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ANALYTICAL METHOD VALIDATION OF GAS CHROMATOGRAPHIC METHOD FOR THE ESTIMATION OF RELATED SUBSTANCES IN TRIMETHYL ORTHO PROPIONATE .

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ABSTRACT

A simple, precise, specific, accurate and reproducible Gas Chromatographic method has been developed for the estimation of related substances in Trimethyl Orthopropionate in bulk sample. A Chemito1000 system comprising with FID detector , Hamilton syringe , Chemito software was used to develop the method. The analyte was resolved by using carrier Nitrogen gas as mobile phase at the flow rate of 30ml/ min.The chemito GC1000 consisting of column 10%Carbowax(Chemito, 1/8" ID, 2meter L, particle size 80/100)at oven temp. 80ºC(2 min.)5ºC/ Min.150ºC; Injector temperature at 210ºC and the Detector temperature at 240ºC respectively. For Validation of Trimethyl OrthoPropionate the µl standard solutions of TMOP were applied at above set temperature programme. The chromatogram were developed with retention time at 2.987 with percent area at 99%.Then validation parameters like, Linearity (CorrelationCoefficient0.9947), Precision study (The determination of RSD,0.1089), Robustness(by changing parameters like temperature and flow rate does not affect the assay values , assay difference NMT 1%),Limit of Detection (at 0.5µl) etc. Were determined respectively. Analytical Method Validation Report for the Determination of Related Substances in Trimethyl OrthoPropionate for these valid parameters was prepared. Present study was undertaken with an objective of developing suitable, sensitive ,and simple analytical approach for estimation of organic impurities present in the API.

KEY WORDS

TMOP, API

INTRODUCTION

Defination

Validation is an integral part of current good manufacturing practice; it is therefore also an element of the quality assurance programme associated with a particular product or process.

Objective of Validation

ANALYTICAL METHOD VALIDATION OF GAS CHROMATOGRAPHIC METHOD FOR THE ESTIMATION OF RELATED SUBSTANCES IN TRIMETHYL ORTHO PROPIONATE .

The objective of Validation of Analytical Procedure is to demonstrate that it is suitable for its intended purpose .

Types of Analytical Procedures to be validated

1. Identification Tests .
2. Quantitative Tests for impurities content.
3. Limit Tests for the control of Impurities .
4. Quantitative Tests of the active moiety in the samples of drug substance or drug product or other selected component (s) in the drug product.

5.Although there are many other analytical procedures such as , dissolution testing for drug products or particle size determination for drug substance these have not been addressed in the initial text on validation of analytical procedures.

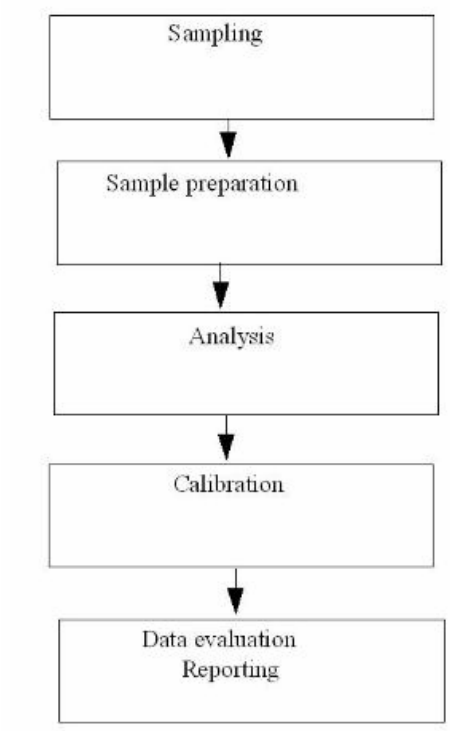
Aim of Validation

Validation process should establish and provide documentary evidence that :

The premises , the facilities , the equipment and the processes have been designed in accordance with the requirements of current GMP that each pharmaceutical company indentifies what qualification and validation work is required to prove control of the critical aspects of the particular operation .

Steps for validating complete Analytical Procedures

ANALYTICAL METHOD VALIDATION OF GAS CHROMATOGRAPHIC METHOD FOR THE ESTIMATION OF RELATED SUBSTANCES IN TRIMETHYL ORTHO PROPIONATE .



Selection Of Analytical Method For Validation

Gas Chromatography
Principle

Seperation is due to differential distribution coefficients.

In this chromatography , moving phase (or mobile phase) is a carrier gas, usually an inert gas such as helium or an unreactive gas such as nitrogen. The *stationary phase* is a microscopic layer of liquid or polymer on an inert solid support, inside a piece of glass or metal tubing called a column. The instrument used to perform gas chromatography is called a gas chrmaograph (or "aerograph", "gas separator").The gaseous compounds being analyzed interact with the walls of the column, which is coated with different stationary phases.

This causes each compound to elute at a different time, known as the retention time of the compound.

ANALYTICAL METHOD VALIDATION OF GAS CHROMATOGRAPHIC METHOD FOR THE ESTIMATION OF RELATED SUBSTANCES IN TRIMETHYL ORTHO PROPIONATE .

Secondly, the column through which the gas phase passes is located in an oven where the temperature of the gas can be controlled, whereas column chromatography (typically) has no such temperature control. Thirdly, the concentration of a compound in the gas phase is solely a function of the vapor pressure of the gas.

Gas chromatography is also sometimes known as **Vapor- Phase Chromatography (VPC),** or **Gas-Liquid Partition Chromatography (GLPC).**

Applications of GC

1) Very minute amounts of a substance can be measured.
2) Various temperature programs can be used to make the readings more meaningful; for example to differentiate between substances that behave similarly during the GC process.
3) Gas Chromatography is used in the separation and analysis of multi component mixtures such as essential oils, hydrocarbons and solvents.
4) Intrinsically, with the use of the flame ionization detector and the electron capture detector (which have very high sensitivities) gas chromatography can quantitatively determine materials present at very low concentrations.
5) The most important application area is in pollution studies ,forensic work and general trace analysis.

USP defines eight steps for validation:

1. Accuracy
2. Precision
3. Specificity
4. Limit of Detection
5. Limit of Quantitation
6. Linearity and range
7. Ruggedness
8. Robustness

Materials and Methods

Selection of product for Analytical Method Validation

The ester product Tri Methyl Ortho Propionate(1, 1 1, Trimethoxy Propane) is selected for the analytical method validation by Gas chromatography for determination of related substances.

ANALYTICAL METHOD VALIDATION OF GAS CHROMATOGRAPHIC METHOD FOR THE ESTIMATION OF RELATED SUBSTANCES IN TRIMETHYL ORTHO PROPIONATE .

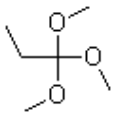


Fig.1 Chemical Structure of TMOP

Reagents and Chemicals

1.Tri Methyl Ortho Propionate.
2.Methanol
3.Propionitrile
4.Solvent A.

Chromatographic System

GC analysis was performed on Chemito GC 1000 system equipped with FID detector .

Other Insruments

1. LOD oven :
2. Analytical Balance.
3. Ultra- Sonicator
4. pH Meter.

Chromatographic conditions as follows

1.Column : 10% Carbowax (Chemito)
2.Oven Temp : 80ºC(2 min.)5ºC/Min.150ºC.
3.Injector Temp. : 210ºC.
4.Detector Temp : 240ºC.
5.Carrier Flow : 30ml/min.
6.Range : 1.

EXPERIMENTAL DETAILS

Calibration of GC

Experimental Conditions

Apparatus	Chemito GC 1000
Sample ID	Calibration Injection : 1
Sample	5% Benzene in Toluene
Blank	Methanol.
Autostop	5.00 Min.
Column	Packed , 5% SE 30,(2.5 m, 48", 80µm/ 100µm)

ANALYTICAL METHOD VALIDATION OF GAS CHROMATOGRAPHIC METHOD FOR THE ESTIMATION OF RELATED SUBSTANCES IN TRIMETHYL ORTHO PROPIONATE .

Carrier Gas	Nitrogen .@constant Pressure 1.2 bar (30ml/min.)
Oven Temperature	80ºC.
Injector Temperature	150ºC.
Detector Temperature	160ºC.
Injection Volume	0.5µL
Detector	FID

Procedure

1) Set Zero GC by injecting Methanol as Blank.
2) Inject 5% Benzene in Toluene solution 5 times into the injector of GC.
3) Observe the peaks & their R.T.
4) Calculate the RSD of 5 replicates from their area and R.T.

Acceptance Criteria

1.RSD OF 5 replicates should be NMT 2 %.

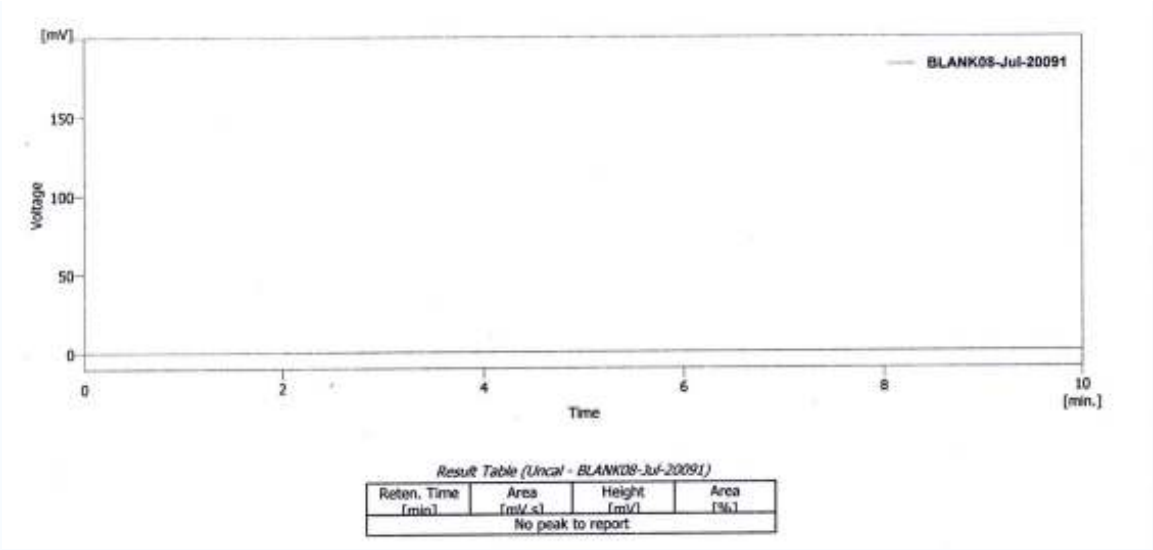
Table 1.

TABLE FOR CALIBRATION OF GC

S .No.	Injection volume	Area	Retention Time
1.	0.5µL	152.985	3.737
2.	0.5µL	148.264	3.747
3.	0.5µL	148.282	3.763
4.	0.5µL	152.266	3.773
5.	0.5µL	146.902	3.787

Chromatograms For Calibration of GC

ANALYTICAL METHOD VALIDATION OF GAS CHROMATOGRAPHIC METHOD FOR THE ESTIMATION OF RELATED SUBSTANCES IN TRIMETHYL ORTHO PROPIONATE .



Result Table (Uncal - BLANK08-Jul-20091)

Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
No peak to report			

Fig.2 Chromatogram For Calibration of GC Blank (Methanol)

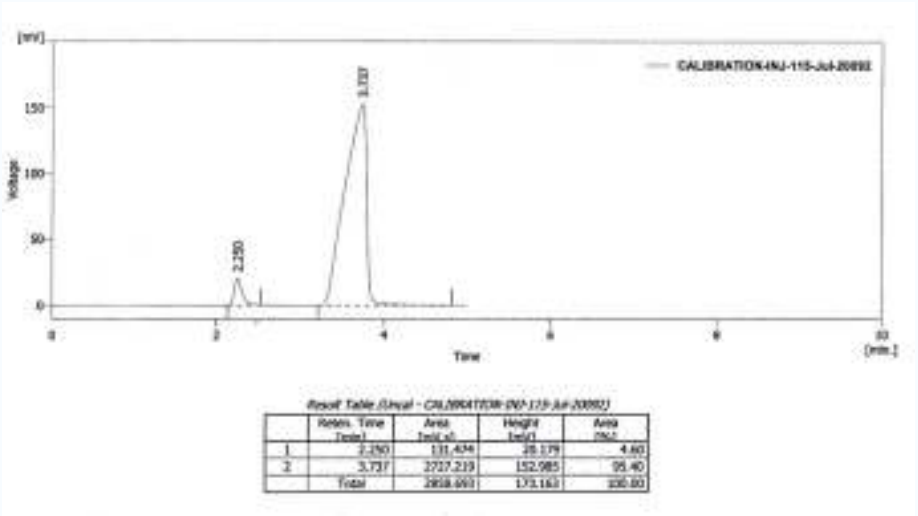


Fig.3 Chromatogram For Calibration of GC.

ANALYTICAL METHOD VALIDATION OF GAS CHROMATOGRAPHIC METHOD FOR THE ESTIMATION OF RELATED SUBSTANCES IN TRIMETHYL ORTHO PROPIONATE .

Remarks:

Table-2

CALIBRATION REPORT OF GAS CHROMATOGRAPH

Make: Chemito	Location : Instrument Room
Model No : GC 1000	Frequency : 1 Month
Serial No : 17	Date of calibration : 15/07/2009
Instrument Code No: INS /GC/002	Next calibration due on: 14/08/2009

Injection No.	Retention Time	Peak Area
1	2.250	131.474
2	2.260	129.964
3	2.267	132.397
4	2.247	136.601
5	2.280	132.325
Mean	2.2608	132.5524
%RSD	0.52 %	1.66 %

Conclusion :

From the above observations, the instrument is working satisfactory / ~~not satisfactory~~

Remarks:

Meets the acceptance criteria. Hence; GC is properly calibrated.

Validation Parameters

The GC method for determination of related substances of Tri Methyl Ortho Propionate has been validated to show the Linearity, Precision , Robustness ,Limit of Detection , Accuracy , in analytical solution .

ANALYTICAL METHOD VALIDATION OF GAS CHROMATOGRAPHIC METHOD FOR THE ESTIMATION OF RELATED SUBSTANCES IN TRIMETHYL ORTHO PROPIONATE .

LINEARITY STUDY

DEFINATION

The linearity of an analytical method is its ability to elicit test results directly proportional to the concentration of the analyte in samples within given range (0.3uL to 0.7 uL).

Procedure

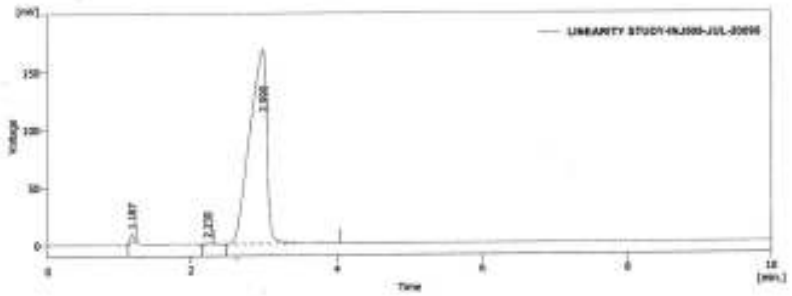
1) Inject the Methanol as Blank.
2) Inject 0.1µL , 0.2µl, 0.3µL,0.4µL, 0.5µL.sample.

Acceptance Criteria

1.Plot a graph of concentration of TMOP verses peak Area . Calculate the correlation coefficient.
2.Correlation Coefficient r = 0.995.
3.Plot of concentrations verses area should indicate linearity.

Observations

Fig.4 Chromatogram For Linearity



	Retention Time [min]	Area [mAU*s]	Height [mAU]	Area [%]
1	3.187	24.430	7.125	0.99
2	3.230	2.066	0.399	0.08
3	3.990	2712.798	168.829	99.04
	Total	2949.294	176.352	100.00

Study.

LINEARITY STUDY

Concentration(X-axis)	Area(Y- axis)
Blank	-
0.1	791.003

ANALYTICAL METHOD VALIDATION OF GAS CHROMATOGRAPHIC METHOD FOR THE ESTIMATION OF RELATED SUBSTANCES IN TRIMETHYL ORTHO PROPIONATE .

0.2	1224.69
0.3	1573.94
0.4	2105.14
0.5	2722.79
0.6	3313.71

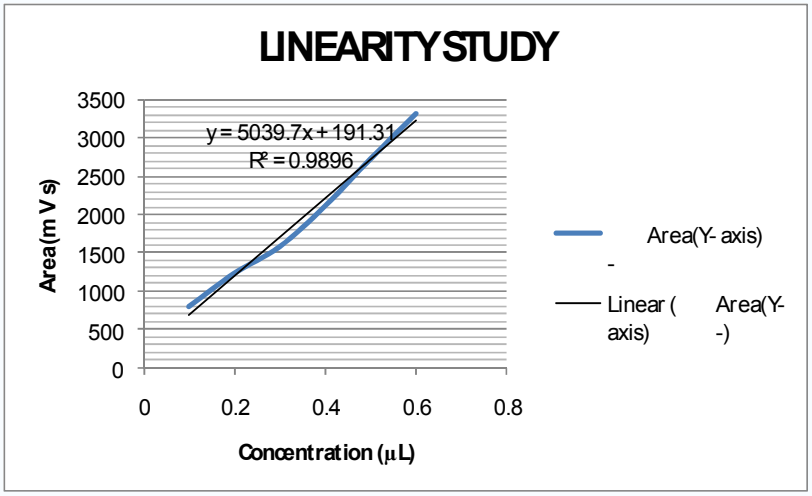


TABLE-3 : FOR LINEARITY STUDY

Level	Concentration(X-axis)	Area(Y- axis)
1.	Blank	-
2.	0.1µL	791.003
3.	0.2µL	1224.687
4.	0.3µL	1573.937
5.	0.4µL	2105.141
6.	0.5µL	2722.788
7.	0.6µL	3313.714
	Correlation Coefficient (r)	0.9896

Remark: Meets the acceptance criteria.

ANALYTICAL METHOD VALIDATION OF GAS CHROMATOGRAPHIC METHOD FOR THE ESTIMATION OF RELATED SUBSTANCES IN TRIMETHYL ORTHO PROPIONATE .

PRECISION STUDY

DEFINATION: The precision of an analytical method is degree of repeatability of the results in a series of experiments run during a single session by single operator with identical reagents and equipments.

Procedure

1) Inject the Methanol as Blank.
2) Inject the five replicates samples and record the chromatogram.

Acceptance Criteria

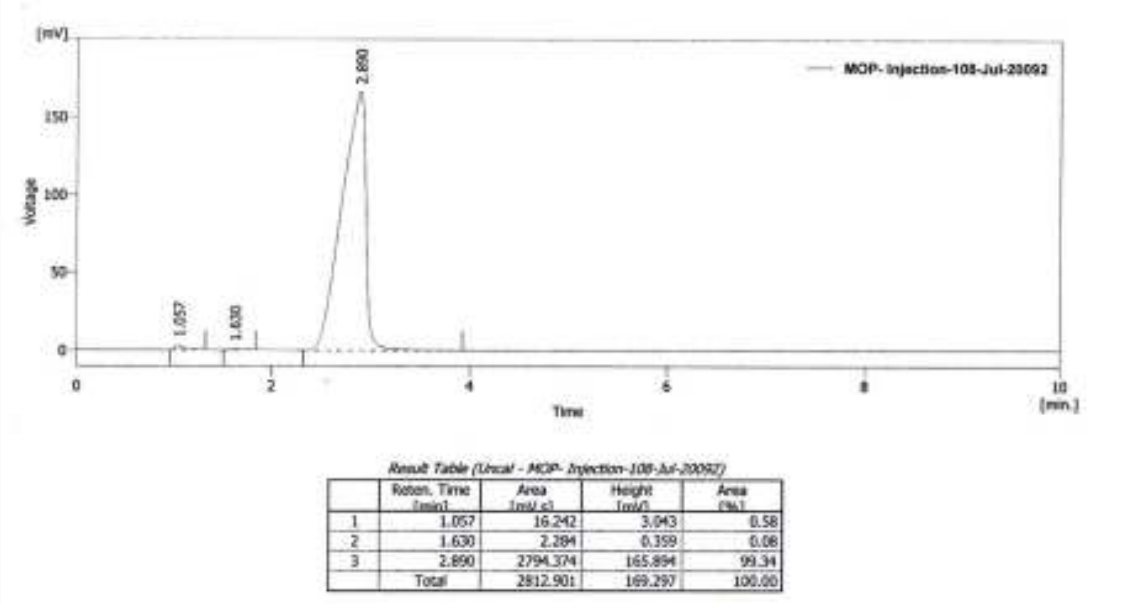
1. Calculate the Relative Standard Deviation .
2. All the results should be within the limit of NMT 2%.

Experimental Conditions:

Apparatus	**Carbowax** Chemito GC 1000
Sample ID	**PRECISION STUDY**
Blank	Methanol.
Autostop	10.00 Min.
Column	(2m.'L, 1/8"ID, : 80/100µm)
Carrier Gas	Nitrogen .@constant Pressure 1.2 bar (30ml/min.)
Oven Temperature	80ºC(2min.)5C/min 150ºC.
Injector Temperature	210ºC
Detector Temperature	240ºC.
Injection Volume:	0 .5µL
Detector :	FID

ANALYTICAL METHOD VALIDATION OF GAS CHROMATOGRAPHIC METHOD FOR THE ESTIMATION OF RELATED SUBSTANCES IN TRIMETHYL ORTHO PROPIONATE .

Fig.5 Chromatogram For Precison Study.



Result Table (Uncal - MOP- Injection-108-Jul-20092)

	Reten. Time [min]	Area [mV s]	Height [mV]	Area [%]
1	1.057	16.242	3.043	0.58
2	1.630	2.284	0.359	0.08
3	2.890	2794.374	165.894	99.34
	Total	2812.901	169.297	100.00

TABLE-4

FOR PRECISION STUDY

Sr. No.	Sample Injected(µL)	Area	% Assay content
1.	Blank	-	
2.	0.5	2794.374	99.34
3.	0.5	2677.687	99.08
4.	0.5	2400.016	99.13
5.	0.5	2282.056	99.08
6.	0.5	2713.633	99.13
		Average (n=5)	99.152
		Standard Deviation	0.108028
		Relative Standard Deviation	0.108924

ANALYTICAL METHOD VALIDATION OF GAS CHROMATOGRAPHIC METHOD FOR THE ESTIMATION OF RELATED SUBSTANCES IN TRIMETHYL ORTHO PROPIONATE .

PRECISION STUDY

Sr. No.	Sample Injected(µL)	Area	% Assay content
1	Blank	-	
2	0.5	2794.37	99.34
3	0.5	2677.69	99.08
4	0.5	2400.02	99.13
5	0.5	2282.06	99.08
6	0.5	2713.63	99.13
		Avg. (n=5)	99.152
		S.D.	0.108028
		R.S.D.	0.108924

Remarks:
The RSD of 6 replicates should be within the limit; hence meets the acceptance criteria.

3.ROBUSTNESS STUDY:

DEFINATION:
The robustness of analytical method defines the capability to retain unaffected by small but deliberate variations in method.

Procedure:

1) Inject the Methanol as Blank.
2) Analyse the sample by changing the flow rate to 25ml/min. and 30ml/min.
3) Analyse the sample by changing the oven temperature to 75ºC and 80 ºC.

Acceptance Criteria:

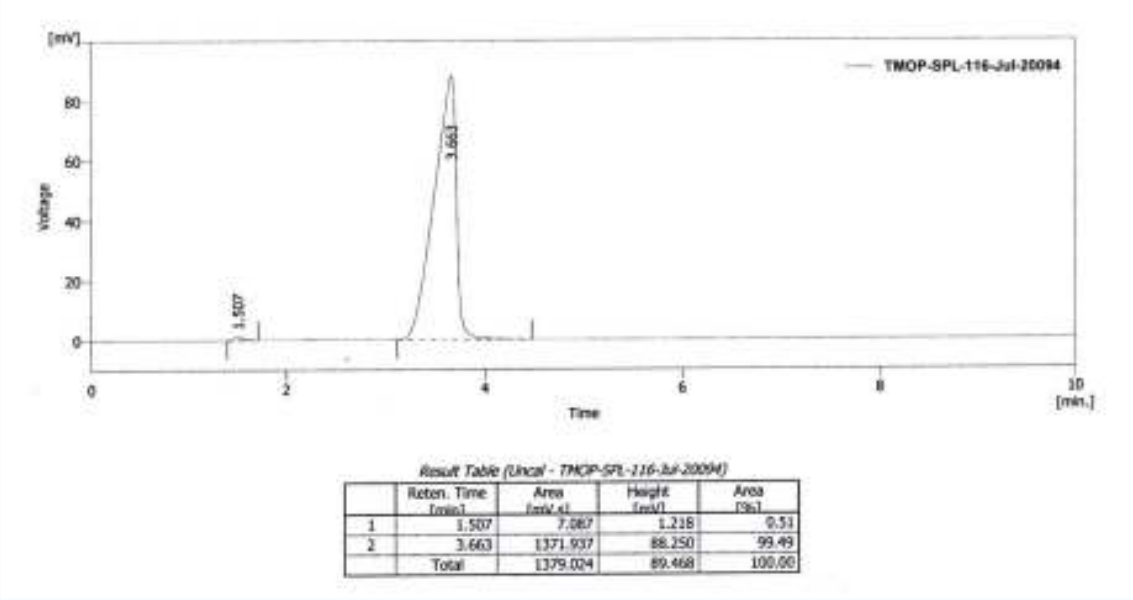
1. Assay difference between two methods should be NMT 1% will be consider acceptable .

ANALYTICAL METHOD VALIDATION OF GAS CHROMATOGRAPHIC METHOD FOR THE ESTIMATION OF RELATED SUBSTANCES IN TRIMETHYL ORTHO PROPIONATE .

2. The chromatography should not be adversely affect when compare to standard chromatogram.

Apparatus:	**Carbowax Chemito GC 1000**
Sample ID:	ROBUSTNESS STUDY
Sample:	SPL-1(VSCL/TMOP/2009/L-01)
Blank :	Methanol.
Autostop:	10.00 Min.
Column:	(2m.'L, 1/8"ID, : 80/100µm)
Carrier Gas:	Nitrogen .@constant Pressure 1.2 bar (25ml/min.)
Flow Rate:	**25ml/min.**
Oven Temperature:	80ºC(2min.)5C/min 150ºC.
Injector Temperature:	210ºC
Detector Temperature:	240ºC.
Injection Volume:	0 .5µL
Detector :	FID

Fig.6 Chromatogram For Robustness Study.



Result Table (Uncal - TMOP-SPL-116-Jul-2009d)

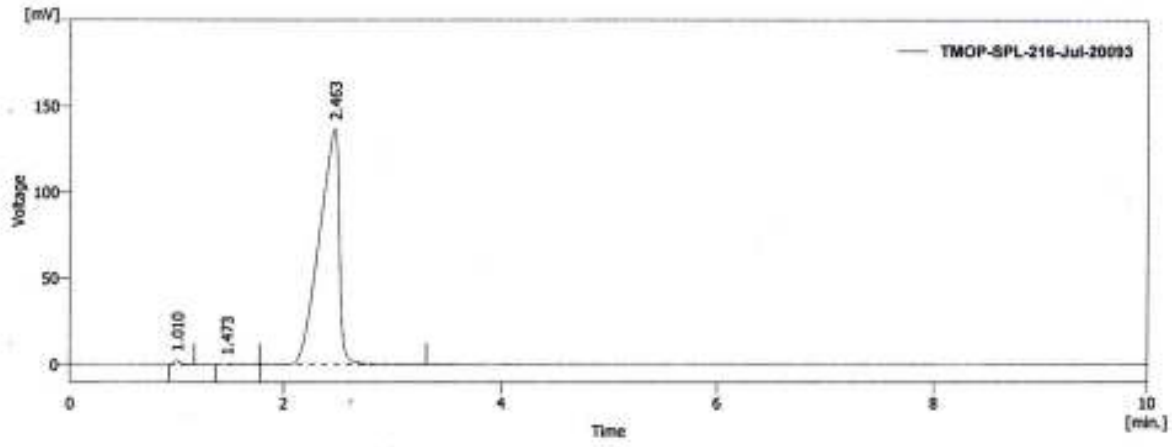
	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	1.507	7.087	1.218	0.51
2	3.663	1371.937	88.250	99.49
	Total	1379.024	89.468	100.00

ANALYTICAL METHOD VALIDATION OF GAS CHROMATOGRAPHIC METHOD FOR THE ESTIMATION OF RELATED SUBSTANCES IN TRIMETHYL ORTHO PROPIONATE .

Apparatus:	**Carbowax Chemito GC 1000**
Sample ID:	ROBUSTNESS STUDY
Sample:	SPL-1(VSCL/TMOP/2009/L-01)
Blank :	Methanol.
Autostop:	10.00 Min.
Column:	(2m.'L, 1/8"ID, : 80/100µm)
Carrier Gas:	Nitrogen .@constant Pressure 1.2 bar (30ml/min.)
Flow Rate:	30ml/min.
Oven Temperature:	**80ºC**(2min.)5C/min 150ºC.
Injector Temperature:	210ºC
Detector Temperature:	240ºC.
Injection Volume:	0 .5µL
Detector :	FID

Fig.7 : Chromatogram For Robustness Study (SPL-2)

ANALYTICAL METHOD VALIDATION OF GAS CHROMATOGRAPHIC METHOD FOR THE ESTIMATION OF RELATED SUBSTANCES IN TRIMETHYL ORTHO PROPIONATE .



Result Table (Uncal - TMOP-SPL-216-Jul-20093)

	Reten. Time [min]	Area [mV s]	Height [mV]	Area [%]
1	1.010	9.403	2.431	0.54
2	1.473	3.685	0.335	0.21
3	2.463	1734.815	136.882	99.25
	Total	1747.903	139.648	100.00

TABLE-5 : FOR ROBUSTNESS STUDY

PARAMETERS	% AREA MODIFIED CONDITION	% AREA STANDARD CONDITION	% DIFFERENCE
FLOW RATE 25 ml/min.	99.49	99.02	0.47
FLOW RATE 30 ml/min.	99.25	99.02	0.23
OVEN TEMPERATURE 75ºC	99.40	99.02	0.38
OVEN TEMPERATURE 80ºC	99.25	99.02	0.23

ANALYTICAL METHOD VALIDATION OF GAS CHROMATOGRAPHIC METHOD FOR THE ESTIMATION OF RELATED SUBSTANCES IN TRIMETHYL ORTHO PROPIONATE .

Remark: Meets the acceptance criteria.

4.LIMIT OF DETECTION:

DEFINATION:

Limit of Detection is the lowest concentration of Analyte that is Detected by given method. It can be determine by practically by lowering the injection volume .It will be established from linearity study.

Procedure:

1) Inject the Methanol as blank .
2) Inject 0.2µL, 0.3µL,0.4µL, 0.5 µL,0.6µL sample.

Acceptance Criteria : Relative standard deviation should not be more than 30.0%.

Experimental Conditions:

Apparatus:	**Carbowax** Chemito GC 1000
Sample ID:	(VSCL/TMOP/2009/L-01)
Sample :	Inj- 1 (VSCL/TMOP/2009/L-01)
Autostop:	10.00 Min.
Column:	(2m.'L, 1/8"ID, : 80/100µm)
Carrier Gas:	Nitrogen .@constant Pressure 1.2 bar (30ml/min.)
Oven Temperature:	80ºC(2min.)5C/min 150ºC.
Injector Temperature:	210ºC
Detector Temperature:	240ºC.
Injection Volume:	0 .2µL
Detector :	FID

Fig.8: Chromatogram For LOD Study.

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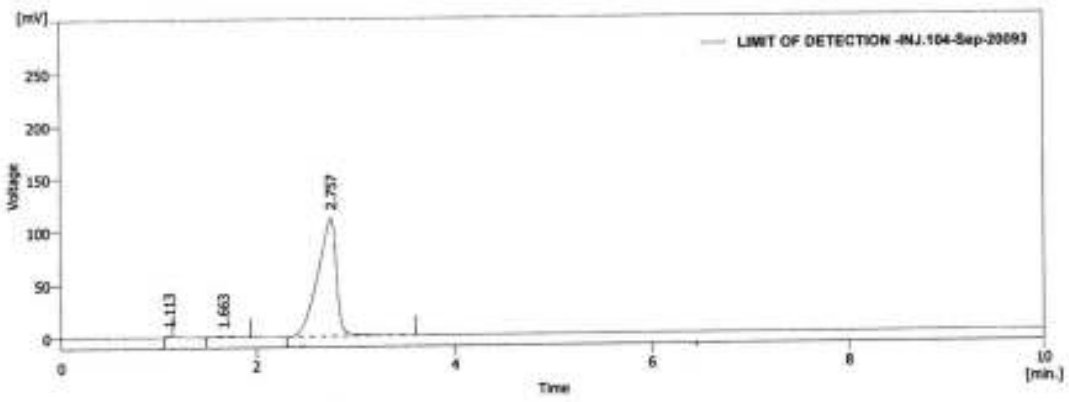


Result Table (Uncal - LIMIT OF DETECTION -INJ.1(4-Sep-2009))

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	1.113	2.586	0.900	0.18
2	1.663	8.976	0.841	0.62
3	2.757	1442.212	111.640	99.20
	Total	1453.774	113.381	100.00

TABLE-6 : FOR LIMIT OF DETECTION STUDY

Sr. No.	Sample Injected	Area	% Assay
1.	Blank	-	-
2.	0.2µL	100.00	99.15
3.	0.3µL	100.00	99.20
4.	0.4µL	100.00	99.30
5.	0.5µL	100.00	99.52
6.	0.6µL	100.00	99.56

LIMIT OF DETECTION

Sr. No.	Sample Injected	Area	% Assay
1	Blank	-	-
2	0.2µL	100	99.15

ANALYTICAL METHOD VALIDATION OF GAS CHROMATOGRAPHIC METHOD FOR THE ESTIMATION OF RELATED SUBSTANCES IN TRIMETHYL ORTHO PROPIONATE .

3	0.3µL	100	99.2
4	0.4µL	100	99.3
5	0.5µL	100	99.52
6	0.6µL	100	99.56

AVERAGE;	99.346
S.D.	0.185688
R.S.D.	0.186833

Remarks:
Limit of Detection was found to be at 0.5µL; Hence meets the acceptance criteria.

5.ACCURACY STUDY:

DEFINATION:
Accuracy should be established across the specified range of the analytical procedure . Accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted as a conventional true value or an accepted reference value and the value found .

Procedure:
1) Inject the Methanol as Blank.
2) Inject 0.5µL of two standards and two samples .
3) Then , determine the closeness of agreement between true value and the value found .

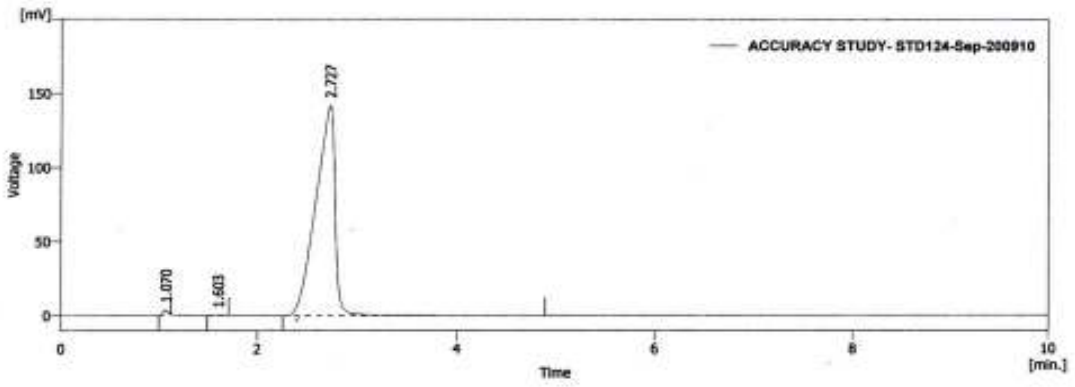
Acceptance Criteria :
1.Closeness of agreement between the experimental result and true or reference value, RSD should be ≤ 2.0.

Apparatus:	**Carbowax Chemito GC 1000**
Sample ID:	ACCURACY STUDY
Sample:	STD-1(FEM/TMOP/2009/L-11)
Blank :	Methanol.
Autostop:	10.00 Min.
Column:	(2m.'L, 1/8"ID, : 80/100µm)
Carrier Gas:	Nitrogen .@constant Pressure 1.2 bar

ANALYTICAL METHOD VALIDATION OF GAS CHROMATOGRAPHIC METHOD FOR THE ESTIMATION OF RELATED SUBSTANCES IN TRIMETHYL ORTHO PROPIONATE .

	(30ml/min.)
Oven Temperature:	80ºC(2min.)5C/min 150ºC.
Injector Temperature:	210ºC
Detector Temperature:	240ºC.
Injection Volume:	1.5 µL
Detector :	FID

Fig.9 : Chromatogram ACCURACY STUDY (STD-1)



Result Table (Uncal - ACCURACY STUDY- STD124-Sep-200910)

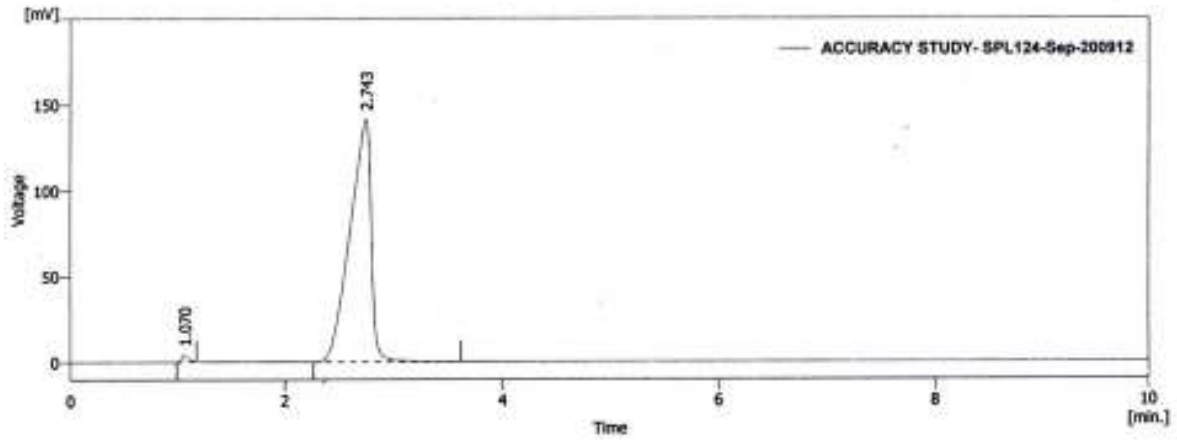
	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	1.070	11.111	3.293	0.58
2	1.603	0.903	0.157	0.05
3	2.727	1894.838	141.782	99.37
	Total	1906.851	145.232	100.00

Apparatus:	**Carbowax Chemito GC 1000**
Sample ID:	ACCURACY STUDY
Sample:	SPL-1(VSCL/TMOP/2009/L-01)
Blank :	Methanol.
Autostop:	10.00 Min.
Column:	(2m.'L, 1/8"ID, : 80/100µm)
Carrier Gas:	Nitrogen .@constant Pressure 1.2 bar (30ml/min.)
Oven Temperature:	80ºC(2min.)5C/min 150ºC.
Injector Temperature:	210ºC

ANALYTICAL METHOD VALIDATION OF GAS CHROMATOGRAPHIC METHOD FOR THE ESTIMATION OF RELATED SUBSTANCES IN TRIMETHYL ORTHO PROPIONATE .

Detector Temperature:	240ºC.
Injection Volume:	1.5 µL
Detector :	FID

Fig. 10: Chromatogram ACCURACY STUDY (SPL-1)



Result Table (Uncal - ACCURACY STUDY- SPL124-Sep-200912)

	Reten. Time [min]	Area [mV s]	Height [mV]	Area [%]
1	1.070	16.571	4.227	0.86
2	2.743	1903.626	141.327	99.14
	Total	1920.197	145.554	100.00

TABLE -7: FOR ACCURACY STUDY

Sr. No.	Sample	Retention Time	Area [%]
1.	STD-1	2.727	99.37
2.	STD-2	2.740	99.36
3.	SPL-1	2.743	99.14
4.	SPL-2	2.763	99.13

ANALYTICAL METHOD VALIDATION OF GAS CHROMATOGRAPHIC METHOD FOR THE ESTIMATION OF RELATED SUBSTANCES IN TRIMETHYL ORTHO PROPIONATE .

ACCURACY STUDY

Sr. No.	Sample	Retention Time	Area [%]
1	STD-1	2.727	99.37
2	STD-2	2.74	99.36
3	SPL-1	2.743	99.14
4	SPL-2	2.763	99.13
		AVG.	99.25
		S.D.	0.132916
		R.S.D	0.133904

Remark:

Meets the acceptance criteria; RSD was within the limit.

4.0 ACCEPTANCE CRITERIA

All the analytical performance parameters tested during validation of analytical method for determination of Trimethyl Ortho Propionate , shall be within following acceptance limit.

Sr. No.	ANALYTICAL PERFORMANCE PARAMETER	ACCEPTANCE LIMIT
3	Linearity Study Correlation Coefficient	Graph should be Linear $r = \geq 0.995$.
2	Precision Study	% RSD :NMT 2

ANALYTICAL METHOD VALIDATION OF GAS CHROMATOGRAPHIC METHOD FOR THE ESTIMATION OF RELATED SUBSTANCES IN TRIMETHYL ORTHO PROPIONATE .

4	Robustness Study	NMT 1%
2	Limit of Detection	Lowest possible concentration
	Accuracy Study	RSD ≤ 2.0

RESULTS AND DISCUSSION:

Results of the analytical method validation shall be tabulated into the Summary Report.

TABLE-8 : FOR SUMMARY REPORT

PARAMETER	ACCEPTANCE CRITERIA	RESULTS
LINEARITY CORRELATION COEFFICIENT	Correlation coefficient was o.9896.	Graph should be Linear r= ≥ 0.995
PRECISION METHOD PRECISION	The RSD of 6 replicates was within the limit; 0.1089	% RSD : NMT 2.0.
ROBUSTNESS	Meets the acceptance criteria.	NMT 1.0% of each other.
LIMIT OF DETECTION	Limit of Detection was found to be at 0.5µL.	Lowest possible concentration.
ACCURACY	Meets the acceptance criteria.	RSD : NMT ≤ 2.0.

REMARKS :

1. The method does not show interference with the peaks .
2. The claimed method is accurate and precise.
3. The tested validation parameters are within specified limits, thus the method is validated.

CONCLUSION :

The claimed method can be used for routine and stability analysis of Tri Methyl OrthoPropionate by GC.

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