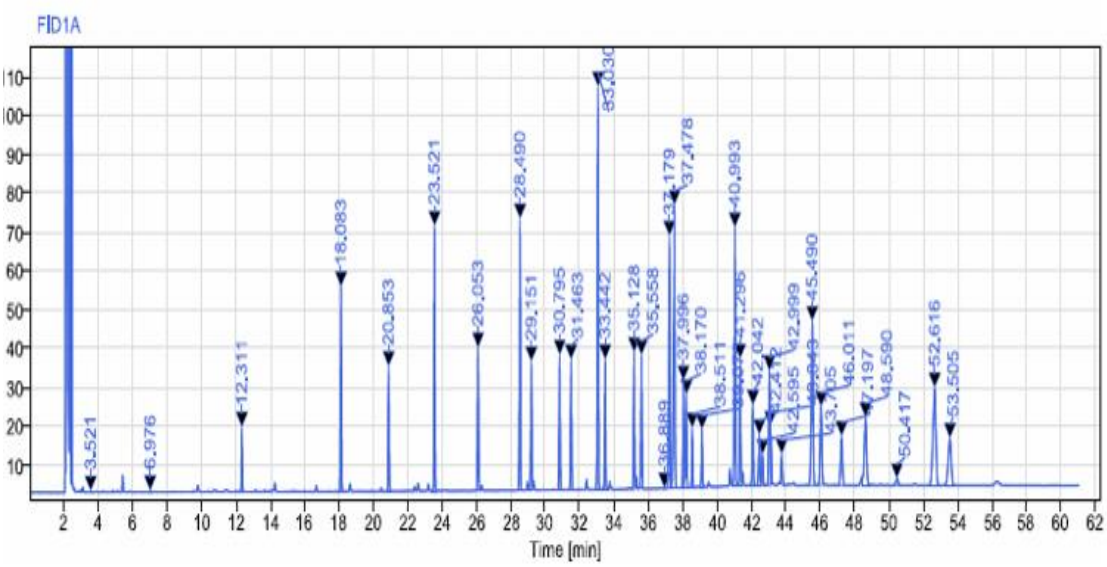
esters. The following table-3 shows the FSSAI standard for fatty acid profile in Mustard oil.

TABLE-3 FSSAI STANDARD FOR FATTY ACID PROFILE IN MUSTARD OIL

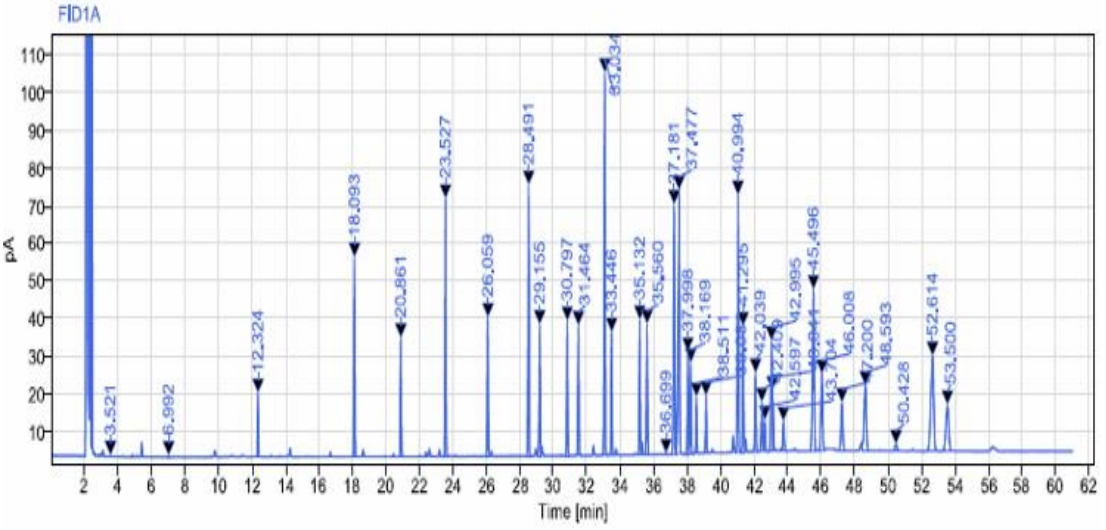
Sr.No	Name of the Fatty Acid	Chemical Identity	Standard as per FSSAI
1	Methyl Hexanoate	C6:0	**ND**
2	Methyl Octanoate	C8:0	**ND**
3	Methyl Deconate	C10:0	**ND**
4	Methyl Laurate	C12:0	**ND**
5	Methyl Myristate	C14:0	**ND-1.0**
6	Methyl Palimitate	C16:0	**0.5-5.0**
7	Methyl palimitoleate	C16:1	**ND-0.5**

Sr.No	Name of the Fatty Acid	Chemical Identity	Standard as per FSSAI
8	Methyl Hepta deconate	C17:0	**ND**
9	CiS-10 Hepta decenoic acid Methyl ester	C17:1	**ND**
10	Methyl sterate	C18:0	**0.5-2.0**
11	Cis-9 Oleic acid Methyl Ester	C18:1 n9 Cis	**8.0-23.0**
12	Methyl Linoleate	C18:2n6 Cis	**10.0-24.0**
13	Methyl Linoleate	C18:3n3	**6.0-18.0**
14	Methyl archidate	C20:0	**ND-1.5**
15	Methyl CIS-11 Eicosenoate	C20:1 (cis)	**5.0-13.0**
16	Cis-11,14 Eicosadienoic acid Methyl Ester	C20:2 (Cis)	**ND-1.0**
17	Methyl Behenate	C22:0	**0.2-2.5**
18	Methyl Erucate	C22:1n9	**40.0-58.0**
19	Cis-13,16-Doco sadienoic acid Methyl ester	C22:2	**ND-1.0**
20	Methyl Lignocerate	C24:0	**ND-0.8**
21	Methyl Nervonate	C24:1	**0.5-2.5**

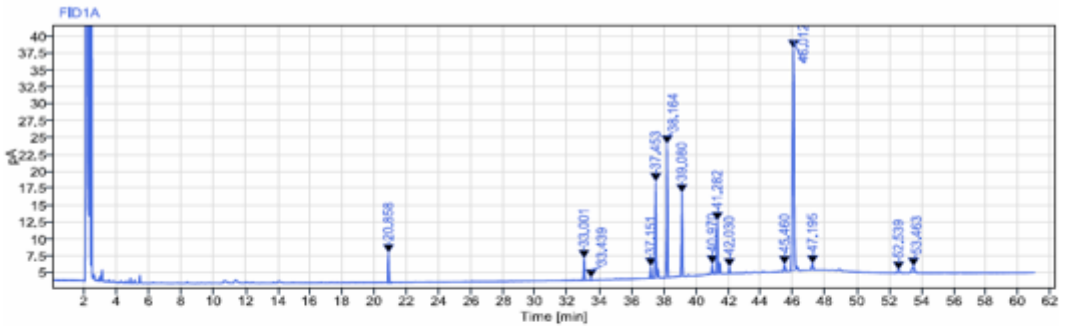
The Graphs 1, 2 represent the chromatograms of FAME Mix. Graph 3, 4 represents the chromatograms of C11 standards Graphs 5 to 9 represents the chromatograms of solutions A to E respectively. Thhe chromatograms FAME mix shows the peaks for 37 fatty acids. The chromatograms of fatty acids in mustard oils are identified only from C11:0 with a total of 16-18 fatty acids are only identified with the solutions.



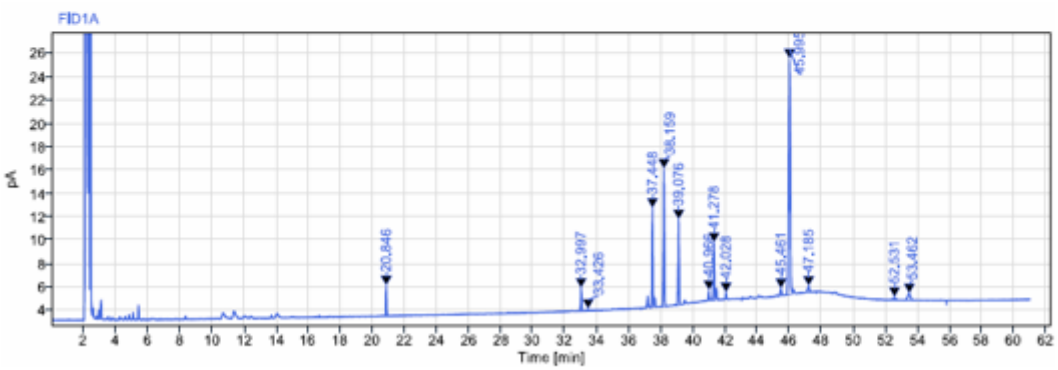
GRAPH-1 FAME MIX STANDARD -I CHROMATOGRAM.



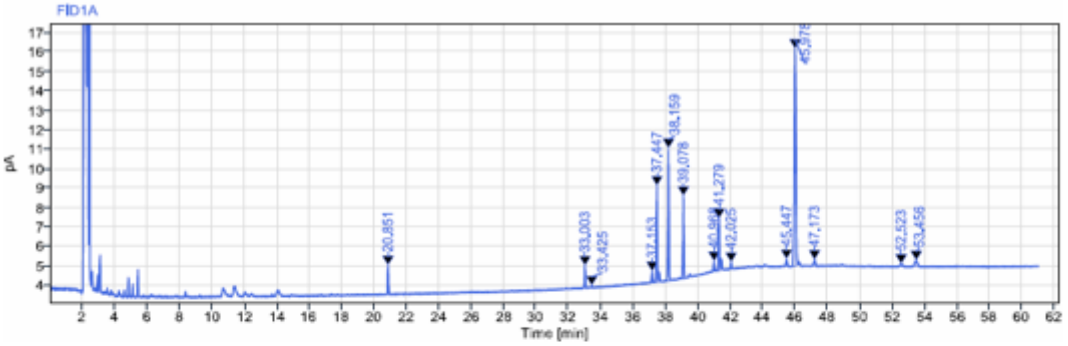
GRAPH-2 FAME MIX STANDARD -II CHROMATOGRAM.



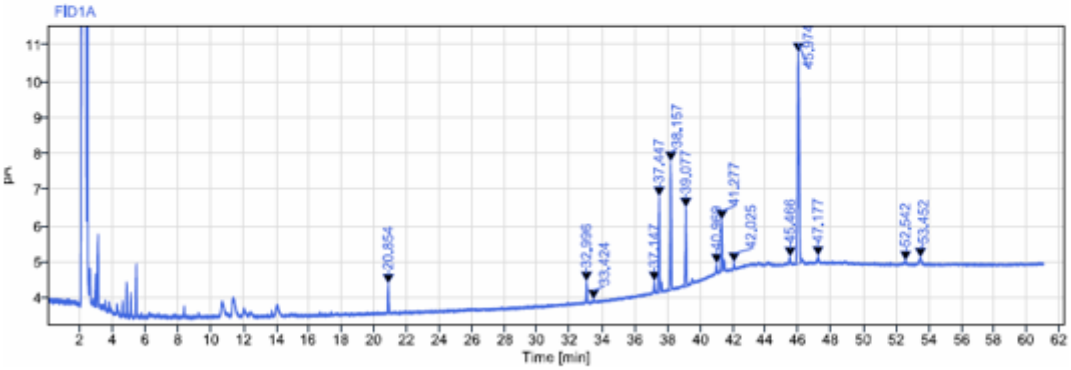
GRAPH-6 CHROMATOGRAM OF SOLUTION B



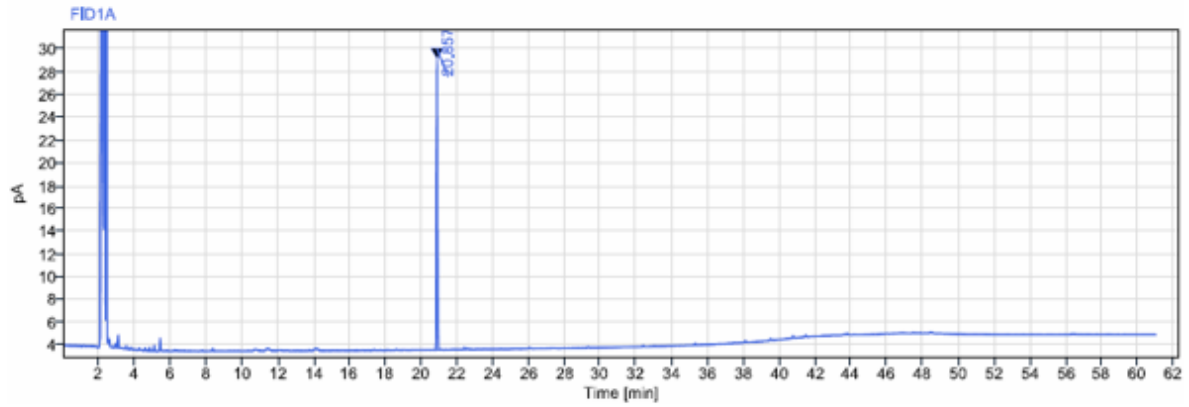
GRAPH-7 CHROMATOGRAM OF SOLUTION C



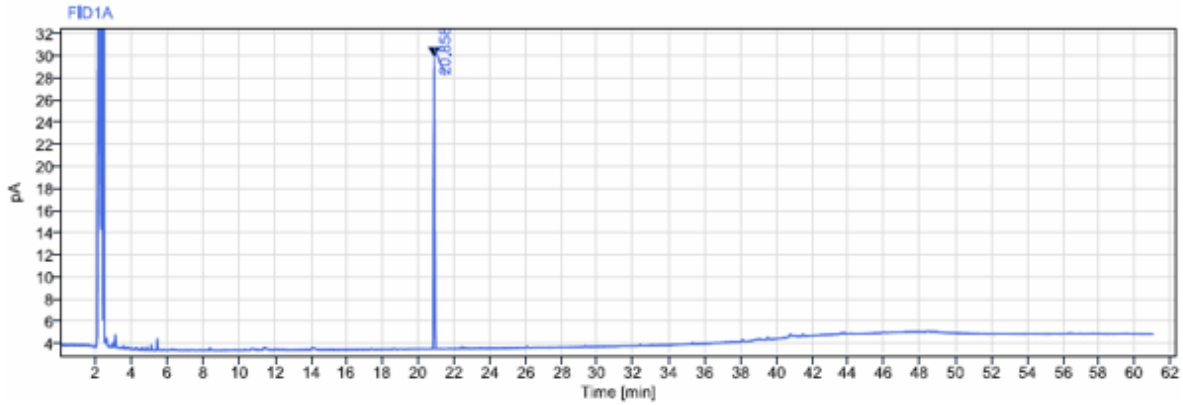
GRAPH-8 CHROMATOGRAM OF SOLUTION D



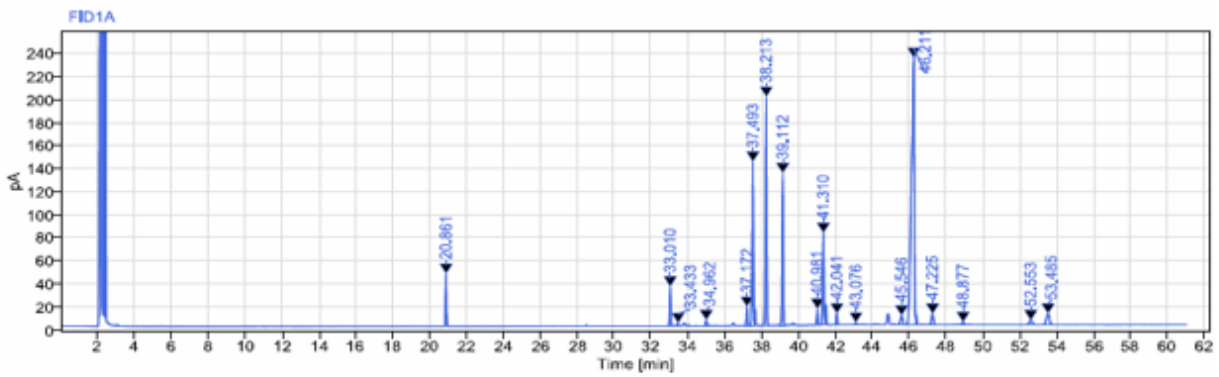
GRAPH-9 CHROMATOGRAM OF SOLUTION E



GRAPH-3 CHROMATOGRAM OF C11 STANDARD



GRAPH-4 CHROMATOGRAM OF C11 STANDARD



GRAPH-5 CHROMATOGRAM OF SOLUTION A

TABLE-3 SUMMARY OF METHOD VALIDATION IN MUSTARD OIL

		Acceptance criteria	Results obtained
	A. C22:0		
1	Linearity Range (µg/mL)	From Calibration Curve	1.25-78.73
	Regression	NLT 0.99	0.9999
	LOD(µg/mL)	From Calibration Curve	1.14

		Acceptance criteria	Results obtained
	LOQ(µg/mL)		3.46
	B. C22:1n9		
	Linearity Range (µg/mL)	From Calibration Curve	64.18-2919.86
	Regression	NLT 0.99	0.9999
	LOD(µg/mL)	From Calibration Curve	44.83
	LOQ(µg/mL)		135.85
2	Precision(% RSD)	NMT 20%	
	C22:0		11.04
	C22:1n9		1.73
3	Recovery	70%-120%	
	Solution-A		83.13
	Solution-B		81.27
	Solution-C		95.76
	Solution-D		101.52
	Solution-E		106.86

The LOD, LOQ and precision studies were done with C22:0 and C22:1n9 fatty acids whish are naturally present in mustard oil. The LOD and LOQ from calibration curve were 1.14 and 3.46 µg/mL for C22:0 standard and 44.83 and 135.85 µg/mL for C22:1n9. The recovery study at Central Agmark Laboratoy, Nagpur was done with spiking of C11:0 standard in mustard oil. The recovery percentage were 83.13, 81.27, 95.76, 101.52,106.86 for solutions A,B,C, D and E respectively.

CONCLUSION

The study conducted at Central Agmark Laboratory, Nagpur showed that the recovery percentage of spiked C11: 0 standard meets the acceptance criteria within 70-120% and ranged between 81.27 to 106.86% . The regression coefficient was 0.9999 for C22:0 and C22:1n9. It is hereby infered that the method validation study of fatty acids in Gas Chromatograph with FID detector, has minimum interference of naturally present fatty acids (C22:0 and C22:1n9) with the spiked standard.

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