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Validation for Residual Solvents in Bisoprolol Fumarate by Gas Chromatographic Technique

Sandip Telavane[1], Seema Kothari[1] and Manohar V. Lokhande[2]*

1. Department of Chemistry, PAHER University, Udaipur-313003, Rajasthan, **INDIA**
2. Department of Chemistry, Sathaye College, Mumbai-400057, Maharashtra, **INDIA**

Email: manohar2210@gmail.com

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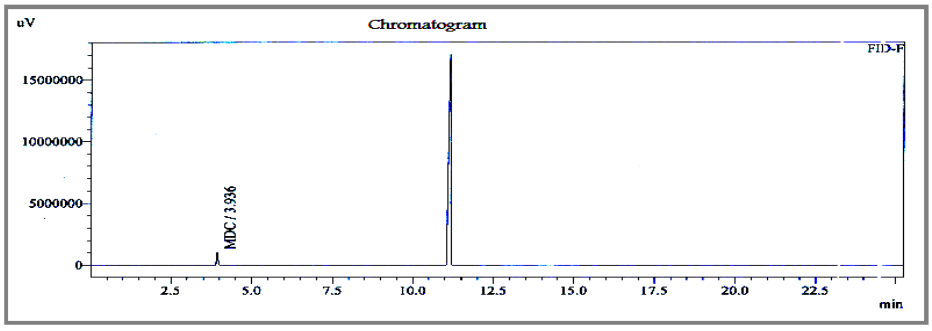
ABSTRACT

Validation is important technique for detection, progress and estimation of drugs for pharmaceutical analysis. Aim of this article was to check the progress and validation of the method employed for the Residual Solvents in Bisoprolol Fumarate by Gas Chromatographic technique. The objective of this protocol is to validate a GC method of analysis for detection and Quantification of Residual Solvents Methanol, Acetone and Methylene dichloride in Bisoprolol Fumarate. In the pharmaceutical industry, validation policy is more important for documented of validation, types of validation and validation policy. The method was developed accurately and validation parameters are explained. Chromatographic condition was GC- 2014, gas chromatograph equipped with FID detector, column: 30 m x 0.32 mm ID x 1.8 µm DB - 624 capillary column or equivalent and column temperature was 45°C (hold 7 minutes) to 250°C @ 40°C/minutes, hold at 250°C for 3 minutes. The parameters such as Accuracy, Specificity, Precision, Linearity and Range, Limit of detection (LOD), Limit of quantitation (LOQ), ruggedness, robustness and system suitability testing with residual solvent such as Methanol, Acetone and methylene dichloride. All validation parameters are used in the routine and stability analysis.

Graphical Abstract



GC of Methylene Dichloride.

Keywords: GC, Validation, Bisoprolol Fumarate, LOD, LOQ and Linearity.

INTRODUCTION

Bisoprolol is used for the treatment of high blood pressure (hypertension). The lower or high blood pressure generates strokes, heart attacks and kidney problems. This drug is recognized for beta blockers [1]. This drug work as blocking agent for positive natural chemicals in the human body such as epinephrine on the heart and blood vessels [2]. The result of lower heart rate, blood pressure and stress on the heart. Bisoprolol is oral supplement is available as a generic medicine [3]. This drug doesn't have its brand-name. it is used for treatment of high blood pressure for those persons. Drug is used in single dose form or in combination with another blood pressure drugs [4, 5].

The various types of pharmaceutical companies have manufacturing same type of pharmaceutical drugs using different types of organic solvents. Thus, the study of residual solvents becomes an interesting investigative mission in pharmaceutical analysis and its control. Unidentified residual solvents are normally noticed through routine quality control analysis. During the analysis an error has occurs while using the routing methods were applicable for analysis. thus, we want to develop such a technique which are a rapid, sensitive technique which categorize and quantitate for all residual solvents in pharmaceuticals [6]. Residual solvents are naturally determined using GC techniques If the solvents are expected to be present, this may be known and measured. If solvents are different it may be present at greater than their limits, hence it may be known and measured. The pharmaceutical drugs must to be available in such a category that quality as well as bioavailability, adequate plasma concentration, desired period, the onset of action, correct dose, safety, effectiveness and stability on storage of product will be assured throughout the storage of the products [7, 8]

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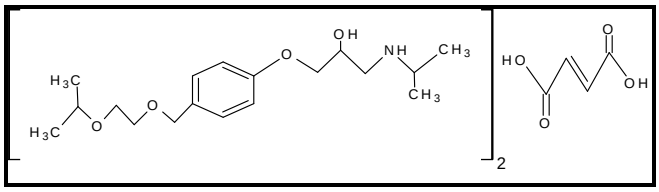
In this paper, we are presenting method of Validation and Determination for Residual Solvents in Bisoprolol Fumarate by Gas Chromatographic Technique with parameters like as Accuracy, Specificity, Precision, Linearity and Range, Limit of detection (LOD), Limit of quantitation (LOQ), ruggedness, robustness and system suitability.

MATERIALS AND METHODS

Following equipment's was used for the validation studies.

Instrumental Name	Instrumental Number	Make	Model Number
Analytical Balance	SLL/QC/50	Mettler	B247544075
GC - 01	SLL/QC/53	Shimadzu	GC-2014
GC- 02	SLL/QC/61	Shimadzu	GC-2010 plus



Chemical name: 2-Propanol, 1-[4-[[2-(1-methyletoxy)ethoxy] methyl] phenoxy]-3-[(1-methyl ethyl) amino]-,(E)-2-butenedioate), Molecular formula is $C_{40}H_{66}N_2O_{12,}$ Molecular weight is: 766.96, Colour is White or almost white, slightly hygroscopic powder, Solubility is Very soluble in water, freely soluble in methanol.

Regent Name	Batch Number	Purity	Make
Methanol	SI4P640642	99.8	Merck
Acetone	SH4SF64460	99.5	Merck
Methylene Dichloride	IG3IF6332	99.5	Merck
Dimethyl Sulfoxide	R133D14	99.0	Rankem
Bisoprolol Fumarate sample	SLL/BF/0315001	99.94	SLL

Residual solvent method of Bisoprolol Fumarate API

Limits for Residual Solvents: Methanol: NMT 3000 ppm, Acetone: NMT 5000 ppm, Methylene Dichloride: NMT 600 ppm

Reagents: Methanol (AR Grade), Acetone (AR Grade), Methylene Dichloride (AR Grade), Dimethyl Sulfoxide (DMSO) (AR Grade), Bisoprolol Fumarate working standard. Bisoprolol Fumarate sample was available from Supriya Life science, Mumbai.

Chromatographic condition:

Instrument	:	GC 2014, gas chromatograph equipped with FID detector.
Column	:	30 m x 0.32 mm ID x 1.8 µm DB - 624 capillary column or equivalent.
Column Temp.	:	45°C (hold 7min) to 250°C@ 40°C min^{-1}, hold at 250°C for 3 min.
Injector/detector	:	250°C/ 260°C
Carrier gas	:	Nitrogen @ 35cm sec^{-1} linear velocity
Split Ratio	:	10:1

Head Space Parameters: Incubation Temperature is 95°C, Incubation Time is 15 min, Syringe Temperature is 115°C, Injection Volume is 1 mL.

Preparation of standard stock solution: Take 760 µL of Methanol, 90 µL of Methylene chloride and 1270 µL of Acetone into 100 mL of volumetric flask, containing 80 mL of Dimethyl sulfoxide (DMSO) and mix. Dilute up to the mark with DMSO and mix.

Preparation of System suitability solution: Transfer accurately 5.0 mL of stock solution into a 100.0 mL volumetric flask and dilute up to the mark with Dimethyl Sulfoxide (DMSO).

Preparation of Test Solution: Weigh accurately about 500 mg of sample into a 20 mL headspace vial and add 5.0 mL Dimethyl Sulfoxide (DMSO).

Procedure: Inject blank solution and sample preparation (in duplicate) records the chromatogram for all injection.

Calculation:

$$\textbf{PPM} = \frac{\text{Area of Sample} \times \mu\text{L in standard} \times 5 \times 5 \times \text{Purity of STD.} \times \text{Density}}{\text{Area of Standard} \times 100\ \text{mL} \times 100 \times \text{Wt. of Sample, mg} \times 100 \times \text{STD}} \times 1000000$$

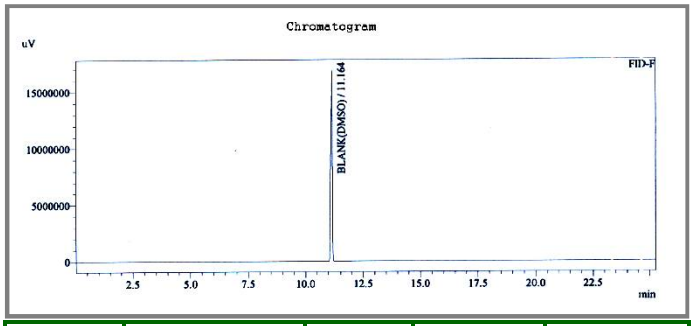
RESULTS AND DISCUSSION

Specificity: The suspension media showed no peaks beyond the void volume, while only one peak was observed for the drug substance samples [7]. However, Co-eluting peaks were observed in the chromatograms of the drug products, likely from the excipients present in these drug products. Due to lack of convenience of the unlike excipients current in these formulations, the excipients eluting at these peak locations could not be investigated (Figure 4). To validate the specificity of the technique, the solvent, the solutions of standards and excipients were injected into the column. The chromatograms show that, peaks of solvent and excipients do not interfere by peaks of dynamic substances listed in table 1.

Identification: Compare the retention times obtained for Methanol, Acetone and Methylene dichloride peaks. Also inject Diluents (Blank). The data will be processed for Methanol, Acetone and Methylene dichloride peaks. Check for the interference from Diluents Dimethyl Sulfoxide (DMSO) at the retention time of main peak.

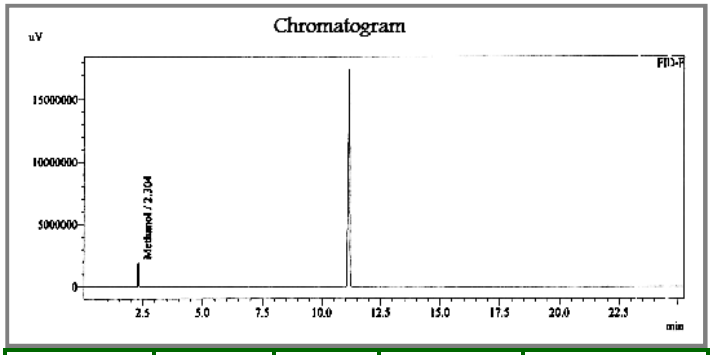
Table 1. Specificity

S. No.	Name of sample	Retention Time of sample
1	Methanol	2.304
2	Acetone	3.408
3	Methylene Dichloride	3.936
4	DMSO	11.164



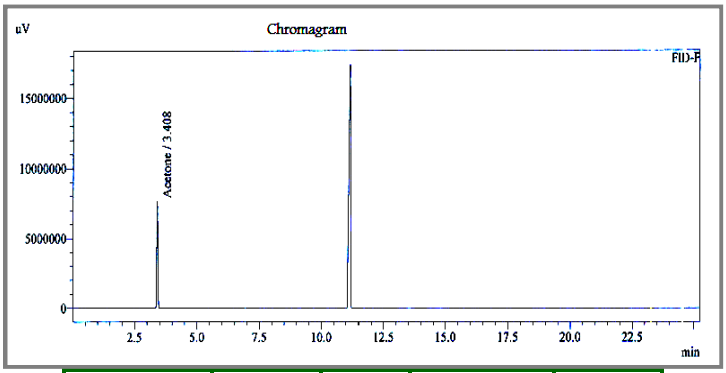
Peak#	Name	RT	Area	Area%
1	Blank (DMSO)	11.164	77862466	100.00
Total			77862466	100.00

Figure 1. GC of Blank DMSO.



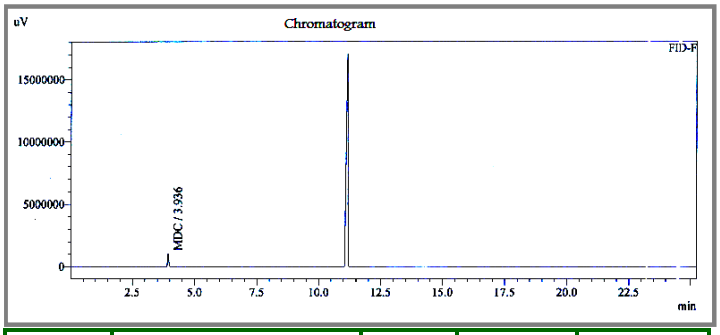
Peak#	Name	RT	Area	Area%
1	Methanol	2.304	5218289	100.00
Total			5218289	100.00

Figure 2. GC of Methanol.



Peak#	Name	RT	Area	Area%
1	Acetone	3.408	23773773	100.00
Total			23773773	100.00

Figure 3. GC of Acetone.



Peak#	Name	RT	Area	Area%
1	Methylene Dichloride	3.936	3496795	100.00
Total			3496795	100.00

Figure 4. GC of Methylene Dichloride.

Limit of Detection and Limit of Quantification: Limit of detection and Limit of quantitation parameters are connected by separate meanings. The determination of a define the lowest concentration of analyte, which may be identified by no agreement nearby the prejudice or fuzziness of the effect with an analyze, the concentrations are different and definite with bias and correctness goals is possible and lastly the concentration at which the analyte can be quantitated with a linear answer [**9**]. Assessment of governing authorities such as USP [**10**] and ICH [**11**] for LOD and LOQ. There are numerous terms that have been used to describe LOD and LOQ. In overall, the LOD is occupied as the lowermost concentration of an analyte in a sample, which may detect, but not essentially quantified, below the stated conditions of the trial. The LOQ is the lowermost concentration of an analyte in an example that may determine by suitable accuracy and precision under the stated situations of test [**12-14**]. While reagent package supplements may state that, an assay has an active choice of ranges from zero concentration to some higher edge, naturally a test was just not accomplished of accurately determining analyte concentrations down to zero. Enough analyte concentration necessity be current to produce an analytical sign have consistently be distinguished from analytical noise, the motion produced in the presence of analyte [**15, 16**].

Preparation of Stock Solution for LOD: Take 25 µL of methanol, 2 5µL of Acetone and 15µL of Methylene dichloride into 100 mL of volumetric flask, containing 80 mL of Dimethyl Sulfoxide and mix. Dilute again 5.0 mL of stock solution into 100 mL volumetric flask and makeup with Dimethyl Sulfoxide (100 ppm). Take below amount of predicated LOD stock solution in 20 mL Headspace Vial (figure 5 and 6, tables 2 to 5). From the above table detection limit Methanol is 0.9 ppm, Acetone is 0.9 ppm and Methylene dichloride is 1 ppm.

Table 2. Data Sheet for Limit of Detection

Concentration in ppm	Response Area		
	Methanol	Acetone	Methylene Dichloride
0.1	Not detected	Not detected	Not detected
0.2	Not detected	Not detected	Not detected
0.3	Not detected	Not detected	Not detected
0.4	Not detected	Not detected	Not detected
0.5	Not detected	Not detected	Not detected
0.6	Not detected	Not detected	Not detected
0.7	Not detected	Not detected	Not detected
0.8	Not detected	Not detected	Not detected
0.9	2914	2151	Not detected
1.0	3312	2517	68

The definition of linearity of sign can in the framework of LC–MS, have two faithfully related senses: (a) linear association among analyte signals and analyte concentrations in standardization of samples and (b) linear association among analyte signals and analyte concentrations in samples

Table 3. Dilution for LOD solution

Sample Name	Amount of Prediction LOD stock solution (mL)	Volume made up with Dimethyl Sulfoxide (mL)	Concentration in PPM
LOD solution-1	0.1	100	0.1
LOD solution-2	0.2	100	0.2
LOD solution-3	0.3	100	0.3
LOD solution-4	0.4	100	0.4
LOD solution-5	0.5	100	0.5
LOD solution-6	0.6	100	0.6
LOD solution-7	0.7	100	0.7
LOD solution-8	0.8	100	0.8
LOD solution-9	0.9	100	0.9
LOD solution-10	1.0	100	1.0
LOD solution-11	2.0	100	2.0
LOD solution-12	4.0	100	4.0
LOD solution-13	5.0	100	5.0

Table 4. Data Sheet for LOQ- System Suitability

Sample Name	Response Area		
	Methanol	Acetone	Methylene Dichloride
LOQ std-1	10159	5879	170
LOQ std-2	9603	5790	184
LOQ std-3	10517	5995	191
LOQ std-4	9966	5852	191
LOQ std-5	9500	5631	187
LOQ std-6	9698	5824	181
Average	**9907**	**5829**	**184**
SD	**385**	**119**	**8**
% RSD	**3.89**	**2.05**	**4.25**

Table 5. Data Sheet for List of Detection –System Suitability

Sample Name	Response Area		
	Methanol	Acetone	Methylene Dichloride
LOD std-1	2918	2530	56
LOD std-2	3087	2531	64
LOD std-3	3339	2659	57
LOD std-4	3112	2549	69
LOD std-5	2921	2638	52
LOD std-6	2578	2605	50
Average	**2993**	**2585**	**58**
SD	**255**	**56**	**7**
% RSD	**8.53**	**2.18**	**12.43**

comprising medium mechanisms. The final sense is attractive progressively further used and is used also in this development. The aim is that, if the analyte signal in samples is linear, then nearly positively it is linear also in calibration solutions, though the reverse is not essentially correct. The most mutual cause for this is the marvel of matrix result. Linearity of the calibration graph is closely related to choosing calibration model and working range [**17, 18**].

Experiment: Prepare six different concentrations of the Methanol, Acetone and Methylene dichloride concentration values LOQ level, 50%, 80 %, 100 % 120 % and 150 % of the working levels. Prepared concentration at each level should be analyzed in duplicate, from the responses obtained for each conc. Level, (y- value) should be plotted against concentration (x-value) using a least squares of test results versus analyte conc. From regression data, calculate the following parameters listed in table 6.

Preparation of Linearity Stock Solution: Take 760 µL of Methanol, 90 µL of Methylene chloride and 1270 µL of Acetone into 100 mL of volumetric flask, containing 80 mL of Dimethyl sulfoxide (DMSO) and mix. Dilute up to the mark with DMSO and mix. A least square fit graph of the individual area counts against the concentration of Methanol, Acetone and Methylene dichloride will be plotted and the correlation coefficient, slope and intercept reported in table 7. The range of the analytical method in concentration (µg per mL) will be reported. Correlation coefficient: Not less than 0.98.

Table 6. Dilutions for Linearity

Linearity solution	Standard stock Solution added mL	Vol. in mL with internal standard
LOQ	Prepared as per LOQ std.	
50 %	2.5	100.0l
80 %	4.0	100.0
100 %	5.0	100.0
120 %	6.0	100.0
150 %	7.5	100.0

Table 7. Table for Linearity

Sample name	Response Area		
	Methanol	Acetone	Methylene Dichloride
LOQ std. solution	11012	5682	186
Lin 50%	169616	1938958	34750
Lin 80%	277241	3163251	56763
Lin 100%	359224	4042540	72735
Lin 120 %	449926	4951978	88349
Lin 150 %	533739	6038360	109704
Correlation coefficient	**0.996**	**0.999**	**0.999**

Correlation coefficient should not be less than 0.98. Correlation coefficient meets acceptance criteria. Therefore, the GC method for the determination of Methanol, Acetone and methylene dichloride is linear.

Precision: The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple samplings of a homogeneous sample. The precision of an analytical method is usually expressed as the standard deviation or relative standard deviation (coefficient of variation) of a series of measurements [**19, 20**].

System Precision: Six replicate injections of the Standard preparation will be made into the GCHS using the method as described. The standard deviation and relative standard deviation (%RSD) of the six replicate injections will be calculated and reported. % RSD for NMT 15% listed in table 8. RSD should not be more than 15.0%. The RSD of system precision Methanol is 2.42%, Acetone is 1.87 and methylene chloride is 2.26 respectively and it meets acceptance criteria. Therefore, the GC method for the determination of Methanol, Acetone and methylene chloride in Bisoprolol fumarate API is precise (figure 7).

Linearity and Range:

Method Precision: Six sample preparations of Bisoprolol Fumarate API are to be prepared and injected into the GCHS using the method as described. PPM levels of Methanol, Acetone and Methylene dichloride will be calculated and reported along with Standard deviation and Relative standard deviation of the six samples. % RSD for NMT 15%. RSD should not be more than 15.0%.

Therefore, the GC method for the determination of Residual Solvents of Bisoprolol fumarate API is reproducible.

Table 8. Table for System Precision

Sample Injection	Response Area		
	Methanol	Acetone	Methylene Dichloride
1	358557	4029994	76157
2	358237	4057385	76655
3	352840	4019452	76000
4	359542	4128576	78215
5	353340	4088878	77106
6	376712	4224290	80649
Average	**359871.3**	**4091429.2**	**77463.7**
SD	**8720.2**	**76357.6**	**1751.7**
%RSD	**2.42**	**1.87**	**2.26**

Accuracy (Recovery): The accuracy of an analytical method is the closeness of test results obtained by that method to the true value. The accuracy of an analytical method should be established across its range [21-23].

Experiment: Bisoprolol Fumarate API, will be spiked with Methanol, Acetone and Methylene dichloride at three different levels 80%, 100% and 120% of specification limit of solvents in triplicate (in total nine determinations). It will be prepared according to the Sample Preparation mentioned below.

Preparation of Solvent Recovery Stock Solution: Take 760 µL of Methanol, 90 µL of Methylene chloride and 1270 µL of Acetone into 100 mL of volumetric flask, containing 80 mL of Dimethyl sulfoxide and mixture. Dilute up to the mark with DMSO and mixture listed in table 9.

Table 9. Preparation of 80, 100 and 120 % standard Solution

Standard Solution	Standard Stock solution added mL	Vol. in ml with Dimethyl Sulfoxide
80 %	4.0	100.0
100 %	5.0	100.0
120 %	6.0	100.0

Preparation of Sample without spiking (Control sample): Take accurately about 500 mg of Bisoprolol Fumarate in 20 mL Headspace Vial and add 5 mL of Dimethyl sulfoxide.

Preparation of Sample with spiking: Weigh 500 mg of Bisoprolol Fumarate in 20 mL Headspace vial and add 5 mL of 80 % std. solution. Inject 1.0 mL using Head space instruments. Calculate the Residual Solvents of Methanol, Acetone and Methylene dichloride. Apply correction if required. (Prepared in triplicate). Same procedure for 100% and 120%. Data level of each replicate will be calculated as a) Amount added in mg, b) % Recovery = Amount recovered/Amount added x 100. The Mean, Standard deviation and RSD % will be computed for the twelve determinations and reported along with the above (a) and (b) listed in table 10 to 12. The Mean recovery should be in the range of 90.0% to 110.0% for 80%, 100% and 120% levels. The Mean Recovery for all Residual Solvents is within limits. Therefore, the GC method for the determination of Residual solvents in Bisoprolol fumarate is accurate.

Ruggedness: The ruggedness of an analytical method is the degree of reproducibility of test results obtained by the analysis of the same samples under a variety of conditions, such as different laboratories, different analysts, different instruments, different lots of reagents, different elapsed assay times, different assay temperature, and in different days.

Table 10. Recovery of Methanol

Sample	Amount added (mg)	Amount recovered (mg)	% Recovery
Acc. 80% -1	2400	2338	97.40
Acc. 80% -2	2400	2329	97.00
Acc. 80% -3	2400	2293	95.60
Acc. 100% -1	3000	3051	101.70
Acc. 100% -2	3000	3070	102.30
Acc. 100% -3	3000	3003	100.10
Acc. 120% -1	3600	3686	102.40
Acc. 120% -2	3600	3782	105.10
Acc. 120% -3	3600	3757	104.40
		Mean	**100.70**
		SD	**3.362**
		% RSD	**3.34**

Table 11. Recovery of Acetone

Sample	Amount added (mg)	Amount recovered (mg)	% Recovery
Acc. 80% -1	3989	3892	97.60
Acc. 80% -2	3989	3892	97.60
Acc. 80% -3	3989	3851	96.50
Acc. 100% -1	4986	5003	100.30
Acc. 100% -2	4986	5072	101.70
Acc. 100% -3	4986	4951	99.30
Acc. 120% -1	5983	6018	100.60
Acc. 120% -2	5983	6190	103.50
Acc. 120% -3	5983	6161	103.00
		Mean	**100.70**
		SD	**2.471**
		% RSD	**2.47**

Table 12. Recovery of Methylene dichloride

Sample	Amount added (mg)	Amount recovered (mg)	% Recovery
Acc. 80% -1	475	459	96.70
Acc. 80% -2	475	457	96.40
Acc. 80% -3	475	452	95.10
Acc. 100% -1	593	592	99.70
Acc. 100% -2	593	601	101.30
Acc. 100% -3	593	577	97.30
Acc. 120% -1	712	708	99.50
Acc. 120% -2	712	732	102.80
Acc. 120% -3	712	726	102.00
		Mean	**99.0**
		SD	**2.730**
		% RSD	**2.76**

Experiment: Three sample preparations of the same lot (as used in 4.2) of Bisoprolol Fumarate API is made and spiked with Methanol, Acetone and Methylene dichloride at 100% levels by a different analyst, using different column on a different day and injected into a different GCHS using the method as described, along with Standard preparation. Recovery of Methanol, Acetone and Methylene dichloride will be calculated and data will be reported for Standard deviation and RSD of spiked samples. Mean recovery should be in the range of 90.0% to 110.0% for 100% levels of spiked residual solvent and overall RSD for results should not be more than 15.0%. The Mean Recovery for all Residual Solvents is within limits and the overall RSD of ruggedness is 1.63 % for Methanol, 1.34% for Acetone and 1.78% Methylene dichloride listed in table 14-16. Therefore, the GC method for the determination of Residual solvents in Bisoprolol Fumarate API is rugged.

Table 13. Ruggedness study of Methanol

Sample	Amount added (mg)	Amount recovered (mg)	% Recovery
Recovery 100%-1	3000	3051	101.70
Recovery 100% -2	3000	3070	102.30
Recovery 100% - 3	3000	3003	100.10
Recovery 100%-1	3000	3096	103.20
Recovery 100% -2	3000	2979	99.30
Recovery 100% - 3	3000	3102	103.40
		Mean	**101.7**
		SD	**1.70**
		%RSD	**1.63**

Table14. Ruggedness study of Acetone

Sample	Amount added (mg)	Amount recovered (mg)	% Recovery
Recovery 100%-1	4986	5003	100.30
Recovery100% -2	4986	5072	101.70
Recovery100%- 3	4986	4951	99.30
Recovery 100%-1	4986	4943	99.10
Recovery100% -2	4986	4877	97.80
Recovery100%- 3	4986	5007	100.40
		Mean	**99.8**
		SD	**1.30**
		%RSD	**1.34**

Table 15. Ruggedness study of Methylene dichloride

Sample	Amount added (mg)	Amount recovered (mg)	% Recovery
Recovery 100%-1	593	592	99.70
Recovery100% -2	593	601	101.30
Recovery100%- 3	593	577	97.30
Recovery 100%-1	593	586	98.90
Recovery100% -2	593	573	96.60
Recovery100%- 3	593	593	100.00
		Mean	**99.0**
		SD	**1.80**
		% RSD	**1.78**

Robustness: The robustness of analytical method is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. A good practice is to vary important parameters in method systematically and measure their effect on separation [**24, 25**].

Experiment: Two sample preparations of the same batch of Bisoprolol Fumarate API is made by a different analyst on a different day and injected into a different Oven temperature GCHS using the method along with Standard preparation.
Condition -1: Normal Method oven temperature.
Condition -2: +10% change in oven temperature w.r.t current oven temperature.
Condition -3: +2% change in carrier gas flow w.r.t current carrier gas flow.
Methanol, Acetone and Methylene dichloride will be calculated and reported along with Standard deviation and Relative standard deviation. % RSD should not be more than 15 %. The cumulative RSD for results should not be more than 15.0%. Robustness of the methods done on same instruments with change in carrier gas flow and Oven temperature with these change methods doesn't show and difference. Therefore, the GC method for the determination of Residual solvents in Bisoprolol Fumarate is robust.

System Suitability: System suitability testing is an integral part of an analytical procedure. The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be evaluated as such. System suitability test parameters to be established for a particular procedure depend upon on the type of procedure being validated [**26, 27**].

Preparation of standard Stock Solution: Take 760 µL of Methanol, 90 µL of Methylene chloride and 1270 µL of Acetone into 100 mL of volumetric flask, containing 80 mL of Dimethyl sulfoxide (DMSO) and mix. Dilute up to the mark with DMSO and mix.

Preparation of standard Solution: Take 5 mL of stock solution in a 100 mL volumetric flask and dilute up to the mark with Dimethyl Sulfoxide (DMSO). Inject standard solution six times. Calculate the area Precision. Calculate % RSD from replicate injections of std. % RSD for peak area NMT 15% for each peak of interest.

Tailing Factor-NMT 2.0 and Resolution-NLT 2.0 listed in table 16. The relative standard deviation should not be more than 15%, and tailing factor should not more than 2.0 and resolution should not less than 2.0. System suitability complies.

Table 16. System Suitability

Standard	% RSD of Area	% RSD of RT	Tailing Factor	Resolution
Methanol	2.42	0.04	1.278	--
Acetone	1.87	0.04	1.180	14.699
Methylene Dichloride	2.26	0.03	1.054	6.405

[RT= Retention Time, RSD= Relative standard deviation]

APPLICATION

Write the application of the work

CONCLUSION

The objective of this protocol is to validate a GC method of analysis for detection and Quantification of Residual Solvents Methanol, Acetone and Methylene dichloride in Bisoprolol Fumarate in table 17. This method is applicable for Quantifying and monitoring the traces of Residual Solvents Methanol listed in table 18, Acetone listed in table 19 and Methylene dichloride listed in table 20. Simultaneously on routine basis using Gas chromatograph in Bisoprolol Fumarate. This validation study is intended to show that the method is suitable for release of Bisoprolol Fumarate for Residual Solvents of manufacturing batches. Following parameters to be validated for Bisoprolol Fumarate to prove the test method is capable to yield consistent and reliable results within the pre-determined acceptance limits. The Residual solvent test method is validated for Specificity, Limit of Detection, Limit of Quantification, Linearity and Range, Precision, Accuracy, Ruggedness and Robustness and found to be meeting the predetermined acceptance criteria. The validated method is Specific, LOD, LOQ, Linear, Precise, Accurate, Rugged and Robust for Residual solvents of Bisoprolol Fumarate.

Table 17. Acceptance Criteria

Validation Parameter	Acceptance Criteria
Specificity Identification	Results should be comparable with respect to Retention time.
LOD/LOQ	
Limit of Detection	Experimental observed PPM
Limit of Quantification	Experimental observed PPM
Linearity and Range	Correlation coefficient should not be less than 0.98
PRECISION	
System Precision	RSD should not be more than 15.0%.
Method Precision	RSD should not be more than 15.0%.
Accuracy (Recovery)	Mean of recovery should be in the range of 90.0 to 110.0 % for 80 to 120 % levels.
Ruggedness	RSD should not be more than 15.0 %
Robustness	RSD should not be more than 15.0 %
System Suitability	RSD should not be more than 15.0%. Tailing Factor – NMT 2.0 Resolution – NLT 2.0

Table 18. Summary Report of Methanol

Validation Parameter	Acceptance Criteria		Results
Specificity	No interference		Meets Acceptance Criteria
	% of RSD Area – NMT 15%		2.42
	% of RSD for RT– NMT 15%		0.04
	Tailing Factor – NMT 2		1.278
System suitability	Resolution – NLT 2		NA
LOD	Experimental observed PPM		0.9 ppm
LOQ	Experimental observed PPM		2.7 ppm
Linearity and Range	Correlation NLT 0.98		0.996
Method Precision	% RSD of Sample -NMT 15%		Below detection limit
	% Recovery –	Level	
	Between 90.0 %	80%	96.67
Accuracy / Recovery	to 110.0 %	100%	101.67
		120%	103.97
Ruggedness	Cumulative RSD NMT 15%		1.63
Robustness	Cumulative RSD NMT 15% for temperature change		Below detection limit
	Cumulative RSD NMT 15% for flow change		Below detection limit

Table 19. Summary Report of Acetone

Validation Parameter	Acceptance Criteria		Results
Specificity	No interference		Meets Acceptance Criteria
	% of RSD Area – NMT 15%		1.87
	% of RSD for RT – NMT 15%		0.04
	Tailing Factor – NMT 2		1.180
System suitability	Resolution – NLT 2		14.699
LOD	Experimental observed PPM		0.9 ppm
LOQ	Experimental observed PPM		2.7 ppm
Linearity and Range	Correlation NLT 0.98		0.999
Method Precision	% RSD of Sample -NMT 15%		Below detection limit
	% Recovery –	Level	
	Between 90.0 %	80%	97.23
Accuracy / Recovery	to 110.0 %	100%	100.43
		120%	100.0
Ruggedness	Cumulative RSD NMT 15%		1.31
Robustness	Cumulative RSD NMT 15% for temperature change		Below detection limit
	Cumulative RSD NMT 15% for flow change		Below detection limit

Table 20. Summary Report of Methylene Dichloride

Validation Parameter	Acceptance Criteria	Results
Specificity	No interference	Meets Acceptance Criteria
System suitability	% of RSD Area-NMT 15%	2.26
	% of RSD for RT-NMT 15%	0.03
	Tailing Factor-NMT 2	1.054
	Resolution-NLT 2	6.405
LOD	Experimental observed PPM	1 ppm
LOQ	Experimental observed PPM	3 ppm
Linearity and Range	Correlation NLT 0.98	0.999
Method Precision	% RSD of Sample -NMT 15%	Below detection limit
Accuracy / Recovery	% Recovery – Between 90.0 % to 110.0 % Level 80%	96.07
	Level 100%	99.43
	Level 120%	101.43
Ruggedness	Cumulative RSD NMT 15%	1.31
Robustness	Cumulative RSD NMT 15% for temperature change	Below detection limit
	Cumulative RSD NMT 15% for flow change	Below detection limit

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