



A Newly Improved Modified Method Development and Validation of Bromofenac Sodium Sesquihydrate in Bulk Drug Manufacturing

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ABSTRACT

The main objective of this study was to develop a simple, efficient, specific, precise and accurate newly improved modified Reverse Phase High Performance Liquid Chromatographic Purity (or) Related substance method for bromofenac sodium sesquihydrate active pharmaceuticals ingredient dosage form. Validation of analytical method is the confirmation by examination and the provision of objective evidence that the particular requirements for a specific intended use are fulfilled as per ICH, USP, BP or any other suitable regulatory guidelines. The Reverse Phase High Performance Liquid Chromatographic Gradient method was developed by utilizing Waters Symmetry C8, 150x4.6mm, 3.5 µm on Waters 2487 series Liquid Chromatograph. The retention time of bromofenac sodium sesquihydrate was found to be 5.973 minutes. Considering all the results of validation parameters simplicity of the method and the cost effectiveness of the overall procedure, it is possible to conclude that the developed method can be suitable for the regular quality control determination of bromofenac sodium sesquihydrate in bulk as well as pharmaceutical dosage form. The developed Reverse Phase High Performance Liquid Chromatographic Purity (or) Related substance method for bromofenac sodium sesquihydrate active pharmaceuticals ingredient method was validated with respect to System Suitability, linearity, precision, Range, Ruggedness, Test Solution and Mobile phase stability, Robustness.

Key words: Bromofenac sodium sesquihydrate, Reverse Phase High Performance Liquid Chromatographic Purity (RPHPLC), Determination, Validation, Pharmaceutical dosage form.

INTRODUCTION

Bromofenac sodium sesquihydrate (which is the sodium salt of 2-amino-3-(4-bromobenzoyl)

phenyl acetic acid, also sometimes referred to as sodium 2-amino-3-(4-bromobenzoyl)phenyl acetate sesquihydrate, having the empirical formula C₁₅H₁₁BrNNaO₃.1.5H₂O) in Figure-1 is

a non-steroidal anti-inflammatory drug (NSAID) with analgesic properties. It was initially marketed as an oral suspension under the trade name DURACT but was withdrawn from the United States market in 1998 due to drug-induced hepatotoxicity leading to acute liver failure. Currently, bromfenac sodium is sold as an ophthalmic solution under the brand name XIBROM. The Food and Drug Administration approved this product in 2005 for use in ophthalmic surgery including postoperative inflammation, reduction of pain after cataract and refractive surgery and management of macular edema after cataract surgery. XIBROM ophthalmic solution contains 1.035 mg bromfenac sodium, equivalent to 0.9 mg bromfenac free acid per mL of solution, giving a 0.09% sterile topical ophthalmic formulation with a pH of 8.3. Anti-inflammatory agents that are non-steroidal in nature. In addition to anti-inflammatory actions, they have analgesic, antipyretic, and platelet-inhibitory actions. They act by blocking the synthesis of prostaglandins by inhibiting cyclooxygenase, which converts arachidonic acid to cyclic endoperoxides, precursors of prostaglandins. Inhibition of prostaglandin synthesis accounts for their analgesic, antipyretic, and platelet-inhibitory actions; other mechanisms may contribute to their anti-inflammatory effects. See a list of PubChem compounds matching this category.¹⁻¹¹

Literature review reveals that few spectrophotometric and LC, LCMS methods have been reported for analysis of Bromofenac sodium sesquihydrate. LC, LCMS simultaneous methods for pharmaceutical dosage and one for injections were reported in the literature review; existing methods were Long cyclic run time chromatographic process method. More over reported methods were not much cost-effective in terms of solvent

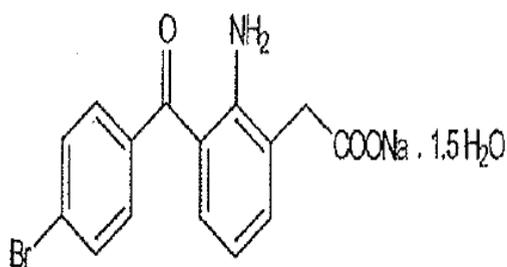


Fig. 1: Chemical structure of Bromofenac sodium sesquihydrate

consumption and total run time of the analysis, so we decided to perform rapid, selective and precise newly improved modified method development and validation of Reverse Phase High Performance Liquid Chromatographic Gradient method for determination of Related impurities present in the Bromofenac sodium sesquihydrate in solid dosage form¹²⁻¹³.

MATERIALS AND METHODS

Chemicals and Reagents

Reference standard of Bromofenac sodium sesquihydrate, Related Impurities are 7-(4-bromobenzoyl)-1,3-dihydro-indol-2-one (Bro-4), (4-Bromo phenyl)-(1H-indol-7-yl) methanone (Bro-2), (3-Bromo-1H-indol-7-yl)-(4-bromo-phenyl)-methanone (Bro-3) & (4-Bromo-Phenyl)-(2,3-dibromo-1H-indol-7-yl)-methanone (Dibromo Impurity) and samples was obtained from well reputed research laboratories and characterized by use of LCMS, NMR and IR. All the chemicals were analytical grade from Rankem Ltd., Mumbai, India, while Methanol, Acetonitrile (HPLC grade), Potassium dihydrogen phosphate and Ortho phosphoric acid (HPLC grade) procured from Merck Pharmaceuticals Private Ltd., Mumbai, India and purchased from Merck Specialties Private Ltd., Mumbai, India. The Liquid Chromatography system was equipped with quaternary gradient pumps with auto sampler and column oven, auto injector connected to a variable wave length programmable ultra Violet visible detector all were controlled by Empower software and Manufactured by Waters Alliance system & 2489 UV detector.

Optimization of mobile phase

Optimization of mobile phase was performed based trial and error method. In this different mobile phase trial was taken like in methanol :water, ACN:water and methanol:ACN:water in different ratio without pH but there are different problem were observed like high tailing factor value and not optimized theoretical plate. When the mixture of Phosphate buffer (pH adjusted to 3.2 using dill. Ortho phosphoric acid) and Acetonitrile, Methanol in ratio of 80:10:10 v/v was selected as mobile phase-A and Acetonitrile, Water in the ratio of 90:10 as mobile phase-B in gradient form Bromofenac sodium sesquihydrate full fill all the criteria of system suitability. The Gradient mobile phase –A consisting

of Phosphate buffer (pH adjusted to 3.2 using dill. Ortho phosphoric acid) and acetonitrile, Methanol in ratio of 90:10:10 v/v and Acetonitrile ,Water in the ratio of 90:10 was selected which gave gradient elution of sharp peak with retention time at 5.954 min. Similarly for the selection of diluent we tried the standard into different solvents like water, methanol, mobile phase and acetonitrile . Finally the selected diluent was Methanol. So finally the above said mobile phase-A ,Mobile Phase-B and diluent was selected for analysis. Optimized chromatographic conditions are shown in Table 1.¹⁴⁻¹⁷

Selection of detection wavelength

For the selection of analytical wavelength 1mg/ml Bromofenac sodium sesquihydrate solution was prepared from standard drug solution and scanned in the range of 198 to 400 nm. From the

UV spectra, the maximum λ_{max} of Bromofenac sodium sesquihydrate is found to be 263 nm. So this wavelength was selected as the detection wavelength for analysis. The selected mobile phase, diluent & wave length has given a sharp peak with low tailing factor 1.18 (<2) .

Instrumentation and analytical chromatographic conditions

The chromatographic analysis of method validation for related substance by High Pressure liquid chromatography determination of Bromofenac sodium sesquihydrate was carried out on Waters alliance High Pressure Liquid Chromatography Model -2690 series containing quaternary pump, variable wave length programmable of 2489 ultra violet visible detector and auto injector with up to 1 μ l-1000 μ l loop, column oven modules. Chromatographic

Table 1: Parameter Chromatographic conditions

Instrument	Water alliance High Pressure Liquid Chromatography with 2489 UV Detector
Column	Waters Symmetry C8,150x 4.6mm,3.5 μ m
Detector	UV detector
Mobile phase-A & Mobile phase-B(Gradient Elution)	A mixture of Phosphate buffer (pH adjusted to 3.2 using dill. Ortho phosphoric acid) and Acetonitrile , Methanol in ratio of 80:10:10v/v & Acetonitrile ,Water in the ratio of 90:10 as mobile phase-B
Flow rate	1 mL/min with Gradient Elution .

W2690/5 Gradient Table

	Time	Flow	%A	%B	%C	%D	Curve
1		1.00	40.0	60.0	0.0	0.0	
2	4.00	1.00	40.0	60.0	0.0	0.0	6
3	8.00	1.00	10.0	90.0	0.0	0.0	6
4	30.00	1.00	10.0	90.0	0.0	0.0	6
5	31.00	1.00	40.0	60.0	0.0	0.0	6
6	35.00	1.00	40.0	60.0	0.0	0.0	6

Detection wave length By UV	At 263 nm.
Run time & Stop time	40Minutes
Temperature Ambient temperature	27°C
Volume of injection loop	10 μ L
Retention time (Rt)	5.954 minutes

analysis was performed using Waters Symmetry C8 column, with 150 x 4.6mm internal diameter and 3.5 μ m particle size. Sartorius electronic balance was used for weighing. The elution was carried out Gradient at flow rate of 1 ml/min using the mixture of Phosphate buffer (pH adjusted to 3.2 using dill. Ortho phosphoric acid) and Acetonitrile, Methanol in ratio of 80:10:10v/v & Acetonitrile, Water in the ratio of 90:10 as mobile phase-B was selected as mobile phases A and B and injection Volume 10 μ L (μ L –micro.litre). The detection wavelength was set at 263 nm with a runtime of 40 min . The mobile phases A and B was prepared freshly and it was degassed by sonication for 5 min before use. The column was equilibrated for at least 30 min with the mobile phases A and B flowing through the system. The column oven module and the High pressure liquid chromatography system were kept at 27°C \pm 2°C temperature and Water: Acetonitrile and Methanol in the ratio of 33:33:33 v/v is used as rinsing solvent.

Mobile phase preparation

Mobile Phase –A

Accurately weighed 1.360 g of Potassium dihydrogen phosphate (KH_2PO_4) dissolved in 1000 ml of Mili-Q-water to get phosphate buffer. pH was adjusted to 3.2 \pm 0.05 with dilute ortho phosphoric acid. Above prepared buffer and acetonitrile, Methanol were mixed in the proportion of 80:10:10 v/v. This mixture was sonicated for 10 minutes and filtered through 0.22 μ m membrane filter and used as mobile phase-A .

Mobile Phase –B

Mixed Acetonitrile and Water in the proportion of 90:10 v/v , this mixture was sonicated for 10 minutes and filtered through 0.22 μ m membrane filter and used as mobile phase-B .

Preparation of standard solutions & system suitability solution

Pure standards of Bromofenac sodium sesquihydrate were used as external standards in the analysis. Different concentrations of the standards were used based on the range required to plot a suitable calibration curve. About 10 mg of the standard Bromofenac sodium sesquihydrate was accurately weighed and transferred in to 10 ml volumetric flask and make up with sufficient diluent. Volumetric flask containing standard solution was sonicated for 10 minutes. Similarly different concentrations of these standards were analysed using the same chromatographic conditions and a calibration curve was generated. The sample chromatogram and results recorded is in Fig. 2, Table2.

Diluent

Used diluent (Methanol) as blank.

System suitability solution (or) 0.20% Impurity Blend Solution : (Prepare fresh solution)

Weigh about 10.0 mg each of Bromofenac sodium sesquihydrate standard, Bro-4, Bro-2 and Bro-3, Dibromo Impurity in a 100 ml volumetric flask. Dissolve it by sonication till the solution is clear and

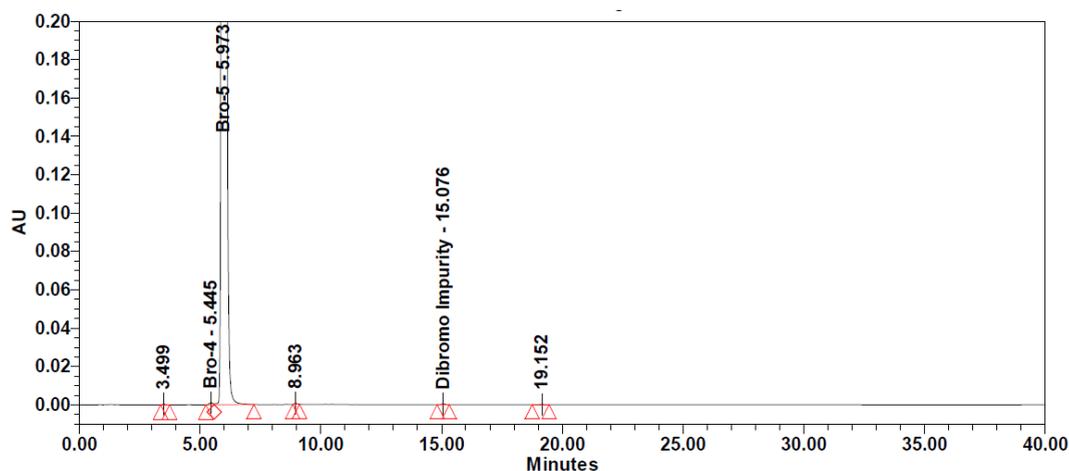
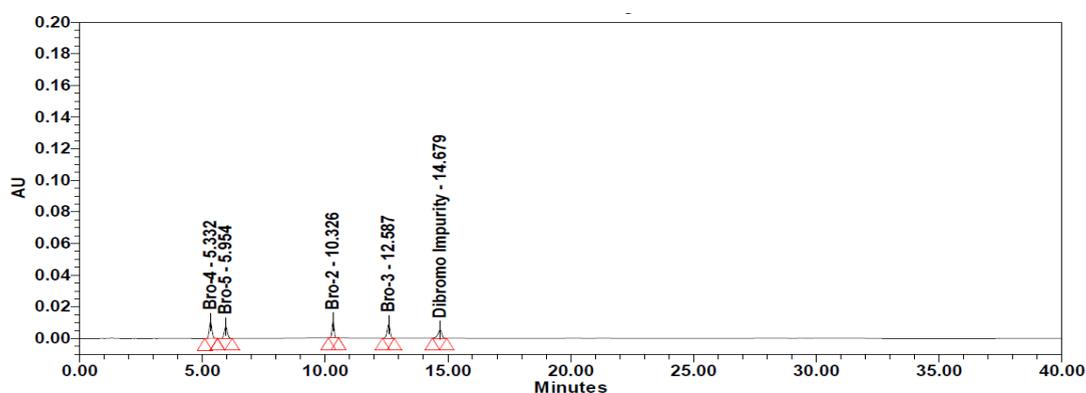


Fig. 2: Standard solution chromatogram

Table 2: Results

Peak Results									
	RT	Height	Area	%Area	RT Ratio	Name	USP Plate Count	USP Resolution	USP Tailing
1	3.499	377	2543	0.011	0.586		6225.84		1.17
2	5.445	1058	11257	0.047	0.912	Bro-4	2584.68		
3	5.973	2640693	23731918	99.898		Bro-5	10062.48		1.18
4	8.963	639	4517	0.019	1.501		35151.54	13.78	1.09
5	10.326					Bro-2			
6	12.587					Bro-3			
7	15.076	386	4010	0.017	2.524	Dibromo Impurity	48202.89	25.65	0.94
8	19.152	113	1929	0.008	3.207		30309.90	11.01	0.82

**Fig. 3: System suitability solution chromatogram****Table 3: System suitability Results**

Peak Results									
	RT	Height	Area	%Area	RT Ratio	Name	USP Plate Count	USP Resolution	USP Tailing
1	5.332	10312	79766	24.154	0.896	Bro-4	10606.44		1.08
2	5.954	7414	63803	19.320		Bro-5	10895.26	2.84	1.08
3	10.326	10427	62605	18.958	1.734	Bro-2	65282.58	22.15	0.98
4	12.587	8603	68922	20.870	2.114	Bro-3	56106.88	11.96	0.92
5	14.679	5160	55143	16.698	2.465	Dibromo Impurity	43807.63	8.28	0.85

make up to the mark with diluent. Dilute 200 μ L of the solution in to 10 ml volumetric flask and dilute up to the mark with diluent. System suitability solution or Impurity blend Solution were analysed using the same chromatographic conditions. The sample chromatogram and results recorded is in Fig. 3, Table 3.

Preparation of Sample solutions

Weigh accurately about 10 mg of Bromofenac sodium sesquihydrate sample to be analysed in 10 ml volumetric flask. Dissolve with about 5 ml of diluent by sonication till the

solution is clear and make up to the mark with diluent. These solutions were analysed using the same chromatographic conditions. The sample chromatogram and results recorded is in Fig. 4, Table.4.

Method validation procedure

After the completion of High pressure liquid chromatography method development, the objective of the method validation is to demonstrate that the method is suitable for its intended purpose as it is stated in International Conference on Harmonisation (ICH) guidelines. The method was validated for

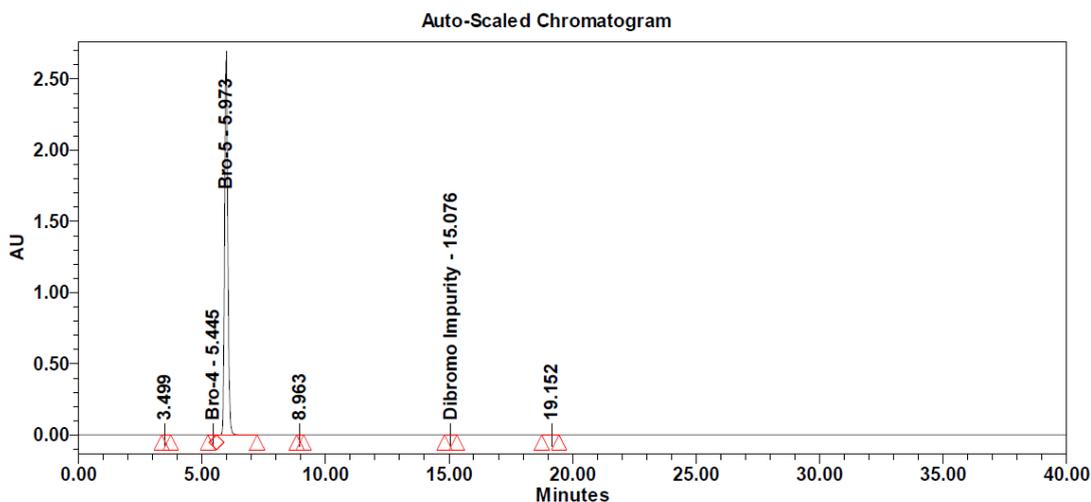


Fig. 4: Sample solution chromatogram

Table 4: Sample solution system suitability parameters

Peak Results						
	RT	Height	Area	%Area	RT Ratio	Name
1	3.499	377	2543	0.011	0.586	
2	5.445	1058	11257	0.047	0.912	Bro-4
3	5.973	2640693	23731918	99.898		Bro-5
4	8.963	639	4517	0.019	1.501	
5	10.326					Bro-2
6	12.587					Bro-3
7	15.076	386	4010	0.017	2.524	Dibromo Impurity
8	19.152	113	1929	0.008	3.207	

system suitability, System precision, specificity, linearity, limit of detection and limit of quantification & Range, Method precision, Robustness.¹⁸⁻²⁰

System suitability Parameter

To verify that analytical system is working properly and can give accurate and precise results,

the system suitability parameters are to be set. System suitability tests were carried out on freshly prepared Weigh about 10mg standard solutions of Bromofenac sodium sesquihydrate and Bro-4 in 100 ml volumetric flask. Dissolve it by sonication till the solution is clear and make up to the mark with diluent. Dilute 200 µL of the solution in to 10

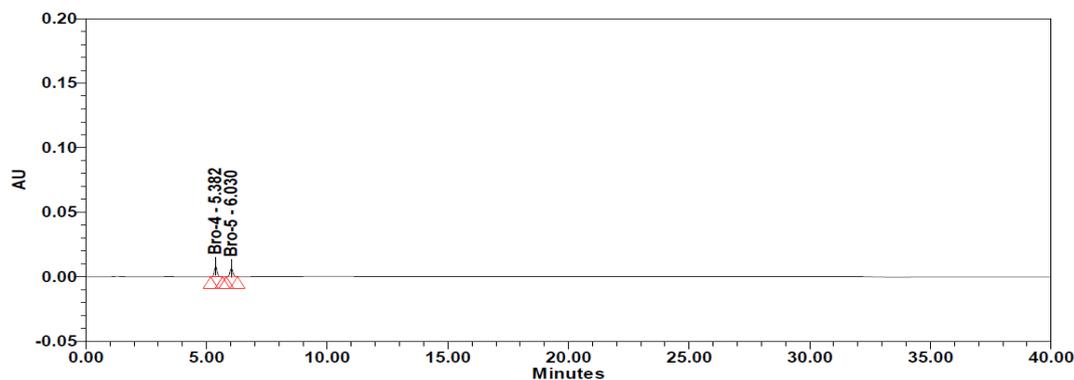


Fig. 5: System suitability solution chromatogram

Table 5: Results

Peak Results									
	RT	Height	Area	%Area	RT Ratio	Name	USP Plate Count	USP Resolution	USP Tailing
1	5.382	8147	63629	53.876	0.892	Bro-4	10629.82		1.08
2	6.030	6313	54475	46.124		Bro-5	11141.45	2.93	1.07
3	10.326					Bro-2			
4	12.587					Bro-3			
5	14.679					Dibromo Impurity			

ml volumetric flask and dilute up to the mark with diluent. System suitability solution were analysed using the same chromatographic conditions. it was calculated by determining the standard deviation of the values were recorded in Table 5. The system suitability method acceptance criteria set in each validation run were tailing factor ≤ 2.0 and theoretical plates >2000 , Resolution >1.5 between two closely eluting peaks (Half width) or product specific. In all cases, the relative standard deviation (R.S.D) for the analytic peak area for two consecutive injections was not More than 2%. A chromatogram obtained from reference substance solution is presented. System suitability parameters were shown in Table.5. System suitability solution Standard chromatogram was given in Figure.5. The total results of system suitability studies summarized in Table 6. In this studies %RSD

value of retention times, peak areas, tailing factor and theoretical plate count, Resolution were found to be less than 2% for Bromofenac sodium sesquihydrate (Bro-5) and 7-(4-bromobenzoyl)-1,3-dihydro-indol-2-one¹⁸⁻²⁰.

RESULTS AND DISCUSSIONS

System suitability parameter System precision

The system precision is checked by using standard chemical substance to ensure that the analytical system is working properly. The retention time and area of Six determinations is measured and % RSD shall be calculated and it is Not More than 1.0%. The total results of system precision studies summarized in Table 7¹⁸⁻²⁰.

Table 6: Results for System suitability parameter

S. no	Name of the Impurity	Retention Time	Area	Resolution	Tailing	Plate count
1	BRO-4	5.382	63629	NA	1.08	10629.82
		5.385	63043	NA	1.09	10663.68
		5.389	63728	NA	1.09	10656.40
		5.386	62527	NA	1.08	10630.68
		5.374	62366	NA	1.08	10659.39
		5.305	62013	NA	1.08	10686.11
%RSD		0.602069	1.112485686	NA	0.4766749	0.4931055
2	BRO-5	6.030	54475	2.93	1.07	11141.45
		6.054	54766	3.02	1.07	11125.80
		6.06	54536	3.02	1.06	11088.16
		6.052	52658	3.04	1.07	11475.89
		6.030	53396	2.99	1.07	11447.37
		5.912	53563	2.9	1.07	11225.84
%RSD		0.927097	1.528861873	1.8802846	0.3821357	1.508197017

Table 7: System Precision parameters

Preparation	Area	Retention time	Tailing Factor
1	23731918	5.973	1.18
2	23531716	5.971	1.17
3	23631615	6.125	1.16
4	23431514	5.865	1.15
5	23519818	6.001	1.16
6	23481645	6.120	1.14
STDEV Average	73982.02155	0.073426	0.014142
STDEV	23519261.6	6.029167	1.16
%RSD	0.31	1.22	1.22

Preparation of standard solution

Accurately weigh & transfer about 30.0mg of Bromofenac sodium sesquihydrate standard into a 10 ml volumetric flask. Dissolve dilute to volume with diluent.

System precision

From the above tabulated data, it can be concluded that the system precision parameters meets the requirements of method validation.

Specificity Parameter

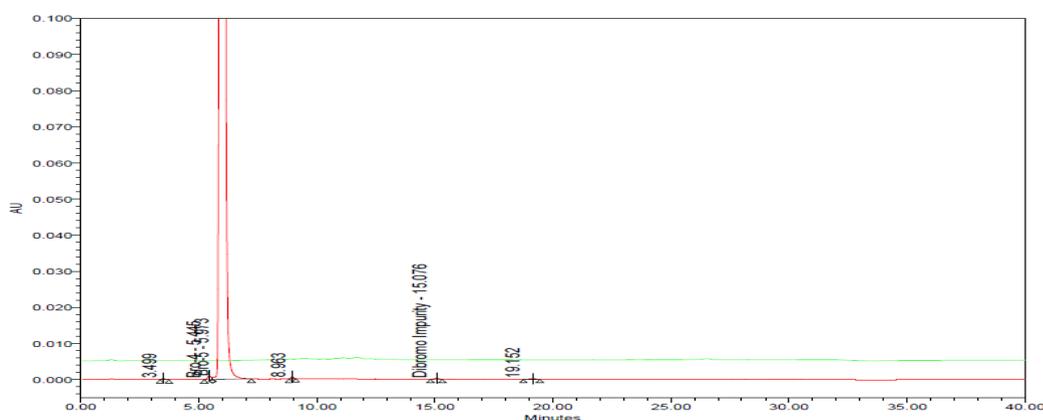
Specificity is the ability of analytical method to assess the analyte in the presence of components that may be expected to be present, such as impurities, degradation products and matrix components¹⁸⁻²⁰.

Specificity tests were carried out on above prepared standard Bromofenac sodium sesquihydrate solution of and it was determined by injecting the mobile phase and placebo solution in triplicate and recording the chromatograms specify solution (standard solution) for Bromofenac sodium sesquihydrate Standard Solution.

Table 8: Specificity Parameters

Peak name	RT
Mobile phase	No peaks
Placebo	No peaks
Bromofenac sodium sesquihydrate standard solution	5.985

From the above data (Table 8), (Fig. 6) Proves that method is specific that is there is no interference of placebo peaks in Bromofenac sodium sesquihydrate Standard solution.

**Fig. 6: Over laid chromatogram for specificity**

Linearity

The linearity of an analytical method is its ability to elicit test results that are directly or by a well-defined mathematical transformation, proportional to the concentration of analyte in sample within a given range¹⁸⁻²⁰.

The developed method has been validated as per International Conference on Harmonisation (ICH) guidelines the Standard test solutions of Bromofenac sodium sesquihydrate in the mass concentration range of 25% to 150% was injected into the chromatographic system. The

Table 9: Linearity different levels of concentrations

Level	Weight of test solution taken with Respect to Maximum impurity level	Dissolved and made up volume with diluent
25%	37.5mg	100ml
50%	75.05mg	100ml
75%	112.5mg	100ml
100%	150.1mg	100ml
125%	187.6mg	100ml
150%	225.1mg	100ml

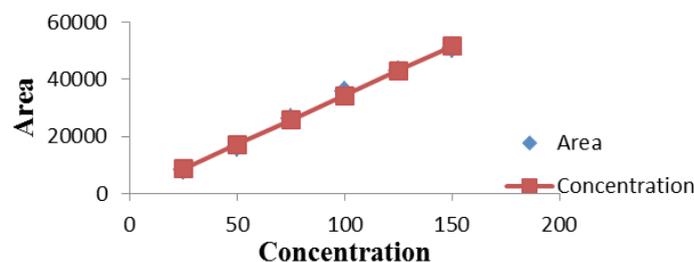
Table 10: Linearity parameters

Level	Concentration in mg/ml	Area
25%	0.3750	8433
50%	0.7505	16378
75%	1.1250	26131
100%	1.5010	35600
125%	1.8760	42840
150%	2.251	50767
Slope	343.4571	
Correlation Co-efficient	0.999	
Regression Coefficient	0.998	

Table 11: Residual output for Linearity parameters

Observation	Residual Output	
	Predicted Area	Residuals
1	8558.76	-125.7619047
2	17145.19	-767.1904762
3	25731.62	399.3809524
4	34318.05	1281.952381
5	42904.48	-64.47619048
6	51490.90	-723.9047619

Linearity for Bromofenac sodium sesquihydrate

**Fig. 7: Calibration curve for Linearity**

chromatograms were developed and the peak area was determined for each concentration of the drug solution. Calibration curve of Bromofenac sodium sesquihydrate was obtained by plotting the peak area ratio versus the applied concentrations

of Bromofenac sodium sesquihydrate. The linear correlation coefficient was found to be 1.0 (0.999). The Values & Calibration curve were recorded in Table 10 & Fig. 7¹⁸⁻²⁰.

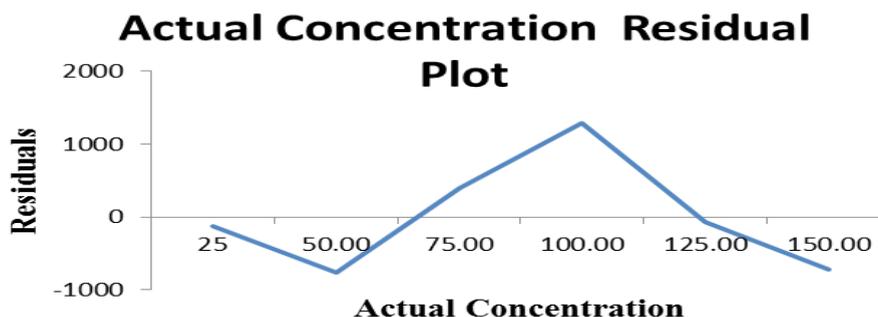


Fig. 8: Residual plot for linearity parameters

Linearity of Solutions from 25% to 150% was prepared with respect to the maximum percentage of known impurity level.

Preparation of linearity solutions

Injected each solution once into the HPLC system and plotted the calibration curve by taking concentration (mg/ml) on X-axis and peak area on Y-Axis and calculated the correlation coefficient .

Table 12: LOD & LOQ Theoretical Results

Levetiracetam	
Theoretical LOD in mg/ml	0.0736mg/ml
Theoretical LOQ in mg/ml	0.2230mg/ml

Table 14: Preparations of 150% test solution

Spiked Prep.	Weight taken	Dissolved in diluent
1	225.1mg	100ml
2	225.2mg	100ml
3	225.4mg	100ml
4	225.3mg	100ml
5	224.9mg	100ml
6	225.5mg	100ml

Table 13: Preparations of 100% test solution

Spiked Prep.	Weight taken	Dissolved in diluent
1	150.1mg	100ml
2	149.9mg	100ml
3	150.5mg	100ml
4	149.8mg	100ml
5	149.9mg	100ml
6	150.2mg	100ml

Table 15: Method precision Results For Peak Responses of 100% and 150% of specification level

Preparation	100%	150%
1	35693	50767
2	35826	50787
3	35888	50757
4	35798	50747
5	35853	50748
6	35805	50999
Average	35834	508000
STDEV	37.00676	98.20064
% RSD	0.10	0.19
Acceptance criteria	The %RSD for impurity area -Not more than 2%	

From the above data, it is clear that the area response of Bromofenac sodium sesquihydrate vs concentration in percentage of Bromofenac sodium sesquihydrate linear in the range of interest. The correlation coefficient and regression coefficient calculated from regular plot is greater than 0.999. Hence the method is linear for the residual determination of Bromofenac sodium sesquihydrate.

Limit of Detection & Limit of Quantification

Limit of detection is the lowest amount of analyte in a sample that can be detected, but not necessarily quantitated, under the stated experimental conditions¹⁸⁻²⁰.

Limit of quantification is the lowest amount of analyte in a sample that can be quantitated with acceptable precision, under the stated experimental conditions¹⁸⁻²⁰.

Limit of detection and Limit of quantitation were calculated using following formula.

$LOD = (3.3 \times \text{Residual standard deviation}) / \text{slope}$.
 $LOQ = (10 \times \text{Residual standard deviation}) / \text{slope}$.
 The LOD and LOQ values are presented in Table 12.

Performed a regression analysis of the linearity data with concentration vs ppm on X-axis. Calculated the residual standard deviation of the Y data. Calculated the slope of the linearity curve generated with concentration on X-axis and area response on Y-axis. The % RSD for area response of Bromofenac sodium sesquihydrate six replicates at LOQ level was found to be 0.10%.

Method precision

Precision is a measure of the degree of repeatability of the analytical method, determined by analyzing sufficient number of aliquots of a homogenous sample solution. To study precision, three triplicate solutions of 100% and 150% Bromofenac sodium sesquihydrate were prepared and analyzed using the proposed method i.e. Injected each solution once in to the chromatograph.

Table 16: Linearity Results for Range

Linearity*	Correlation:0.999
Acceptance criteria	Correlation Coefficient- Not less than 0.999

Table 17: Precision Results for Range

Precision(%RSD)*	
At 100% level	0.10%
At 150% level	0.19%
Acceptance criteria	The % RSD for impurity content-Not more than 2.0%

Table 18: Solution Stability result

Impurity	Solution Stability			Variation %		Variation		Acceptance Criteria
	Initial	1 st day	2 nd day	1 st day	2 nd day	1 st day	2 nd day	
Purity	99.898	99.890	99.895	0.008	0.003	0.00001	0.00003	Within±15%

Table 19: Mobile Phase Stability results

Impurity	Solution Stability			Variation %		Variation		Acceptance Criteria
	Initial	1 st day	2 nd day	1 st day	2 nd day	1 st day	2 nd day	
Purity	99.898	99.889	99.897	0.009	0.001	0.0001	0.00001	Within±15%

Calculated %RSD for area. The percentage of relative standard deviation (% RSD) for peak responses was calculated and it was found to be 0.10 & 0.19% which is well within the acceptance criteria of not more than 2.0%. Results of Method precision studies are shown in Table.15¹⁸⁻²⁰.

Preparation of Method precision solutions for test solution

Results of Method precision

Range

Range is defined as the range of concentration in which method is linear, precise and accurate. For range, data was considered from

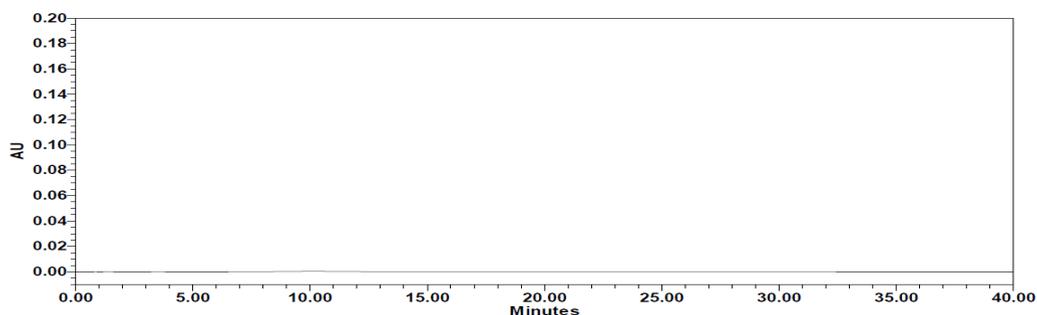


Fig. 9: Blank Solution Initially prepared

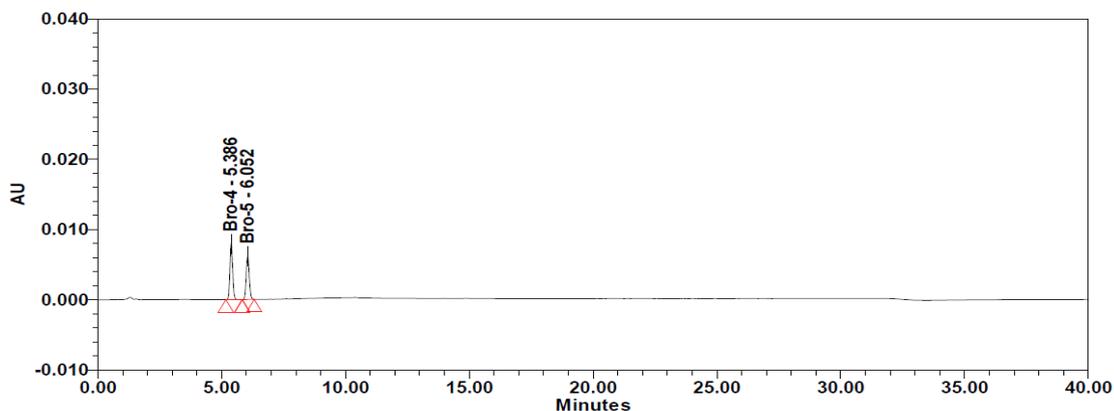


Fig. 10: System Suitability Solution Initially prepared

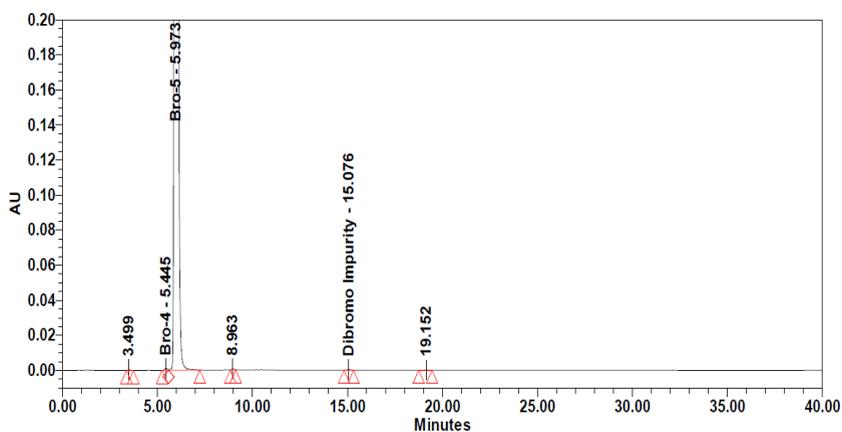


Fig. 11: Standard Solution Initially Prepared

linearity and precise sections. Range was performed for the test solution at 25% to 150% of specification level and found it to be precise, accurate and linear¹⁸⁻²⁰.

Test solution and mobile phase stability

Established the stability of standard solution, test solution and mobile phase which was

used in estimation of % of purity, over a period of 2 days. Prepared the standard solution and test solution at 100% of specification. Prepared the mobile phase as per the test method and kept it well-closed condition. Injected blank, Standard solution and test solution freshly and injected into HPLC system by following the conditions described in test method. Calculated the % purity for test solution as

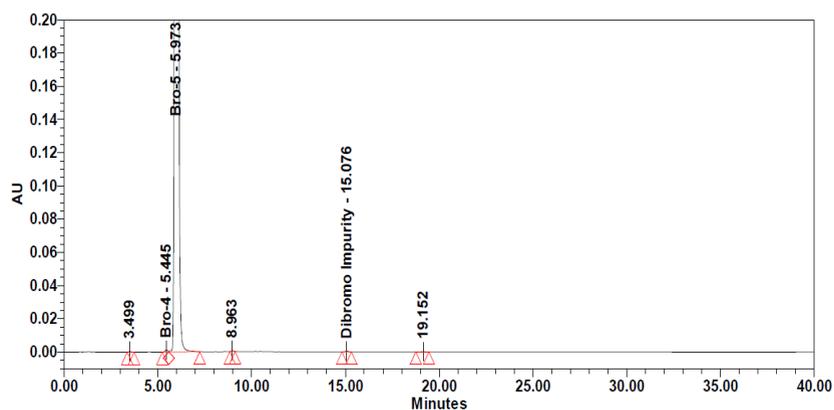


Fig. 12: Sample Solution Initially Prepared

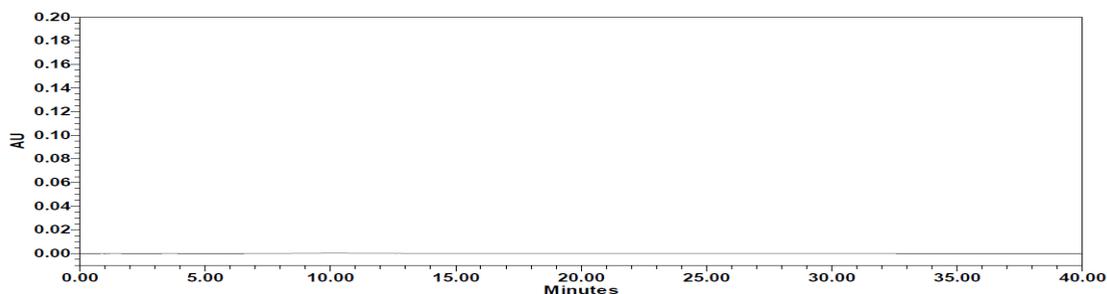


Fig. 13: Blank After 24 hours injected chromatogram

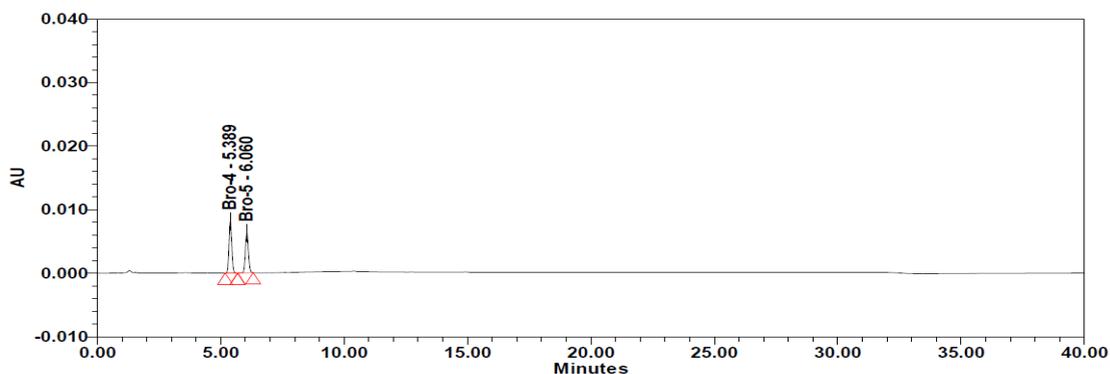


Fig. 14: System Suitability Solution After 24 hours injected chromatogram

per the test method. Stored the mobile phase on bench top .Stored the standard solution and test sample solution on bench top¹⁸⁻²⁰.

On day 1 and day 2, used the stored mobile phase and injected stored system suitability

solution and test solutions followed by injected freshly prepared standard solution and test solution. Test solution and mobile phase are found to be stable for 48hours from the time of preparation .System suitability results of resolution solution are within the acceptance criteria up to 48hours from the time of

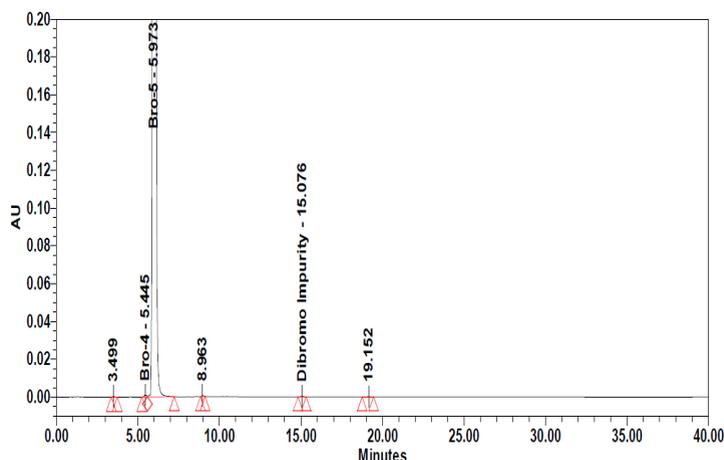


Fig. 15: Standard After 24 hours

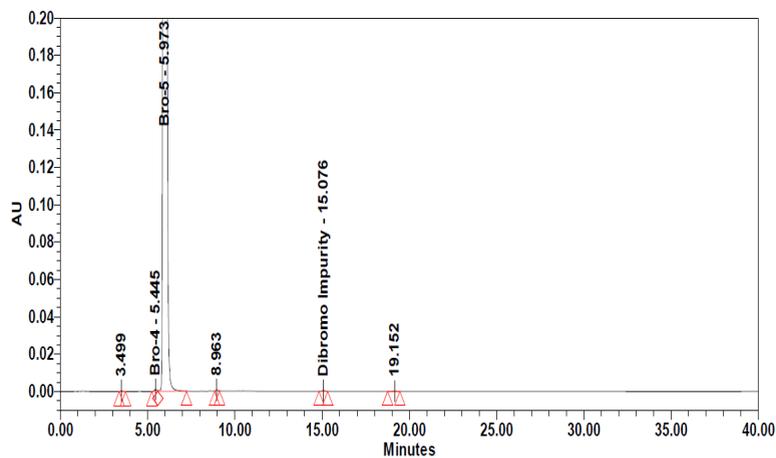


Fig. 16: Sample After 24hours

Table 20 :

S.no	Parameter	Condition	Area
1	Standard	Standard conditions	23731918
2	Mobile phase –B	Acetonitrile85% & Water15%	23741028
3	Mobile phase	pH 5.0	23751214
4	Wavelength	261 nm	23781354

preparation. The results from these studies indicated, the standard & sample solution was stable at room temperature for at least 48(48h). Calculated the % of

purity for stored test solutions and freshly prepared solutions as per the test method for estimation of test sample solution and mobile phase stability. Results

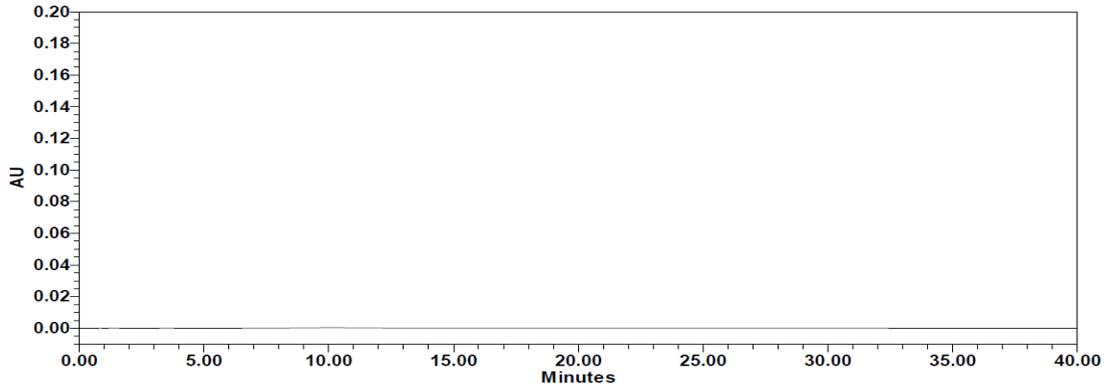


Fig. 17: Blank After 48 hours injected chromatogram

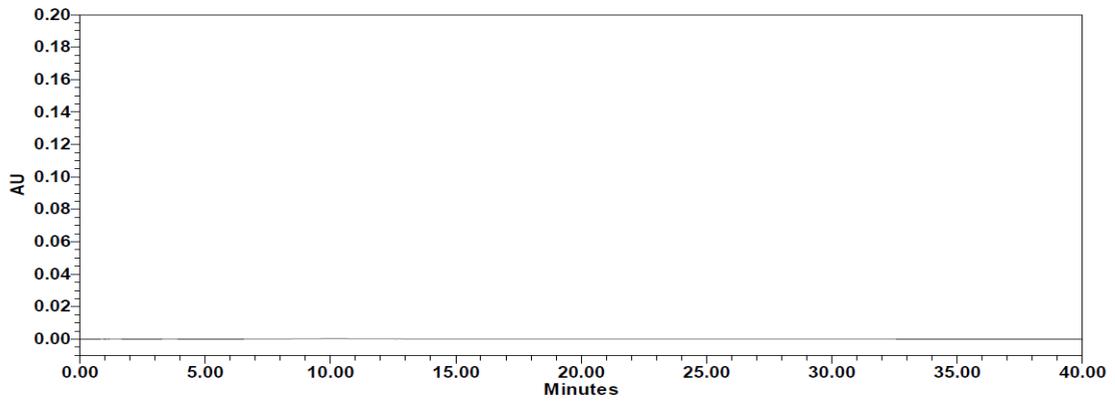


Fig. 18: Blank

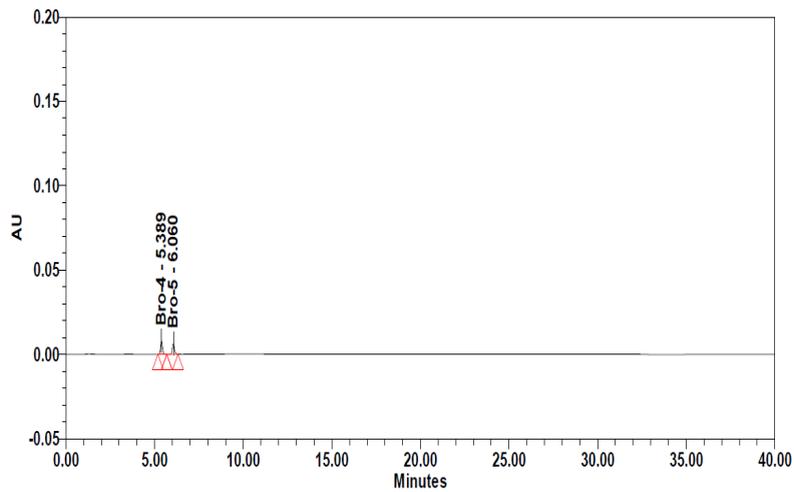


Fig. 19: Standard chromatogram

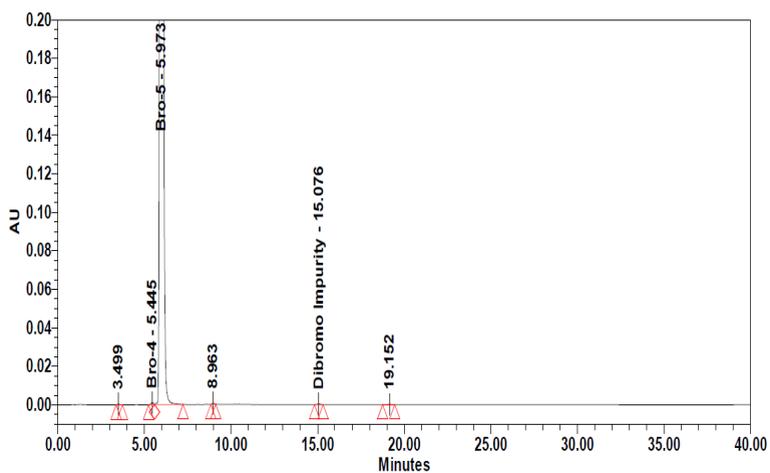
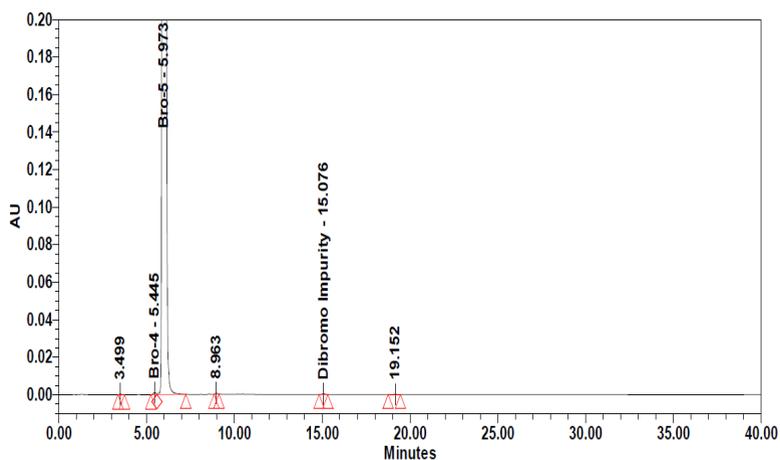
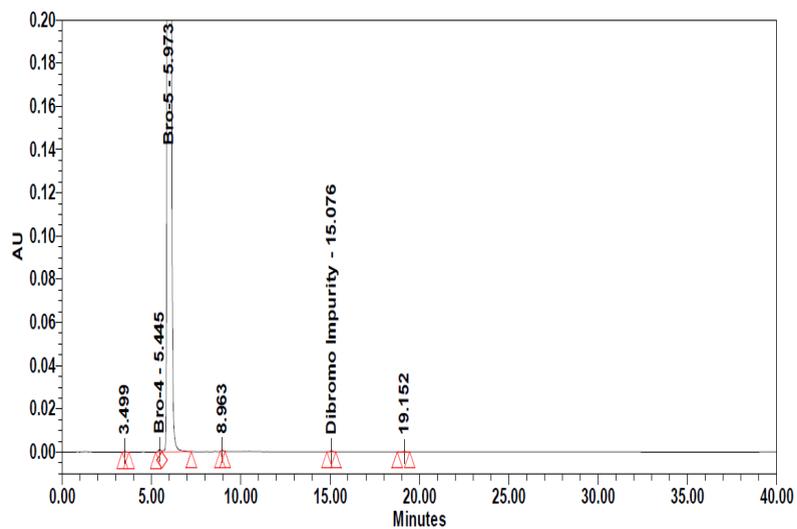
Fig. 20: 1st Sample chromatogramFig. 21: 2nd Sample chromatogramFig. 22: 3rd Sample chromatogram

Table 21: Summary and Evaluation of Results

Validation parameter	Acceptance criteria	Results
System suitability	The system suitability method acceptance criteria set in each validation run were tailing factor ≤ 2.0 and theoretical plates >2000 , Resolution >1.5 between two closely eluting peaks (Half width) or product specific. In all cases, the relative standard deviation (R.S.D) for the analytic peak area for two consecutive injections was not More than 2%.	System suitability parameter meets the criteria and %RSD value of retention times, peak areas, tailing factor and theoretical plate count, Resolution were found to be less than 2%.
System precision	The % RSD of retention time and area of 10 determinations should not more than 1.0%	System precision parameters meets the requirements of method validation. Method is specific that there is no interference of placebo peaks in Bromofenac sodium sesquihydrate Standard solution
Specificity	No interference of placebo peaks in Bromofenac sodium sesquihydrate Standard solution	Method is specific that there is no interference of placebo peaks in Bromofenac sodium sesquihydrate Standard solution
Linearity	The correlation coefficient and the regression coefficient between concentration and area response of Bromofenac sodium sesquihydrate should be NLT 0.999	The correlation coefficient and regression coefficient calculated from regular plot is greater than 0.999
LOD/LOQ	The% of RSD for area response of Bromofenac sodium sesquihydrate from six replicates at LOQ level should be NMT 10.0%	LOD/LOQ parameters meets the requirements of method validation.
Method Precision	% of RSD for purity and Area at 100% of specification and 150% of specification level not more than 2.0%	Method precision parameter meets the criteria and %RSD value of retention times, peak areas were found to be less than 2%.
Range	At 25% to 150% of specification level to be precise , accurate and linear.	Meet the criteria At 25% to 150% of specification level and found it to be precise , accurate and linear.
Test solution and Mobile phase Stability	Established the stability of standard solution , test solution and mobile phase which was used in estimation of % of purity, over a period of 2 days.	The results from these studies indicated, the standard & sample solution , Mobile phase was stable at room temperature for at least 48(48h).
Robustness	A small deliberate variations in method parameters like Mobile Phase –B ($\pm 5\%$ of organic Phase), Wave length ($\pm 2\text{nm}$), and pH (± 0.2).	From the results reveal that the method is robust.

of test solution and mobile phase stability are shown in Table.18 & 19 .The sampled chromatograms are recorded as below in Fig.9, Fig.10, Fig.11, Fig.12, Fig.13, Fig.14, Fig.15, Fig.16 & Fig.17

Robustness

Typical variations in liquid chromatography conditions were used to evaluate the robustness of the assay method. In this study, the chromatographic parameters monitored were retention time, area, capacity factor, tailing factor and theoretical plates. The robustness acceptance criteria set in the validation were the same established on system suitability test describe above¹⁸⁻²⁰.

Record of analysis for levetiracetam samples

Triplicate Bromofenac sodium sesquihydrate samples are run successfully by using this method and the experimental results & chromatograms are recorded in (Fig. 18), (Fig. 19), (Fig. 20), (Fig. 21) and (Fig. 22)¹⁸⁻²⁰.

Significance of the developed method

Developed Gradient High Performance Liquid Chromatographic indicating method has many advantages over reported methods: (a) the method was simple because mobile phase used was cheap and easily available; (b) total run of chromatogram was 40min and Bromofenac sodium sesquihydrate (Bro-5),7-(4-bromobenzoyl)-1,3-dihydro-indol-2-one(Bro-4), (4-Bromo phenyl)-(1H-indol-7-yl) methanone (Bro-2),(3-Bromo-1H-indol-7-yl)-(4-bromo-phenyl)-methanone(Bro-3) & (4-Bromo-Phenyl)-(2,3-dibromo-1H-indol-7-yl)-methanone (Dibromo Impurity) were eluted within 20 min indicating that very less amount of mobile phase was consumed. (c) the limit of detection for Bromofenac sodium sesquihydrate was 0.0736mg/ml and the limit of quantification was 0.2230mg/ml, respectively, indicating that the method was sensitive and rapid; (d) specificity study(Figs. 1 and 2) and indicate that the method was very specific, stable in the proposed method.(e) mode of separation is Gradient which mean sit is easy to operate throughout the process with out any complications (f) simultaneous estimation of different brands

gives precise results indicating that developed method is compatible to estimate in different active pharmaceuticals ingredient (g) validation of the developed method as per ICH guideline indicates that the method was highly precise, rapid, simple, economical, sensitive accurate, robust and specific for determination of related impurities of Bromofenac sodium sesquihydrate in bulk and pharmaceutical dosage form.

RESULTS

The primary target in developing & Validate Gradient Related Substance High Performance Liquid Chromatographic method developed for the analysis of Bromofenac sodium sesquihydrate in their pharmaceutical preparations method is to achieve the optimum resolution between products with other products to supply high purity of drug i.e. Based on the above observed results the developed Rapid Resolution High Performance Liquid Chromatographic (RRHPLC) validation method for Levetiracetam is valid and run successfully the summary and evaluation of results are in below (Table 21)

CONCLUSION

The Gradient Related Substance High Performance Liquid Chromatographic method developed for the analysis of Bromofenac sodium sesquihydrate in their pharmaceutical preparations is precise, accurate, and with a reasonable run time. The developed method was validated as per ICH Guidelines shows that the developed method was highly specific and robust so that it can be effectively applied for routine analysis in research institutions, in quality control department of pharmaceuticals industries, and in approved testing laboratories, from the above experimental data on the various method validation parameters, it is proved that this method which was designed to determine the related impurities in Bromofenac sodium sesquihydrate is precise, accurate, linear, rugged, robust and range from 25% to 150% of the Specification . Hence, the method can be used for routine application.

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