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Research Article

RAPID UPLC METHOD FOR ESTIMATION OF BROMFENAC AND APPLICATION TO EYE DROPS

BHAVANI BODDEDA[1,*], LANKA A.RAMA PRASAD[1], J VIJAYA RATNA[1], ASIM BISWAL[2]

[1]Department of Pharmaceutical Technology, A.U. College of Pharmaceutical Sciences, Andhra University, Visakhapatnam 530003, India. Email: bhavani2008@gmail.com

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ABSTRACT

Objectives: New stability-indicating reverse phase UPLC method developed and validated for the estimation of Bromfenac and successfully applied for the estimation of it in commercially available eye drops.

Methods: The chromatographic conditions were optimized using the samples generated from forced degradation studies. The chromatographic separation was achieved on C18 UPLC column. The method employed a linear gradient elution and the detection wavelength was set at 230 nm. The mobile phases consists of buffer and acetonitrile delivered at a flow rate of 0.5 mL·min^{-1}. Proposed method was extensively validated as per ICH guidelines.

Results: Regression analysis shows an r value (correlation coefficient) of greater than 0.999 for individual active drug substances. The samples were assayed against a qualified reference standard and the mass balance was found to be close to 98.3%.

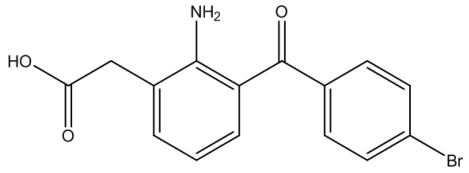
Conclusion: The developed method is also stability-indicating and can be used for the routine analysis of bromfenac crude drug and also check the purity and stability of the active substance in marketed eye drops.

Keywords: Bromfenac, UPLC, Eye drops and Stability indicating

INTRODUCTION

Ocular drug delivery is one of the most fascinating and challenging tasks facing the pharmaceutical researchers. Enhancement of ocular penetration of eye drops remains one of the most challenging tasks in ophthalmology [1-4]. One of the most common disorders in ophthalmic therapy is the ocular inflammatory disease affecting any part of the eye or the surrounding tissues [5, 6]. Topically applied non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used in the management and prevention of ocular inflammation and cystoid macular edema (CME) related to cataract surgery and the maintenance of mydriasis during cataract surgery [7, 8]. Although steroidal agents have been the standard treatment for ocular inflammation [9, 10], the use of NSAIDs has increased over the past two decades [9, 11-13], because of its several advantages over topical NSAIDs [14].

Bromfenac sodium (BFC; hereafter referred to as bromfenac), 2-amino-3- (4-bromobenzoyl) benzene acetic acid sodium salt sesquihydrate, is a potent non-steroidal, non-narcotic analgesic agent that has anti-inflammatory and antipyretic properties and has been shown to be effective and well tolerated (Fig.1) [15,16]. The advantages of non narcotic analgesicsin postoperative care lie in their good short-term tolerability [17] with a lower incidence of nausea and vomiting and no inhibition of respiration or intestinal motility [18]. The absence of abuse potential and the resulting easier dispensing procedures and documentation are additional benefits.



2-[2-amino-3-(4-bromobenzoyl)phenyl]acetic acid

Fig. 1: Structure of bromfenac

Bromfenac exhibits prostaglandin synthetase- inhibiting properties in the animal models. Because of the relatively narrow therapeutic indices of most non-steroidal anti-inflammatory compounds, it is important to monitor their concentrations in plasma in order to facilitate optimum dosage adjustment. Methods to assay non-steroidal anti-inflammatory drugs have been developed which range from direct spectrophotometric determination to the more selective gas chromatographic or high-performance liquid chromatographic (HPLC) procedures [19]. A literature survey reveals that analytical methods based on HPLC is available for the determination of this drug individually in plasma [20], there is no analytical method reported for stability-indicating [21] reverse phase UPLC method [22] The aim of the present work is develop and validate as per ICH [23, 24] a new simple, precise, accurate, and rapid method and application of the same for the estimation of bromfenac bulk form and eye drop formulation.

MATERIALS AND METHODS

Chemicals and Reagents

All the reagents were of ACS or HPLC grade unless stated otherwise. Milli-Q-water was used throughout the experiment. orthophosphoric acid (Merck, Mumbai, India), Methanol (J.T.Baker, Germany) and acetonitrile (J.T.Baker, Germany), were used. Bromfenac drug substance was procured from FDC Limited, Mumbai, eyedrops (Unibrom & Megabrom) manufactured by Ajanta Pharma & Sun Pharmacuticals Ltd, were bought from the local market.

Instrumentation

The LC system, used for method development, forced degradation studies and method validation was Waters Acquity H-Class (manufactured by Waters corporation, USA) LC system with a diode array detector. The output signal was monitored and processed using Empower 2 software (designed by Waters Corporation, USA) on Pentium computer (Digital Equipment Co).

Optimization of Chromatographic conditions

The analysis was carried out on Waters, Acquity HSS C18, 100 mm x 2.1 mm, column with 1.8µm particles, column maintained at 30°C. The mobile phase 0.01% v/v ortho phosphoric acid in water (pH 3.5±0.05) and acetonitrile in the ratio of 55:45%v/v. Flow rate was set of 0.5 mL/min in isocratic elution mode. Before delivering the mobile phase into the system, it was degassed and filtered through 0.22 µm PVDF filter using vacuum. The injection volume was 2 µL and the detection was performed at 230 nm using a photo diode array (PDA) detector. Various compositions of solution A and solution B with different ion-pairing agents were tested for this study. The typical retention time of bromfenac is about 3.2 minutes. The criticality of this method are to elute the active ingredient with optimum separation and symmetric peak shape with no interference

due to placebo or any potential impurities arising due to degradation or during shelf life. A model chromatogram for standard is shown in (Fig. 2). This method was applied for the quantification of Bromfenac in commercially available eye drops.

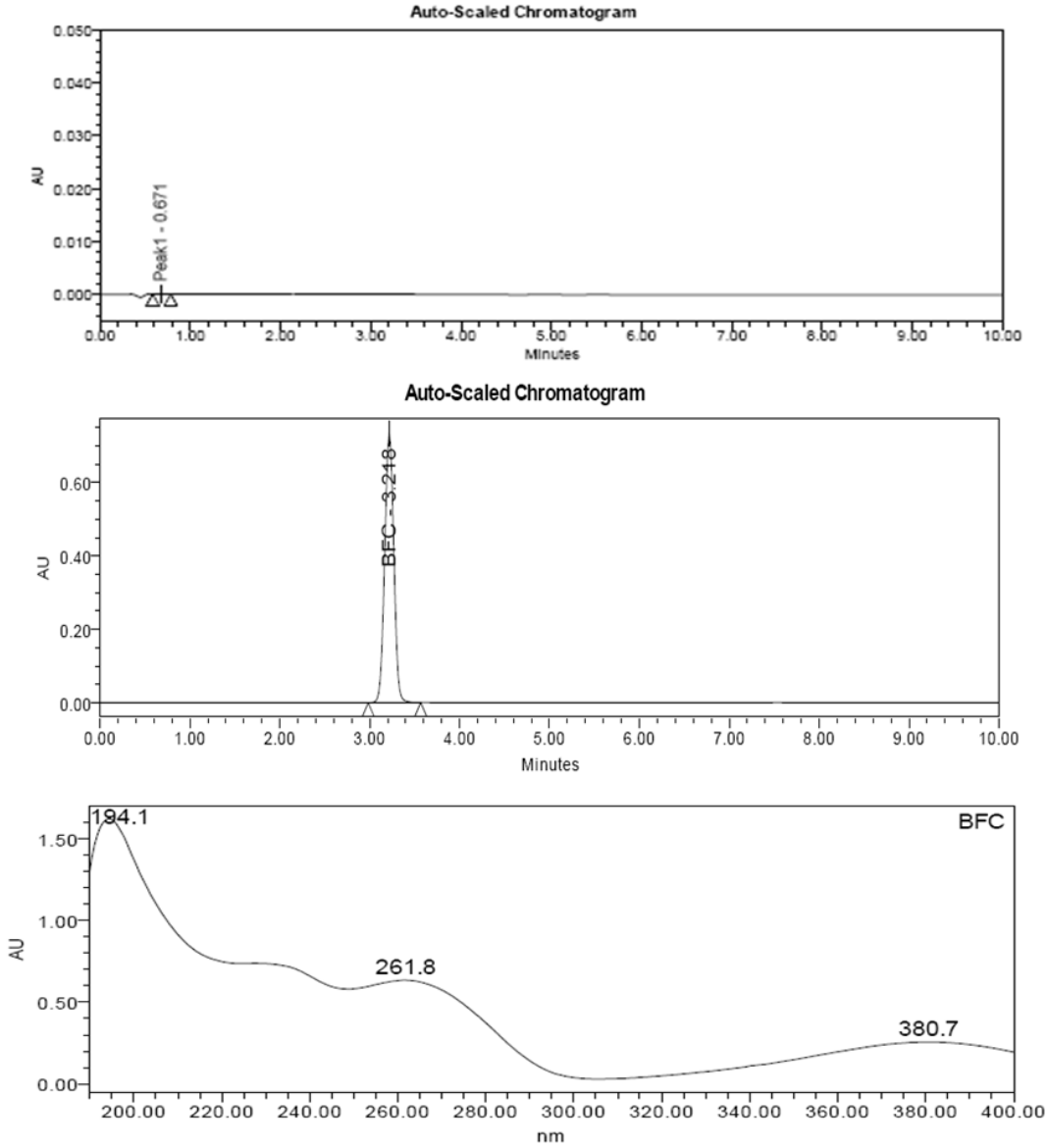


Fig. 2: Chromatograms of A. dilent as blank B. standard C. UV spectrum

Standard solution preparation

Prepared a solution of bromfenac in mobile phase to obtain a concentration of 500 µg/ mL

Sample Preparation

Sample solution was prepared in mobile phase using commercially available eye drops bought from local market, having a concentration of 500 µg/mL. This solution was filtered through 0.22µm membrane filter and discarded first few mL of the filtrate.

RESULTS AND DISCUSSION

Optimum separation between active ingredients from potential degradation impurities was achieved with the proposed conditions. The pharmaceutical formulation along with individual active ingredient was subjected to stress conditions of hydrolysis (acid and base), oxidation and thermal degradation as per International Conference on Harmonization (ICH) to show the stability-indicating power of the method. It was found Bromfenac is sensitive to various stress conditions and readily degrades into various degradation products. The chromatographic conditions were optimized using a solution from forced degradation studies.

Method validation

The aim of method validation was to confirm that the present method was suitable for its intended purpose as described in ICH guidelines Q2 (R1) [24, 25]. The described method has been extensively validated in terms of specificity, precision, linearity, accuracy and robustness. The precision was expressed with respect to the intra- and inter-day variation in the expected drug concentrations. The accuracy was expressed in terms of percent recovery of the known amount of impurities added to the sample preparation.

System suitability

System suitability tests are an integral part of a liquid chromatographic method, and they were used to verify that the proposed method was able to produce good resolution between the peaks of interest with high reproducibility. The system suitability was determined by injecting six replicate injections from freshly

prepared standard solutions and analyzing each injection for their peak area, theoretical plates (N), and tailing factors (T). System suitability requirements for the proposed method are (i) the theoretical pates (T) should not be less than 10000 for (ii) the % of RSD for peak area of Bromfenac from replicate injections of standard solution is not more than 2.0 (iii) the tailing factor is not more than 1.5. The results of the system suitability test in comparison with the required limits are shown in Table 1. According to the results presented, the proposed method fulfills these requirements within the accepted limits.

Table 1: System suitability data

Parameter	Result
Tailing	1.0
Theoretical Plates (T)	20565
%RSD	0.2

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix (placebo), etc. Specificity was tested by injecting the artificial tear drops prepared, saline solution, and solution obtained from degradation studies.

Preparation of artificial tear fluid (ATF)

The artificial tear fluids were prepared by dissolving 0.670g of sodium chloride, 0.200g, of sodium bicarbonate, 0.008g of calcium chloride dehydrate in about 100g of water; pH of resulting solution is 7.4

Forced degradation studies

Forced degradation studies were performed to provide an indication of the stability indicating property and specificity of the proposed method. Intentional degradation was attempted to stress conditions like acid hydrolysis (using 1 N HCL at 70°C for 1 hr), base hydrolysis (using 0.1 N NaOH at 70°C for1 hr), and oxidative degradation (using 3.0% H_2O_2 at 70°C for1 hr) to evaluate the ability of the proposed method to separate degradation products from each other and active ingredient as well. The forced degradation of samples are shown in the Fig. 3 & 4.To check and ensure the homogeneity (peak purity) of peaks in the stressed sample solutions, photodiode array detector was employed. In forced degradation it was observed that bromfenac is susceptible for degradation in acid and base stress conditions, results are tabulated in Table 2.

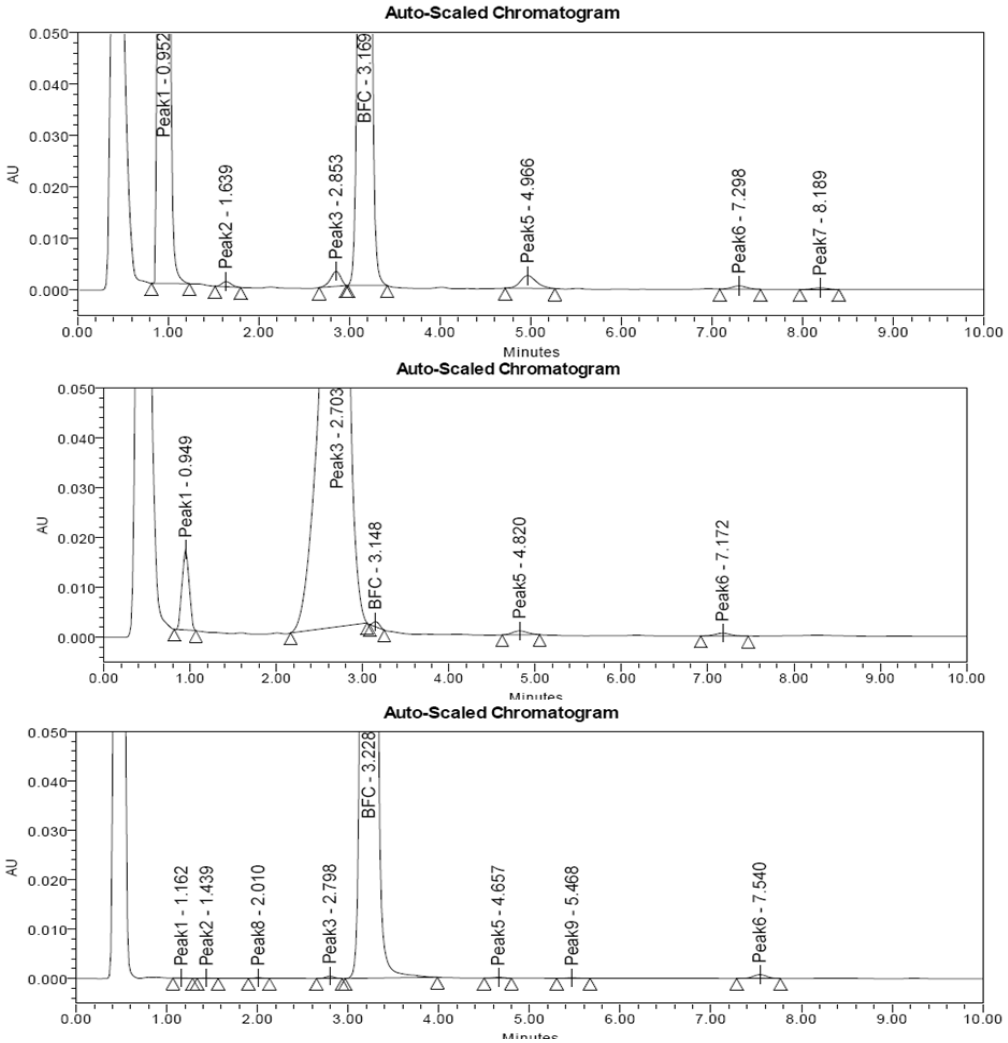


Fig. 3: Auto-scaled Chromatogram A. acid degradation B. base degradation C. oxidative degradation

Table 2: Forced degradation data

Stress condition	% Degradation	Observation	Purity Angle	Purity threshold
Acid stress condition	50.24	No interference at RT of analyte peak	0.110	0.297
Base stress condition	97.26	No interference at RT of analyte peak		
Oxidative stress condition	0.28	No interference at RT of analyte peak	0.396	0.494

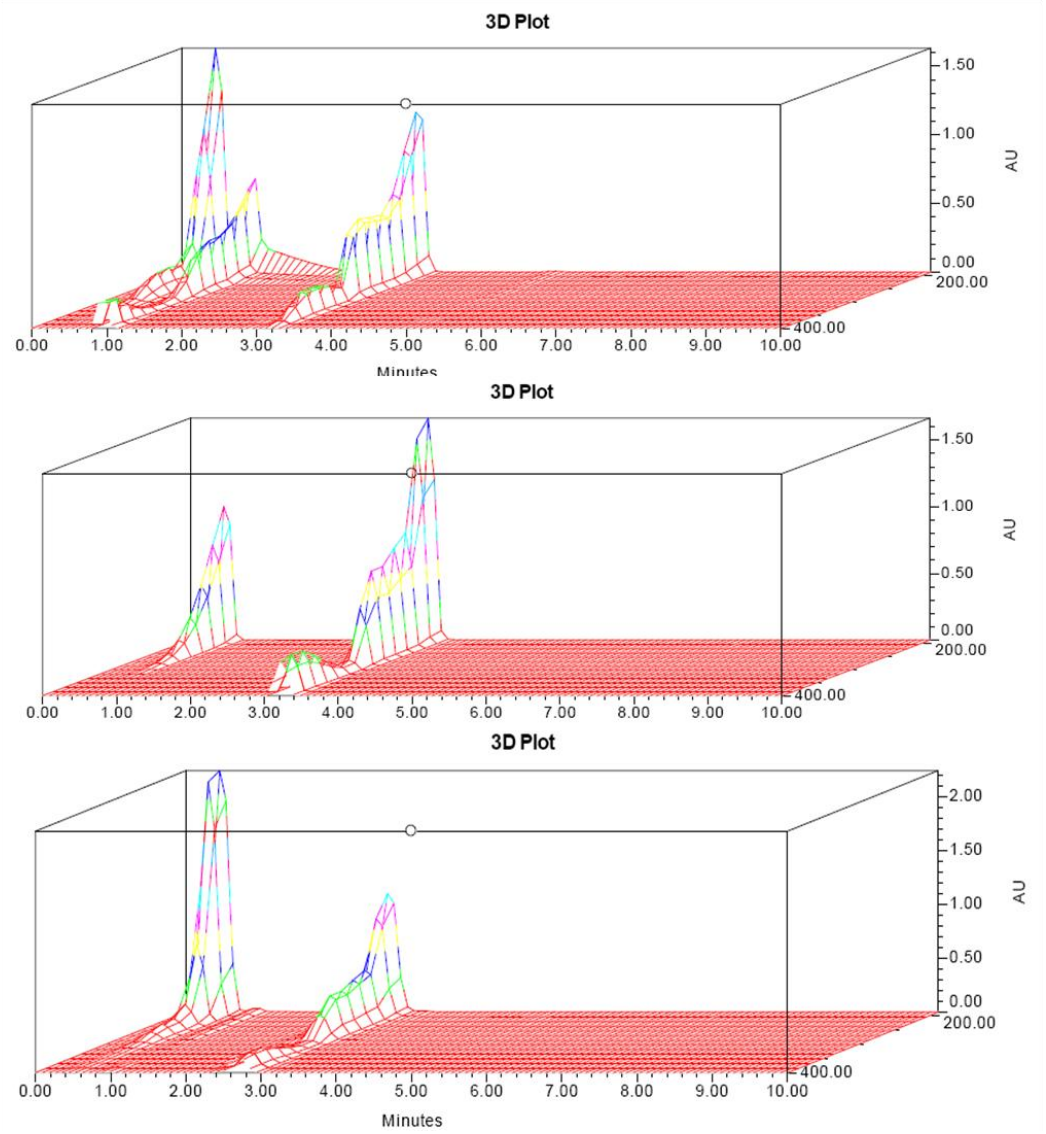


Fig. 4: 3D plots of typical chromatograms of A. acid degradation B. base degradation C. oxidative degradation

Linearity

The linearity of the method was tested in order to demonstrate proportional relationship of response versus analyte concentration over the working range. It is usual practice to perform linearity experiments over a wide range of analyte. This gives confidence that the response and concentration are proportional and consequently ensures that calculations can be performed using a single reference standard/working standard, rather than the equation of a calibration line. The linearity of detector response to different concentrations studied by preparing a series of solutions using bromfenac. The data were subjected to statistical analysis using a linear-regression model; the regression equations and coefficients (r^2) are given in Table 3. The results have indicated good linearity.

Table 3: Linearity data

Linearity Level	Concentration ppm	Average area	Statistical Analysis	
10%	50.28	670189	Slope	13162.24
20%	100.31	1310063	y-Intercept	27793.16
50%	250.44	3401130	% of y- Intercept	0.42
100%	500.25	6602459	Correlation Coefficient	0.9999
150%	750.38	9903689		
200%	1000.45	13186930		

Precision

Six sample solutions were prepared using single sample and the precision of the method was tested. The % RSD indicates that proposed method has got acceptable level of repeatability.

Ruggedness (Intermediate precision)

Ruggedness is the intraday variation obtained at different concentration levels, and is expressed in terms of RSD calculated for each day. The RSD values were found to be below 0.45%. The

intermediate precision is the interday variations calculated for six sample preparations in each set expressed in terms of % RSD values. Results indicate the proposed method has got a good intermediate precision. The ruggedness of the method was determined by analyzing the same samples in triplicate for 2 days by another instrument by a different analyst with different lots of reagents and columns. Results are tabulated in Table 4**.**

Accuracy

Accuracy of the proposed method was established by recovery experiments. This study was employed by spiking of known amounts of Bromfenac into the placebo samples of at 50%, 100% and 150% of targeted concentration, in triplicate and injected into the chromatographic system. The resulting mixtures were analyzed as described in proposed method. Results obtained from recovery studies are given in Table 5.

Robustness

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. In the present study, an experimental design was planned for robustness testing varying some conditions, e.g. Flow rate, column temperature, variation of buffer pH in the mobile phase and filter variability. The results are shown in Table 6. It can be seen that, with every employed condition, there were no dramatic changes in the chromatographic behavior. All parameters have been observed within the limits required for system suitability tests.

Table 4: **Precision and Intermediate Precision data**

Sample ID	% RSD	
	Precision data	Ruggedness data
Sample - 1	99.6	98.9
Sample - 2	97.8	99.6
Sample - 3	100.3	97.8
Sample - 4	99.8	100.1
Sample - 5	98.9	98.8
Sample - 6	99.2	99.5
Mean	99.26	99.11
SD	0.79	0.73
RSD	0.79	0.74

Table 5: Accuracy data

Concentration% of spiked level	% Recovery	Statistical Analysis of % Recovery	
50%Sample 1	98.7	MEAN	98.06
50%Sample 2	97.6	SD	0.46
50%Sample 3	97.9	%RSD	0.469
100%Sample 1	98.2	MEAN	98.46
100%Sample 2	98.5	SD	0.21
100%Sample 3	98.7	%RSD	0.213
150%Sample 1	98.3	MEAN	98.09
150%Sample 2	98.1	SD	0.16
150%Sample 3	97.9	%RSD	0.163

Table 6: Robustness data

Parameter	Deliberate change	Minimum theoretical plates	Maximum tailing factor
Flow rate (0.5mL/min)	0.4mL/min	14063	1.2
	0.6mL/min	13980	1.0
Temperature (30°C)	25°C	14110	1.1
	35°C	14032	1.0
pH of buffer (3.5)	3.3	14008	1.0
	3.7	14180	1.1

Stability of Analytical solutions

The stability of the resolution, standard and sample solutions is tested at regular intervals. The stability of solutions was determined by comparing results with freshly prepared standard solutions. The differences in values were within 0.3% up to 48 hrs.

Application for Eye drops

Optimized method was successfully applied for the assaying of commercially available eye drops Unibrom and Megabrom were tested. These were also tested for other physic chemical properties like Osmolality, pH and clarity test and results were tabulated in Table 7**.**

Table 7: Results of commercial samples

Stress condition	Unibrom	Megabrom
pH	7.6	7.4
Osmolality (mOsm/kg)	302	306
% Assay	96.3	97.1

CONCLUSION

The validated stability-indicating UPLC method has proved to be simple, accurate, precise and reliable. The proposed method provides a good resolution between all the three active ingredients and potential degradants. The developed method reported herein was validated by evaluation of the validation parameters as described in ICH guidelines. System suitability, specificity, linearity, precision, accuracy and robustness of the proposed technique were obtained during the validation studies. The developed method is also stability-indicating and can be used for the routine analysis of bromfenac crude drug and also check the purity and stability of the active substance in marketed eye drops.

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