Research Journal of Chemical Sciences ISSN 2231-606X
Vol. **16(1),** 52-62, February **(2026)** *Res. J. Chem. Sci.*

Assay Method Development and Validation of Biclotymolby High Performance Liquid Chromatography (HPLC)

Sunil Kumar Pandey[1*], Manisha Sheoran[1] and R.N. Sharma[2]
[1]Department of Applied Science, Dr. K.N. Modi University, Newai, Rajasthan, India
[2]Department of chemistry, S.H.S Government P.G College, Dhanapur, Chandauli, India
sunilpandey7057@gmail.com

Available online at: www.isca.in, www.isca.me
Received 28th November 2025, revised 18th January 2026, accepted 10th February 2026

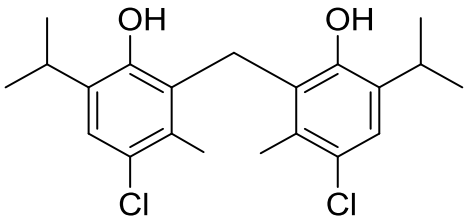
Abstract

Biclotymol is a phenolic antiseptic API molecule which generally use for the treatment of mouth and throat infection. It is antibacterial, anti-inflammatory and analgesic. Due to phenolic antiseptic it kills bacteria, Biclotymol have fast acting and long lasting molecule. The Method Validation is applicable for demonstration of method accuracy, method precision, linearity, repeatability, reproducibility, limit of detection(LOD) and limit of quantitation(LOQ) for analysis of Biclotymol for assay. High Performance Liquid Chromatography(HPLC) equipped with VWD detector and stainless steel column 25 cm×4.6 mm, C18 (5µm). From the analytical method validations report, it is concluded that the Assay of Biclotymol is (96.0%- 101.0%).

Keywords: HPLC, Analytical Method Development, Analytical Validation, pH-Meter and Balance.

Introduction

Biclotymol[1], 2,2-methanediylbis[4-chloro-3-methyl-6-(propan-2-yl) phenol], C21H26Cl2O2, is used in human therapeutics as a pulmonary antiseptic pharmaceutical industry demands. The present mapper describes analytical methods[2-5] that are easily implemented in the laboratory practice and are time/cost-effective, as well as valid for their purpose. For this reason, in our laboratory a variety of methods have been tested for determination of Biclotymol (Figure-1) and its Assay method. Biclotymol belongs to the pharmacological group of a phenolic antiseptic API molecule which is generally used for the treatment of mouth and throat infection. It is antibacterial, anti-inflammatory and analgesic.



Biclotymol

Figure-1: Molecular Structure of Biclotymol.

There are various methods used in literature for the synthesis of biclotymol[6-9] but only few analytical methods available in literature for Assay Method Development and Validation of Biclotymol, such as the optimized HPLC methods for analysis / synthesis ofBiclotymol[10-12] and its related Assay.

Methodology

Method of Analysis: Before taking analytical validation, HPLC qualification was performed, calibrated and test- calibration and standardization performed and found system is suitable for validation. The Installation Qualification (IQ), Operational Qualification (OQ), and Performance Qualification (PQ) are performed.

Optimization: An Additional parameter optimized during validation of Assay method like accuracy and precision are part of the method development and optimization process, these parameters include limits of detection and quantification, linearity of the method, range, recovery, robustness, and selectivity.

Chromatographic Condition: Column- Eclipse Plus C18,250×4.6 mm, C18 (5µm), Flow Rate: - 1.0 ml/min, Wave Length: 288 nm, Injection Volume:20µl, Column Temperature: 30°C, Run Time: 12 min, Diluent: Mobile Phase (pH 3.0 Buffer): Acetonitrile- (150:850), Mobile Phase A: Buffer (pH 3.0), Mobile Phase B: Acetonitrile (HPLC Grade).

Mobile Phase Preparation (Buffer): Aqueous solution of 1.53 gm of sodium dihydrogen phosphate in 1000ml Adjusted to pH 3.0 with phosphoric acid.

Results and Discussion

In present study several mobile phase compositions and several HPLC column were tried. A satisfactory separation and good peak symmetry was found at column Eclipse Plus C18, 250

×4.6 mm, C18 (5µm) in a mixture of Buffer: ACN (15:85% v/v) at pH 3.0 at flow rate of 1ml/min. The optimum wavelength for detection was set at 285nm at which much better detector response for Biclotymol.

Validation parameters: Following validation parameters are performed for establishing the method. 4.1 Applicability (Scope), 4.2 System Suitability, 4.3 Selectivity/Specificity, 4.4 Calibration, 4.5 Linearity (Range), 4.6 Accuracy, 4.7 Repeatability (Intra Day), 4.8 Reproducibility Precision (%RSD), 4.9 Robustness1, 4.10 Robustness2.

Applicability (Scope): The method validation is applicable for demonstration of Method accuracy, Method precision, Repeatability and Reproducibility for analysis of Biclotymol for assay. Labhasdone all relative parameters of method validation as per ICH Q_2(R2) Guidelines for details summery of each parameter are given in Validation Report. Lab will consider method for analysis after getting satisfactory result in each validation parameter and after that the validated method will be applicable for Analysis of assay contain in Biclotymol.

System Suitability: System suitability is explained as the instrument is used for validation is fit for analysis. Lab has ensured that instrument is working properly at the time of analysis, for that lab prepared 100% concentration of biclotymol (2000 PPM) standard and run into instrument and evaluate RSD, also check consistency of readings produced by instrument. So lab was evaluate % RSD of area (biclotymol 0.07%) and %RSD of RT.

(Biclotymol 0.04%) is found NMT 2.0% and NMT 0.5%. On the basis of data of system suitability, Lab is found satisfactory results and instrument fit for analysis.

Selectivity/Specificity: Selectivity is the degree to which the method can quantify the target analyte in the presence of other analytes, matrices, or other potentially interfering materials. For selectivity, lab has ensured the selection of reagents used in the preparation of sample by evaluating concentration of targeted analyte in the reagent blank used in sample analysis. Lab has prepared 5 replicate of reagents blank used in sample preparation and evaluate concentration of biclotymol. Lab has consider/select those reagents which does not show any peak at the retention time of Biclotymol in the reagent blank. Lab is found satisfactory data of replicate analysis of Reagent Blank and found no any peak observed attheretention time of Biclotymol which may interfere in the Analysis of Biclotymol for assay content.

Note: No any peak observed at the retention time of Biclotymolin the reagent blank solution.

As per the Data observed in replicate analysis of Reagent Blank, no any peak observed at the retention time of Biclotymolin the reagent blank solution so it will have used for further analysis of sample. On apply of these selected reagents and Reagents Blank, Result of sample is found satisfactory with respect to label claim.

Calibration: A calibration curve is constructed in ratio between the standard concentrations. A sufficient no. of standards should be used to adequately define the relationship between concentrations. Concentration of standard should be chosen on the basis of the concentration range expected in a particular Sample also based upon the characteristic concentrations provided by Instrument user manual.

Table-1: Selection of column.

Description	Make/Grade	Catalogue/Part No	Remark
Phenyl-Hexyl , 250×4.6mm, 5µm	XSelect CSH	186005401	Peak Response is good, Linearity Fail
Capcell Pak , 150×4.6mm, 5µm	Osaka Soda	92044	Peak Response is Low
CMB PSC C8/C18 150×4.6mm, 3µm	PSC	#135121-PSC	Peak Obtained with Low Response
Hypersil Gold, 150×2.1mm, 1.9µm	Thermo Scientific	25002-152130	Linearity Pass but Reproducibility Fail
Eclipse Plus C18, 250 ×4.6 mm, C18 (5µm)	Agilent	959990-906	Peak Response is good , Validation Parameter Pass

Table-2: List of columns and Regents.

Discription	Make/Grade	Catalogue/Part No
Eclipse Plus C18, 250 ×4.6 mm, C18 (5µm)	Agilent	959990-906
Acetonitrile	Merck or Equivalent	NA
Sodium Dihydrogen Phosphate	Merck or Equivalent	1063422500

Table-3: Gradient Programing.

Time (min)	A(%)	B(%)
0	15	85
12	15	85

Table-4: Sample Set Table.

Sample Name	vial	Inj #	Run Time	Injection Volume	Acquisition Method Set	Sample Weight	Proceed Channel Descr	Dilution
Blank	1	1	12.00	20.00	Biclotymol_Assay	1.00000	2487 Channel 1	1.00000
System Suit	2	1	12.00	20.00	Biclotymol_Assay	1.00000	2487 Channel 1	1.00000
System Suit	2	2	12.00	20.00	Biclotymol_Assay	1.00000	2487 Channel 1	1.00000
System Suit	2	3	12.00	20.00	Biclotymol_Assay	1.00000	2487 Channel 1	1.00000
System Suit	2	4	12.00	20.00	Biclotymol_Assay	1.00000	2487 Channel 1	1.00000
System Suit	2	5	12.00	20.00	Biclotymol_Assay	1.00000	2487 Channel 1	1.00000
Blank	1	1	12.00	20.00	Biclotymol_Assay	1.00000	2487 Channel 1	1.00000
Linearity 500 PPM	3	1	12.00	20.00	Biclotymol_Assay	1.00000	2487 Channel 1	1.00000
Linearity 500 PPM	4	1	12.00	20.00	Biclotymol_Assay	1.00000	2487 Channel 1	1.00000
Linearity 500 PPM	5	1	12.00	20.00	Biclotymol_Assay	1.00000	2487 Channel 1	1.00000
Linearity 500 PPM	6	1	12.00	20.00	Biclotymol_Assay	1.00000	2487 Channel 1	1.00000
Blank	1	1	12.00	20.00	Biclotymol_Assay	1.00000	2487 Channel 1	1.00000
Range 500 PPM	7	1	12.00	20.00	Biclotymol_Assay	1.00000	2487 Channel 1	1.00000
Range 500 PPM	8	1	12.00	20.00	Biclotymol_Assay	1.00000	2487 Channel 1	1.00000
Range 500 PPM	9	1	12.00	20.00	Biclotymol_Assay	1.00000	2487 Channel 1	1.00000
Blank	1	1	12.00	20.00	Biclotymol_Assay	1.00000	2487 Channel 1	1.00000
Repeatibility STD	10	1	12.00	20.00	Biclotymol_Assay	1.00000	2487 Channel 1	1.00000
Repeatibility STD	10	2	12.00	20.00	Biclotymol_Assay	1.00000	2487 Channel 1	1.00000
Repeatibility STD	10	3	12.00	20.00	Biclotymol_Assay	1.00000	2487 Channel	1.00000
Repeatibility STD	10	4	12.00	20.00	Biclotymol_Assay	1.00000	2487 Channel 1	1.00000
Repeatibility STD	10	5	12.00	20.00	Biclotymol_Assay	1.00000	2487 Channel 1	1.00000
Repeatibility Test1	11	1	12.00	20.00	Biclotymol_Assay	1.00000	2487 Channel 1	1.00000
Repeatibility Test2	12	1	12.00	20.00	Biclotymol_Assay	1.00000	2487 Channel 1	1.00000
Repeatibility Test3	13	1	12.00	20.00	Biclotymol_Assay	1.00000	2487 Channel 1	1.00000
Repeatibility Test4	14	1	12.00	20.00	Biclotymol_Assay	1.00000	2487 Channel 1	1.00000
Repeatibility Test5	15	1	12.00	20.00	Biclotymol_Assay	1.00000	2487 Channel 1	1.00000
Repeatibility Test6	16	1	12.00	20.00	Biclotymol_Assay	1.00000	2487 Channel 1	1.00000

Linearity (Range): The range of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity. The specified range is normally derived from linearity studies and depends on the intended application of the procedure. It is established by confirming that the analytical procedure provides an acceptable degree of linearity, accuracy and precision when applied to samples containing amounts of analyte within or at the extremes of the specified range of the analytical procedure. Here for determination of range is detected from 25% value to 100% value of sample. It is achieved by weight variation of sample taken for analysis.

Table-5: Table for 5 replicate injection of standard sample of Biclotymol for System Suitability.

Conc. of Standard	Area of Standard	Mean Area of Standard	RT of Standard	Mean RT of Standard	SD of Area	RSD of AREA	SD of RT	RSD ofRT
2000µg/ml	23573522	23562217.40	4.027	4.03	17172.54	0.07	0.00	0.04
2000µg/ml	23532836		4.027					
2000µg/ml	23571014		4.029					
2000µg/ml	23572692		4.03					
2000µg/ml	23561023		4.03					

Acceptance Criteria: RSD of Area should not more than 2%, RSD of RT should not more than 0.5%.

Table-6: Table for Coefficient of correlation (R^2 Value).

Biclotymol				
Name of Standard	Range	Coefficient of Correlation	Limit	Interpretation
Biclotymol	500to2000 ppm	0.995	NLT0.995	Satisfactory

Table-7: Calibration Curve Table.

S.No.	Level of Standard (PPM)	Area of Standard
1	500	5621666
2	1000	11939516
3	2000	23620805
4	4000	43580551

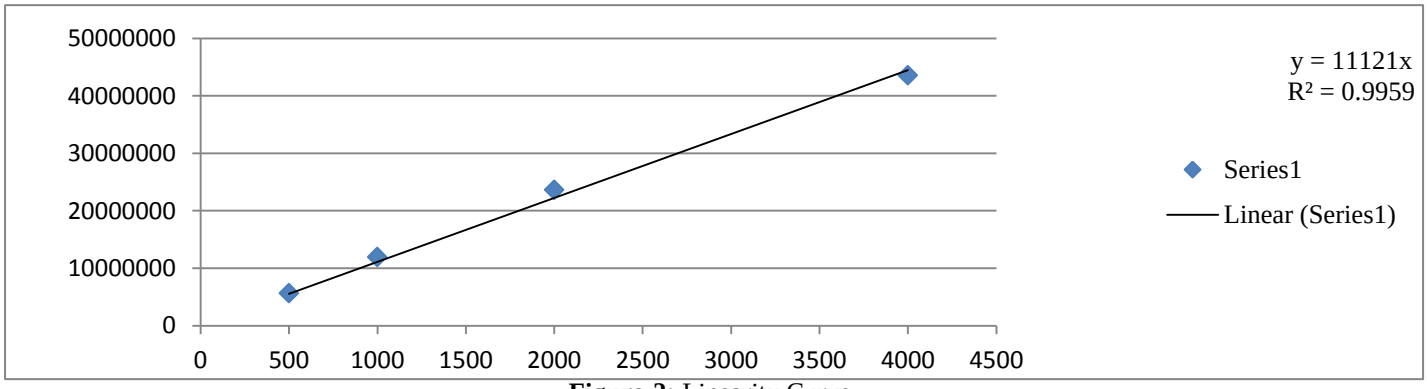


Figure-2: Linearity Curve.

Table-8: Observed Concentration Table for Linearity.

Level	Sample Weight (mg)	Dissolved in (ml)	Sample Area	Standard Slop Area	Conc. Observed
Range at 25%	5.1	10	5594071	11121	503.02
Range at 50%	10.2	10	11938879	11121	1073.54
Range at 100%	20.1	10	23616330	11121	2123.58

Table-9: Table for level (%) of Biclotymol.

Level	Sample weight taken (mg)	Observed Conc. (µg/ml)	R^2Value	Acceptance Criteria
1 at 25%	5.1	503.02	0.999	NLT0.990
2 at 50%	10.2	1073.54	0.999	NLT0.990
3 at 100%	20.1	2123.58	0.999	NLT0.990

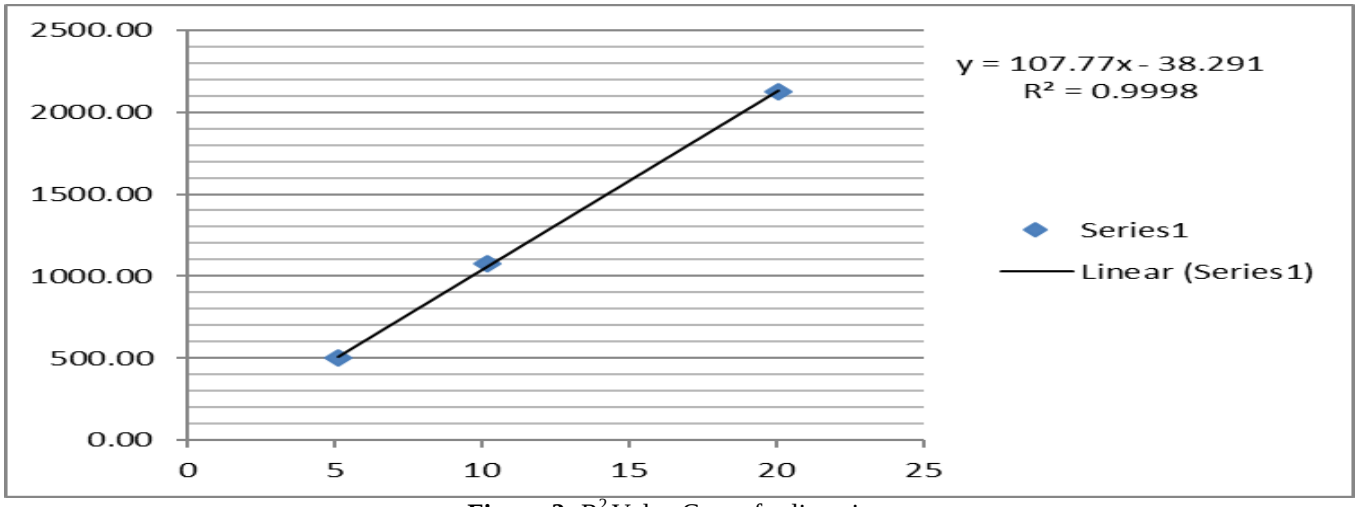


Figure-3: R^2 Value Curve for linearity.

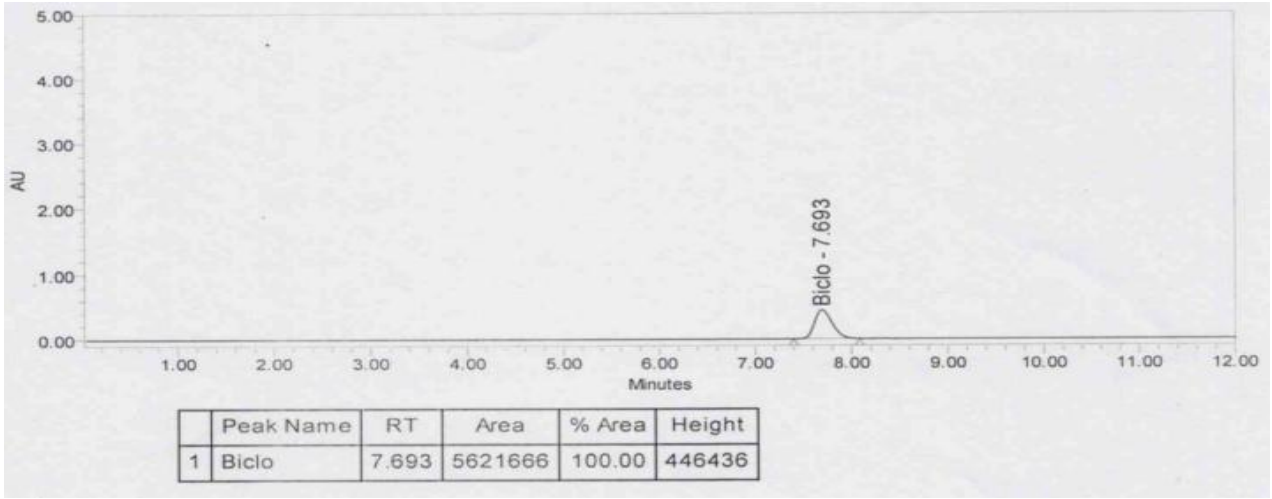
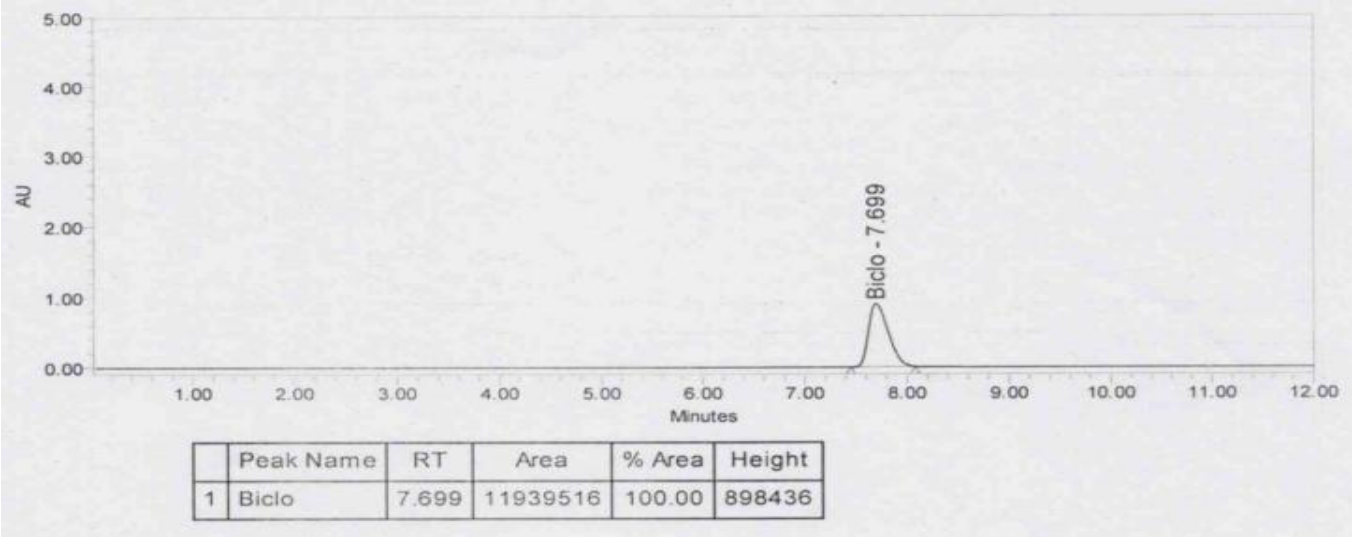
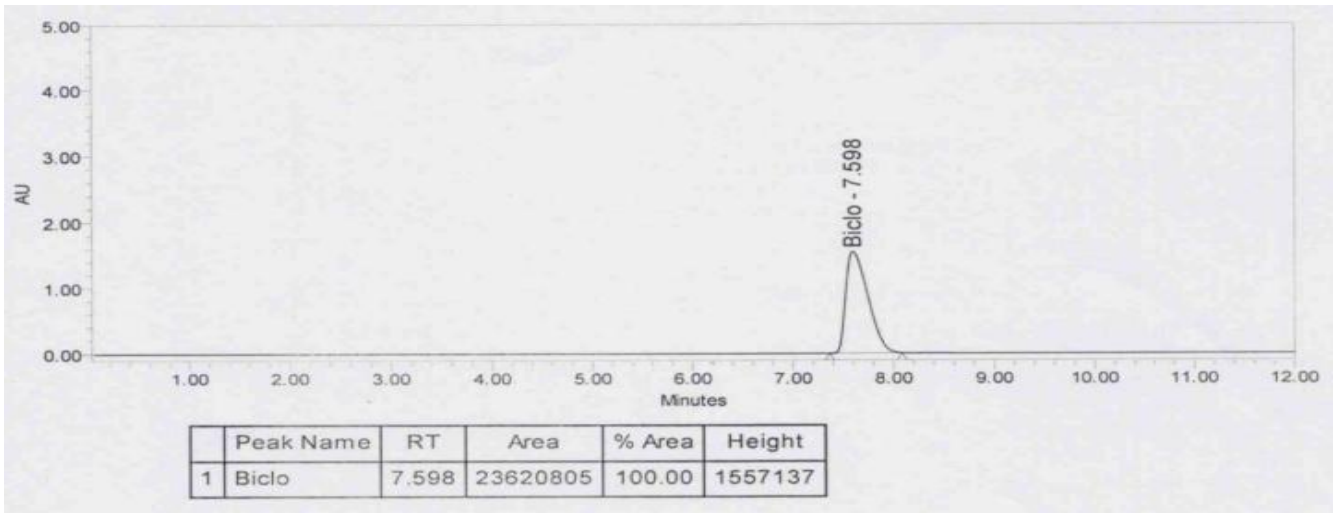


Figure-4: HPLC chromatograph of 500ppm Sample.



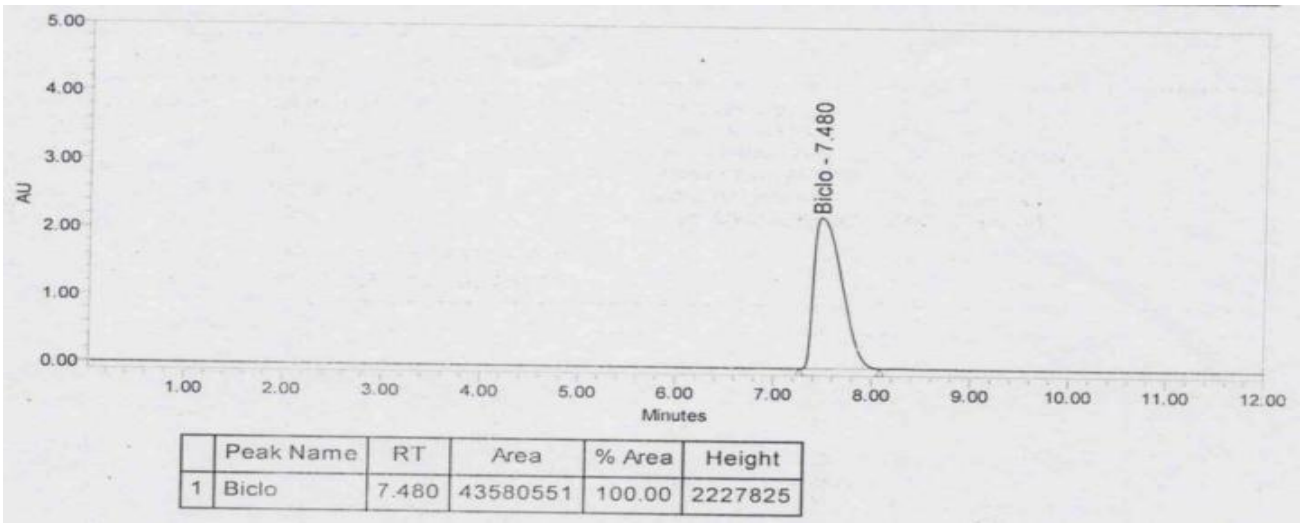
	Peak Name	RT	Area	% Area	Height
1	Biclo	7.699	11939516	100.00	898436

Figure-5: HPLC chromatograph of 1000ppm Sample.



	Peak Name	RT	Area	% Area	Height
1	Biclo	7.598	23620805	100.00	1557137

Figure-6: HPLC chromatograph of 2000ppm Sample.



	Peak Name	RT	Area	% Area	Height
1	Biclo	7.480	43580551	100.00	2227825

Figure-7: HPLC chromatograph of 4000ppm Sample.

Accuracy: Accuracy should be established across the specified range of the analytical procedure. It is calculated by results obtained from the experiment done in the Range. % Accuracy was calculated by the mean value of the result obtained in Range and True value (Claim) of the Sample.

Repeatability Precision (Intra Day): The Precision of an analytical procedure expresses the closeness of agreement between a series of measurement obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Repeatability refers to the degree of agreement of results when condition is maintained as constant as possible with the same analyst, reagents, equipment, and instruments performed within a short period of time. The precision of an analytical procedure is usually expressed as the variance, standard deviation or coefficient of variation of a series of measurements. This is also called degree of repeatability. Here we are calculating the % RSD from 6 Replicate analysis at 100% label claim. % RSD was found satisfactory that is NMT 2%.

Reproducibility Precision (%RSD): Reproducibility Precision refers to the degree of agreements of results when operation conditions are as different as possible. It usually refers to the standard deviation (S_R) or the relative standard deviation (RSD_R) of results on the same test sample within laboratory by different analyst. The same sample was analyzed by different analyst on different day at 100% level. % RSD was estimated by the analysis. % RSD found satisfactory that is NMT 2%.

Table-10: Accuracy Table for Biclotymol.

Result (%)	Result Mean	Acceptance Criteria.	% Accuracy of mean Value
98.43	97.75	80% to120%	97.75%
98.39			
98.40			
96.78			
97.49			
96.99			

Table-11: Table for RSD (%) of 5 Replicate Injection of standard sample.

Name of Analyte	Conc. of Analyte	Area of Anlyte	Mean Area of Analyte	SD	RSD (%)
Biclotymol	2000 µg/ml (100%)	23638698	23690677	37433	0.16
		23689501			
		23730234			
		23722421			
		23672531			

Table-12: Table for RSD (%) of 6 Replicate Injection of sample

Sample Weight	Std Weight	Dissolved in (ml)	Area of Sample	Mean Area of Standard	Potency of WRS	Result %	Mean result %	SD	RSD %
20.2	20.1	10	23672531	23690677	0.99	98.43	97.75	0.73	0.78
20.1	20.1	10	23545011	23690677	0.99	98.39			
20.1	20.1	10	23547698	23690677	0.99	98.40			
19.9	20.1	10	22928670	23690677	0.99	96.78			
19.8	20.1	10	22981285	23690677	0.99	97.49			
19.9	20.1	10	22978339	23690677	0.99	96.99			

Robustness-1: The robustness of an analytical procedure 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. Here we have change the sample analysis method by different analyst. Record the variation of the sample preparation and collect the data of 6 replicate analysis of the same test solution.

Table-13: Table for RSD (%) of 5 Replicate Injection of standard sample for Reproducibility.

Name of Analyte	Conc. of Analyte	Area of Anlyte	Mean Area of Analyte	SD	RSD (%)
Biclotymol	2000 µg/ml (100%)	23955394	23858109	56319.6	0.24
		23859001			
		23825697			
		23823068			
		23827384			

Table-14: Table for RSD (%) of 6 Replicate Injection of sample for Reproducibility.

Sample Weight	Std Weight	Dissolved in (ml)	Area of Sample	Mean Area of Standard	Potency of WRS	Result %	Mean result %	SD	RSD%
20.2	20.2	10	23737154	23858108.8	0.99	98.43	97.76	0.93	0.95
20.1	20.2	10	23655853	23858108.8	0.99	98.39			
20.2	20.2	10	23735792	23858108.8	0.99	98.40			
19.9	20.2	10	23057223	23858108.8	0.99	96.78			
19.8	20.2	10	23023187	23858108.8	0.99	97.49			
20.1	20.2	10	23107304	23858108.8	0.99	96.99			

Acceptance Criteria: RSD of 6 replicate analysis should be NMT 2%

Table-15: Chromatographic Condition Table for Robustness 1.

Parameter	In General Method (Routine Analysis)	In Robust Method (For Validation)
Chromatographic Condition	FlowRate-1.0ml/min Column Temp.-25°C	FlowRate-0.8ml/min Column Temp.-25°C

Table-16: Table for RSD (%) of 5 Replicate Injection of standard sample for Robustness 1.

Name of Analyte	Conc. of Analyte	Area of Anlyte	Mean Area of Analyte	SD	RSD (%)
Biclotymol	2000 µg/ml (100%)	29564469	29586050	21068.6	0.0712
		29596526			
		29585291			
		29568223			
		29615743			

Robustmess 2: The robustness of an analytical procedure 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. Here we have change the sample analysis method by different analyst. Record the variation of the sample preparation and collect the data of 6 replicate analysis of the same test solution.

Table-17: Table forRSD (%) of 6 Replicate Injection of sample for Robustness 1.

Sample Weight	Std Weight	Dissolved in (ml)	Area of Sample	Mean Area of Standard	Potency of WRS	Result %	Mean result %	SD	RSD%
20.1	20	10	29206485	29586050	0.99	97.24	96.43	0.63	0.65
20.2	20	10	29282356	29586050	0.99	97.01			
20.3	20	10	29304759	29586050	0.99	96.61			
20	20	10	28602333	29586050	0.99	95.71			
19.8	20	10	28439390	29586050	0.99	96.12			
19.9	20	10	28508429	29586050	0.99	95.87			

Acceptance Criteria: RSD of 6 replicate analysis should be NMT 2%.

Table-18: Chromatographic Condition Table for Robustness 2.

Parameter	In General Method (Routine Analysis)	In Robust Method (For Validation)
Chromatographic Condition	FlowRate-1.0ml/min ColumnTemp.-25°C	FlowRate-1.2ml/min ColumnTemp.-25°C

Table-19: Table for RSD (%) of 5 Replicate Injection of standard sample for Robustness 2.

Name of Analyte	Conc. of Analyte	Area of Anlyte	Mean Area of Analyte	SD	RSD (%)
Biclotymol	2000 µg/ml (100%)	19782913	19816043	27500.1	0.14
		19814765			
		19803601			
		19821118			
		19857819			

Table-20: Table for RSD (%) of 6 Replicate Injection of sample for Robustness 2.

Sample Weight	Std Weight	Dissolved in (ml)	Area of Sample	Mean Area of Standard	Potency of WRS	Result %	Mean result %	SD	RSD%
20.1	19.9	10	19536297	19816043	0.99	96.63	96.15	0.45	0.46
20.3	19.9	10	19601134	19816043	0.99	96.00			
20.2	19.9	10	19631554	19816043	0.99	96.62			
19.7	19.9	10	19081367	19816043	0.99	96.30			
19.8	19.9	10	19021115	19816043	0.99	95.51			
19.8	19.9	10	19092721	19816043	0.99	95.87			

Acceptance Criteria: RSD of 6 replicate analysis should be NMT 2%.

Table-21: Table for Results obtained in Repeatability, Robustness1 and Robustness 2 of Biclotymol.

Results obtained in Precision (Repeatability)	Results obtained in Robustness1 method	Results obtained in Robustness2 method
98.43	97.24	96.63

98.39	97.01	96.00
98.40	96.61	96.62
96.78	95.71	96.30
97.49	96.12	95.51
96.99	95.87	95.87
97.75	96.43	96.15
0.76	0.63	0.45
0.78	0.65	0.46

Conclusion

Analytical performance parameters for the developed method for determination of Assay method of biclotymol using stainless steel column Eclipse Plus C18, 250 ×4.6 mm, C18 (5µm) have confirmed good results in point of all validation parameters which confirms that this method is suitable for its intendance.

Acknowledgement

The authors are also thankful to Dr.K.N. Modi University Newai Rajasthan for providing necessary equipment, facility and chemicals to complete research work and sincere thanks to my highly respected and esteemed Principal, Dr. Ram Naresh Sharma and HOD SHS Govt. P.G.College Dhanpur, Chandauli. I would like to express thanks to my parents without their encouragement love and blessings I would not have reached this level manuscript.

References

1. Karpova E, Giacomelli L, Tumietto F (2017). Role of biclotymol-based products in the treatment of infectious sore throat. *European Review for Medical and Pharmacological Sciences*, 21(16), 3736–3743.

2. British Pharmacopoeia (2018). Stationary Office on behalf of the Medicines and Healthcare Products Regulatory Agency (MHRA). Monograph for Bisoprolol fumarate.

3. European Pharmacopoeia (2019). European Directorate for the Quality of Medicines and Healthcare, Council of Europe, Strasbourg. Monograph for Biclotymol

4. ICH Guideline (1998). Photostability Testing of New Active Substances and Medicinal Products, ICH Topic Q1B, European Medical Agency. CPMP/ICH/279/95, January 1998. ICH Guideline: Stability Testing of New Drug Substance and Product,

5. ICH Q1A (R2). European Medical Agency, CPMP/ICH/2736/99, August 2003. ICH Guideline: Validation of analytical procedures: Text and Methodology, ICH Q2(R1), European Medical Agency, CPMP/ICH/381/95, November 2005.

6. H. Eldridge Faith (1950). Aldehyde-Phenol Reaction Products and Derivatives. *Journal of the American Chemical Society*, 72, 837-839, DOI:10.1021/ja01158a049.

7. Jakub K. Sypien, Thomas A. Zevaco , Annette Flicker, Olaf Walter (2013). New aluminum 2,2-methylenebis(4-chloro-3-methyl-6-(isopropyl)phenoxides): Structural characterization of an unusual ionic aluminum bisphenoxide [Al(THF)4(Cl)2] +[Al(mcmip)2] −·x THF. *Inorganic Chemistry Communications*, 30, 69–73. DOI: https://doi.org/10.1016/j.inoche.2013.01.021

8. Chen, Hsuan-Ying; Liu, Mei-Yu; Sutar, Alekha Kumar; Lin, Chu-Chieh (2009). Synthesis and structural studies of heterobimetallic alkoxide complexes supported by Bis(phenolate) ligands: Efficient catalysts for Ring-Opening polymerization of L-Lactide. *Inorganic Chemistry* , 49(2), 665 – 674.

9. S. Rantsordas, M. Perrin, and A. Thozet. (1978). Crystal and molecular structures of 2,2-Methylenebis(4-chloro-3-methyl-6-isopropylphenol). *Acta Cryst*., B34, 1198-1203. DOI: https://doi.org/10.1107/S0567740878005208.

10. ICH Topic Q2 (R1) (1996). Validation of Analytical Procedures; Methodology, Q2 (R1). *International Conference on Harmonization*, IFPMA, Geneva 1996.

11. Lisa (Song) Liu, Ariel Mouallem, Kang Ping Xiao, Jerry Meisel (2021). Assay of active pharmaceutical ingredients in drug products based on relative response factors: Instrumentation insights and practical considerations. *Journal of pharmaceutical and biomedical analysis*, 194, 113760.

12. Lloyd R. Snyder, Joseph J. Kirkland, Joseph L. Glajch. (1997). Practical HPLC Method Development. Second Publication, A Wilew Interscience Publication, ISBN: 0-471-00703-X.

13. Manual (2013). Agilent 1200 Infinity Series Diode Assay Detectors User Manual. Agilent HPLC publication.

14. Huafu Wang, Gordon J. Provan, Keith Helliwell (2003). HPLC determination of catechins in tea leaves and tea extracts using relative response factors. *Elsevier*, *Food Chemistry*, 307–312, DOI: https://doi.org/10.1016/S0308-8146(02)00510-1.

15. Sunil K Pandey and R.N. Sharma (2023). Analytical method development and method validation for the Assay of 5-Chloro-2,3-dihydro-1-one by gas chromatography (GC). *J. Chemtracks*, 25(1&2) 225-230.

16. Naseef Hani and Ramzi Moqadi (2024). HPLC Method Development and Validation for the Determination of Apixaban and Clopidogrel in Novel Fixed-Dose Combination Tablets. *Journal of Chemistry*, DOI: http://.doi.org10.1155/2024/26755736.

17. Nidhal S. Mohammed (2016). Development and Validation of RP-HPLC Method for the Determination of Hydrochlorothiazide in Bulk Drug and Pharmaceutical Dosage Form. *Chromatography Research International*, DOI: https://doi.org/10.1155/2016/1693024.

18. Prafulla Kumar Sahu (2011). Development and Validation of Stability Indicating RP-HPLC Method for the Determination of Metaxalone in Bulk and its Pharmaceutical Formulations. *Journal of Chemistry*, DOI: http://.doi.org 10.1155/2011/645710.

19. FDA (2000). Analytical Procedures and Methods Validation: Chemistry, Manufacturing and Controls. *Federal Register (Notices)*, 65(169), 52, 776–777.

20. The European Medicines Agency, Pre-Authorization Evaluation of Medicines for Human Use. ICH Q2(R1): Validation of Analytical Procedures: Text and Methodology, EMEA/410412/2007: London, 2009.

21. Z. Vujic, N. Mulavdi ´ c, M. Smaji ´ c, J. Brbori ´ c, and P. Stankovic, (2012). Simultaneous analysis of irbesartan and hydrochlorothiazide: an improved HPLC method with the aid of a chemometric protocol. *Molecules*, 17(3), 3461–3474.

22. R. Céolin, J. L. Tamarit, M. Barrio, D. Ó. López, B. Nicolaï, N. Veglio, M-A. Perrin and P. Espeau, J. Overall. (2008). Monotropic Behavior of a Metastable Phase of Biclotymol, 2,2′-Methylenebis(4-Chloro-3-Methyl-Isopropylphenol), Inferred from Experimental and Topological Construction of the Related *P-T* State Diagram. *Journal-of-pharmaceutical-sciences*, 97(9), 3927-3941.

23. Nathalie Mahe, Beatrice Nicolai and Rene Ceolin (2008). Crystal Structure of Biclotymol-DMSO 1:1 Solvate: 2,2c-Methylenebis- (4-chloro-3-methyl-6-isopropylphenol) dimethylsulfoxide Solvate.. *The Japan Society for Analytical Chemistry*, *Analytical Sciences*, 24.

24. L.J. Farrugia, (1999). J. Appl. Cryst. 32, 837. 7. J. Bernstein and A. T. Hagler, J. Am. Chem. Soc., 1978, 100, 673.