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Analytical Method Development and Validation for the Quantification of Bromofenac Sodium Sesquihydrate in Bulk and Its Formulation by Gaussian Distribution and Area Under the Curve Spectrophotometric Methods

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Abstract

The aim of the present work was to determine the validation characteristics for the developed methods of the quantification of Bromofenac Sodium Sesquihydrate (BFS) by absorption spectrophotometry. The method is based on Gaussian distribution (Zero, First and Second Order) and Area under the Curve (AUC) method. The entire study was explained in four methods which includes three gaussian methods i.e., Zero order (Method 1), First order (Method 2), Second order (Method 3) and other was AUC (Method 4) spectroscopy. Methanol was used as a diluent for all the methods. The amplitudes for the three Gaussian distribution methods were measured at wavelengths of 260 nm (Method 1), 257.5 nm (Method 2) and 241.5 nm (Method 3) respectively. AUC (Method 4) was studied at the integrated areas of 256 and 266 nm. The developed methods showed linearity in the range of 2-12 µg/ml. The correlation co-efficient was found to be ≥ 0.999 and the results from all the spectrophotometric methods proved that the technique developed by us is accurate, reproducible and meets the current requirements.

Key words: Bromofenac Sodium, Gaussian distribution, AUC, Validation.

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1. Introduction

Bromofenac Sodium Sesquihydrate (BFS) (Figure 1), chemically, it is a sodium salt of 2-amino-3-(4-bromobenzoyl) phenyl acetic acid [1]. It is a non-steroidal anti-inflammatory drug (NSAID) [2-3] acts by inhibiting the prostaglandin synthesis by blocking the cyclooxygenase (COX) enzymes [4-5]. BFS is indicated during ophthalmic surgery including postoperative inflammation, reduction of pain after cataract and refractive surgery [6-7], and management of macular edema after cataract surgery [8]. In addition to anti-inflammatory action, BFS also have antipyretic, analgesic and platelet-inhibitory actions [9].

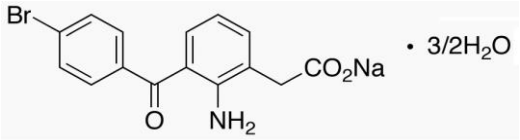

Figure 1. Structure of Bromofenac Sodium.

Literature reveals that few UV spectrophotometric methods in combination [10-11], Colorimetric [12] and HPLC [13-15] methods have been reported for the analysis of BFS. All the reported works were carried out in combination with other drugs and followed simultaneous methodology for pharmaceutical dosage forms and one for injections reported. No analytical method has been reported by using derivative spectroscopy, hence, we have decided to develop selective and precise new method and perform validation for the determination of BFS in dosage form by spectrophotometry.

2. Experimental

2.1. Materials

Reference standard of BFS (99.99%) was kindly provided by Enaltec Lab Pvt. Ltd., Mumbai, India. AR grade Methanol was procured from Merck Pvt. Ltd, Mumbai, India. Distilled water was prepared using Milli Q system in laboratory. All glass wares used were calibrated for Class A type. Instrument used was an UV-Visible double beam spectrophotometer, Shimadzu, Japan (model UV-1800, software-UV probe, version 2.52) with a pair of 1 cm matched quartz cells. All weighing was done on Mettler Toledo electronic analytical balance.

2.2. Method Development

The analytical method was developed by employing the drug in suitable solvent based on solubility profile. The method optimization is done by selecting the absorption maxima at its wavelength.

2.3. Selection of detection wavelength

For the selection of analytical wavelength, 1 mg/ml BFS solution was prepared from the standard drug solution and scanned in the range of 190 to 400 nm. From the UV spectra, the maximum λmax of BFS was measured at different amplitudes for all the methods. The obtained λmax was 260 nm, 257.5 nm and 241.5 nm for method 1, method 2 and method 3 respectively. AUC (Method 4) was studied at the integrated areas of 256 and 266 nm.

2.4. Preparation of calibration standards of BFS

Ten milligrams of BFS was accurately weighed and dissolved in 10 ml of methanol to get 1000 µg/ml. From this stock, stock solution working standards ranging from 2-12 µg/ml were prepared using methanol. The concentration (x-axis) versus mean absorbance (Y-axis) served as a calibration curve for quantification of BSF. Regression coefficient was used to validate the concentration range and regression equation was used to quantify BFS.

2.5. Preparation of Sample solution

From the Ophthalmic formulation, drug equivalent to 10 mg of BFS was drawn accurately and transferred to a 10 ml volumetric flask. A small quantity of methanol was added to dissolve and sonicated for 5 min. The volume was made up to the mark with the same solvent

to give 1000 µg/ml of BFS. 1 ml of the above solution was further diluted to 10 ml with distilled water and analyzed.

2.6. Method Validation

The method was validated for all validation parameters as per ICH Q2 guidelines [16]. The specificity of the method was good and it was proven by analyzing the sample.

2.6.1. *Linearity and Range*

The linearity of analytical method is its ability to elicit test results which are directly proportional to the concentration of analyte in sample within a given range. The range of analytical method is the interval between the upper and lower levels of analyte that has been demonstrated to be determined within a suitable level of precision, accuracy and linearity.

2.6.2. *Precision*

Standard solutions of BFS were prepared of linearity range and spectrums were recorded. Absorbance was measured for all the methods. The absorbance of the same concentration solution was measured six times and RSD was calculated.

2.6.3. *Accuracy*

Accuracy was determined by calculating recovery of BFS by the standard addition method. Known amounts of standard solutions of BFS were added to a pre-quantified test solution. Each solution was measured in triplicate, and the recovery was calculated by measuring absorbance.

3. Results and discussion

From overlain spectra of BFS, it is clear that BFS exhibited λmax at respective wavelengths for all four methods. For estimation of BFS, Gaussian distribution method and AUC spectroscopic method was selected and used. The spectra for the Gaussian distribution Methods 1, 2 and 3 were shown in Figure 2, 4 and 6 respectively. Spectrum for AUC spectroscopy was shown in Figure 8. The developed method was validated in terms of linearity, precision, accuracy, LOD and LOQ by spectrophotometry.

3.1. Linearity and Range

All the four methods showed good linearity in the range of 2-12 µg/ml satisfying beer's law. The correlation coefficient was found to be ≥0.999. The linearity data was shown in table 1 and calibration curve in Gaussian distribution methods was plotted across concentration vs absorbance and concentration vs AUC in method 4. The calibration curves were shown in figures 3, 5, 7 and 9.

3.2. Precision

Both repeatability and intermediate precision were studied and all the four methods showed acceptable limit with % RSD ≤ 2. This proves all the developed methods were more precise.

3.3. Accuracy

The recovery studies were carried in triplicate levels with standard addition method at 80%, 100% and 120% levels of the test concentration showed good results with a mean recovery. From the results, it was found that the developed methods were accurate and mean % recovery was shown in table 2.

3.4. Assay

The assay for BFS formulation was calculated by using calibration curve method and was found to be in limits. The consolidated results of the validation parameters of the developed method were depicted in table 3.

Table 1. Linearity of BFS.

S. No	Concentration (µg/ml)	Absorbance ± SD			Area ± SD
		Method 1 (Zero order)	Method 2 (First order)	Method 3 (Second order)	Method 4 (AUC)
1	2	0.186±0.0061	0.015 ±0.0010	0.003 ±0.0005	0.160 ±0.0064
2	4	0.352 ±0.046	0.027 ±0.0035	0.006 ±0.0009	0.280 ±0.045
3	6	0.505 ±0.0027	0.039 ±0.0074	0.009 ±0.0008	0.394 ±0.0035
4	8	0.647 ±0.038	0.051 ±0.0088	0.012 ±0.0002	0.489 ±0.0072
5	10	0.824 ±0.0085	0.064 ±0.0061	0.0152 ±0.0006	0.601 ±0.0023
6	12	0.976 ±0.0054	0.078 ±0.0065	0.0178 ±0.0007	0.720 ±0.0035

SD: Standard Deviation; AUC: Area Under the Curve.

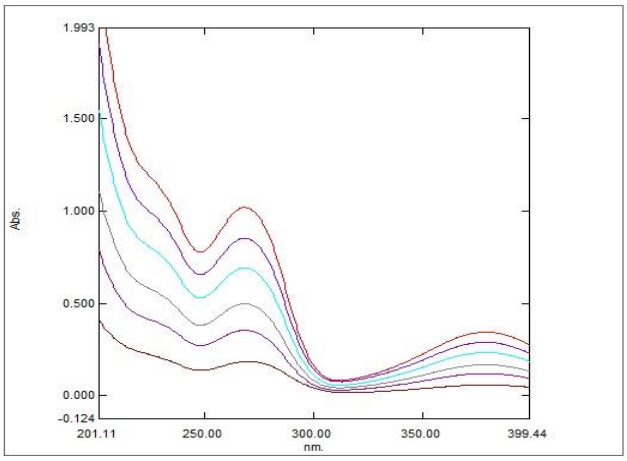

Figure 2. Overlay spectra of BFS zero order derivative (Method 1).

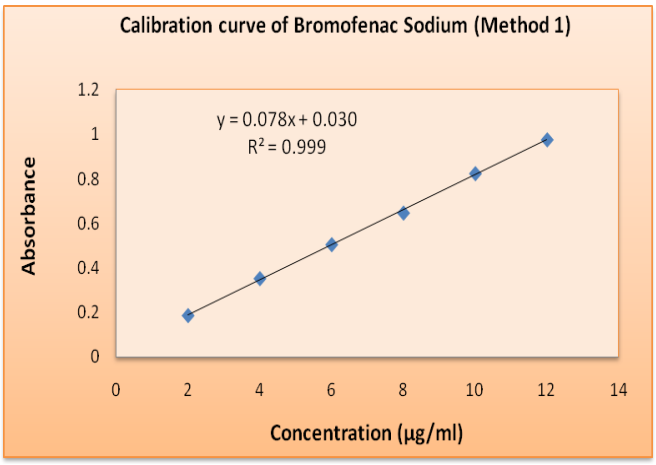

Figure 3. Calibration Curve of BFS zero order derivative (Method 1).

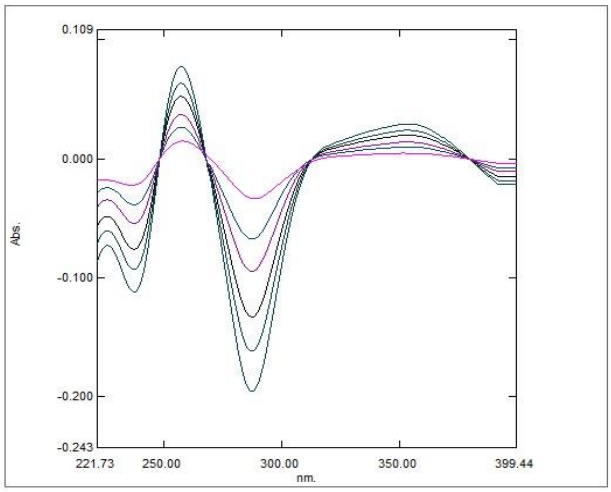

Figure 4. Overlay spectra of BFS first order derivative (Method 2).

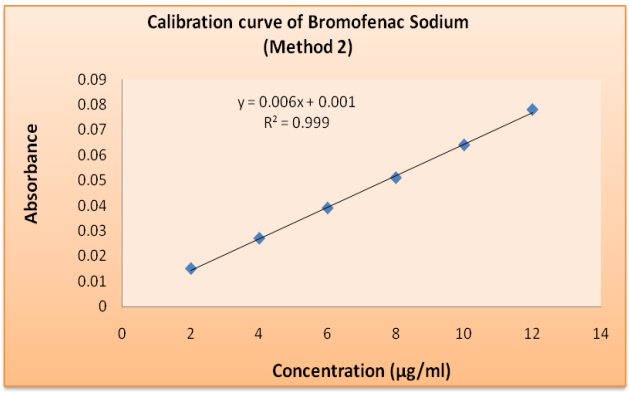

Figure 5. Calibration Curve of BFS first order derivative (Method 2).

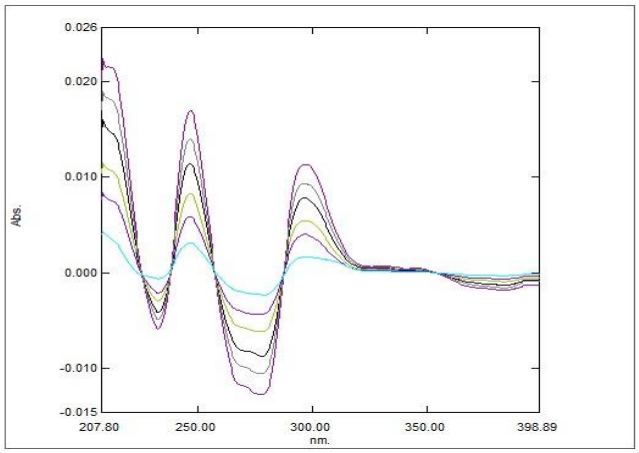

Figure 6. Overlay spectra of BFS second order derivative (Method 3).

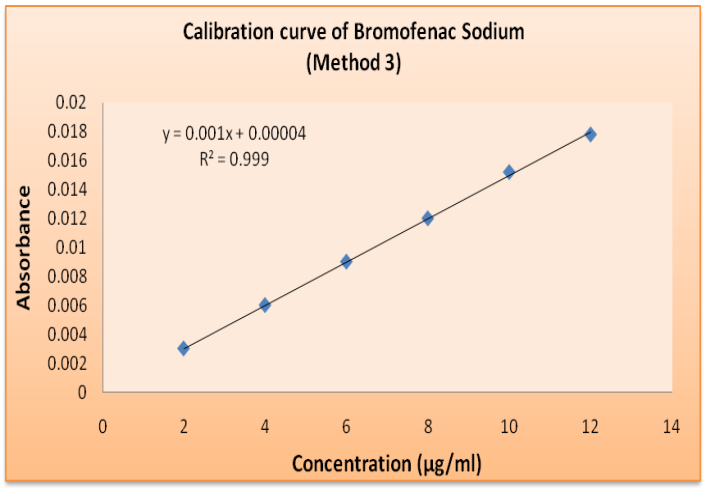

Figure 7. Calibration Curve of BFS second order derivative (Method 3).

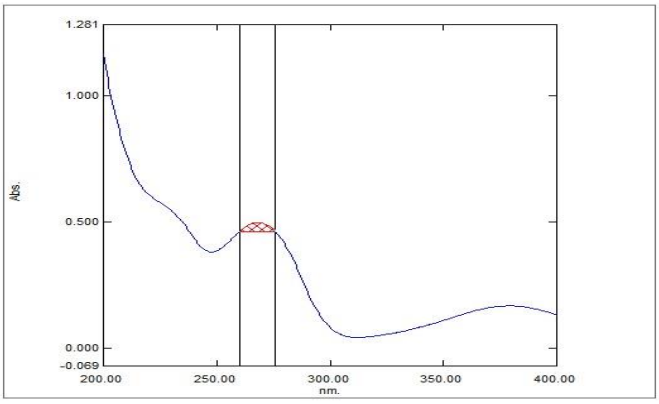

Figure 8. Spectrum of BFS AUC spectroscopy (Method 4).

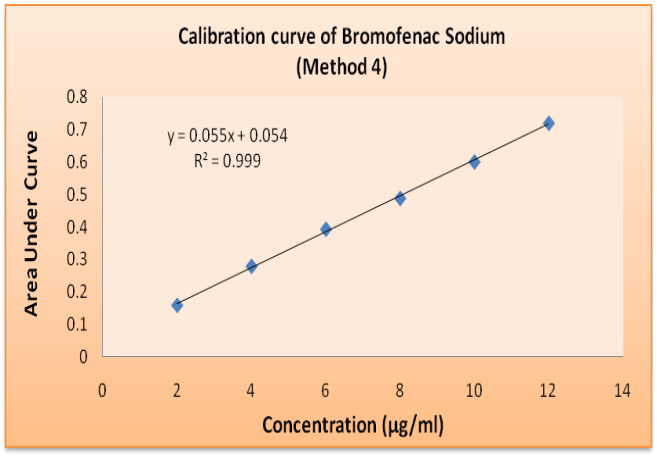

Figure 9. Calibration Curve of BFS AUC spectroscopy (Method 4).

Table 2. % Recovery of BFS.

Range	Spiked concentration (µg/ml)	Amount of sample concentration found (µg/ml)				% mean recovery			
		Method				Method			
		(1)	(2)	(3)	(4)	(1)	(2)	(3)	(4)
80%	4	4.83	4.92	4.80	4.87	99.08	100.37	99.05	98.54
		4.81	4.78	4.93	4.72				
		4.82	4.94	4.73	4.79				
100%	6	6.03	6.16	6.20	6.01	98.99	100.28	100.79	99.18
		6.01	6.01	6.13	6.06				
		6.02	6.12	606	6.03				
120%	8	7.30	7.45	7.33	7.44	100.66	101.26	100.73	101.1
		7.36	7.29	7.46	7.34				
		7.37	7.42	7.26	7.37				

The overall summary of optical characteristics and other validation parameters of zero order, first order, Second order derivative and AUC spectroscopic methods were presented in table 3.

Table 3. Validation parameters of BFS for Zero order, First order, Second order UV spectroscopy and AUC methods.

Validation Parameters	Gaussian Distribution Methods			AUC (Method 4)
	Method 1	Method 2	Method 3	
Absorption Maxima (nm)	260 nm	257.5 nm	241.5	256-266
Linearity (µg/ml)	2-12	2-12	2-12	2-12
Regression equation (Y)	Y = 0.078x + 0.030	Y = 0.006x + 0.001	Y = 0.001x + 0.00004	Y = 0.055x + 0.054
Slope (b)	0.078	0.006	0.001	0.055
Intercept (a)	0.030	0.001	0.00004	0.054
Correlation coefficient (r^2)	0.999	0.999	0.999	0.999
Sandell's sensitivity (µg/cm^2)	0.011	0.153	0.66	0.015
Intraday precision (% RSD)	0.99	1.01	1.40	0.62
Interday precision (% RSD)	1.43	1.47	1.39	0.84
Accuracy (% mean recovery)	99.08 – 100.66	100.28–101.26	99.05 – 100.79	98.54 – 101.10
Limit of detection (µg/ml)	0.21	0.20	0.41	0.14
Limit of quantification (µg/ml)	0.64	0.62	1.26	0.44
Assay (% Purity)	100.80	99.19	99.44	101.29

4. Conclusion

The proposed study describes a validated novel UV spectrophotometric method for the estimation of BFS in bulk and its formulation. From the results, the method was found as specific, accurate and precise when compared to other methods. Percentage of recovery reveals uninterference of excipients. The most striking feature of these methods is its simplicity and cost effective. Therefore, the proposed methods

could be used for routine analysis of BFS in its bulk and dosage form.

5. Conflict of Interest

The author(s) report(s) no conflict(s) of interest(s). The author along are responsible for content and writing of the paper.

6. Acknowledgement

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