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# A novel analytical method development and validation for the estimation of Bromfenac sodium by using reverse phase- HPLC

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**Abstract**
The research work aims to develop a RP-HPLC method for the estimation of Bromfenac Sodium. The Reverse Phase High Performance Liquid Chromatographic Isocratic method was developed by utilizing Phenomenex luna (250×4.6mm, 5µ) column. The retention time of Bromfenac Sodium was found to be 3.63minutes. Considering all the results of validation parameters of the method as per the ICH guidelines. It is possible to conclude that the developed method can be suitable for the regular quality control determination of Bromfenac Sodium.

**Keywords:** Bromfenac sodium, RPHPLC, estimation, ICH, validation

## Introduction
Bromfenac Sodium (Fig.No.1) is chemically 2-[2-amino-3-(4- bromobenzoyl) phenyl] acetic acid[1]. It is a Nonsteroidal anti-inflammatory drug (NSAID) for ophthalmic use which has the ability to block prostaglandin synthesis by inhibiting cyclooxygenase 1 and 2 (COX-1 and -2). From extensive survey it has been evaluated that only few methods were reported for determination of Bromfenac by RP-HPLC method[2-4]. So the present emphasis was given to develop, selective, precise and accurate HPLC method for determination of Bromfenac Sodium. The developed method to be validated in accordance to ICH Q2 ($R_1$) guidelines. [5-6]

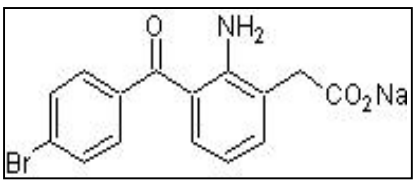


**Fig 1:** Structure of Bromfenac Sodium

## Materials and Methods
### Materials

**Table 1:** Chemicals used for RP-HPLC method.

| S. No. | Chemical Name | Manufacturer |
|---|---|---|
| 1 | HPLC Grade Water | Merck, Mumbai |
| 2 | HPLC Grade Methanol | SD fine chem. Limited, Mumbai India. |
| 3 | Ammonium Dihydrogen ortho phosphate (HPLC Grade) Phosphate | SD fine chem. Limited, Mumbai |

### Instrument Used

**Table 2:** Instruments used for RP-HPLC method

| S. No | Instrument | Model | Manufacturer/ company |
|---|---|---|---|
| 1 | UFLC | LC 20 AD PDA Detector | Shimadzu |
| 2 | HPLC column | C18 Phenomenex Luna (250x4.6 mm;5µ) | - |
| 3 | Ultra Sonicator | 2200 MH SOLTECH | Spincotech Pvt., Ltd |
| 4 | Electronic balance | NO-HT220 | Vibra shinko denshi co. ltd |
| 5 | pH Meter | LI 120 | Elico |
| 6 | Ultra Filtration | Millipore pvt.ltd. | Pall India Pvt Ltd. |

**Methodology**

**Preparation of 0.01M Ammonium Dihydrogen Ortho Phosphate Buffer**

0.132gm of Ammonium Dihydrogen Ortho phosphate buffer was weighed accurately and dissolved in 100mL HPLC grade water for preparation of 0.01M Ammonium Dihydrogen Ortho Phosphate Buffer.

**Preparation of mobile phase**

A mixture of Methanol: 0.01M Ammonium Dihydrogen Ortho Phosphate Buffer in the ratio of 60:40%v/v was prepared and used. pH of the mobile phase was adjusted to 6.0 using Ortho Phosphoric acid. Before proceeding for analysis mobile phase was sonicated, filtered and degassed by 0.45 µ membrane filter.

**Preparation of standard stock solution**

10 mg of Bromfenac Sodium pure drug was weighed and transferred into 10mL volumetric flask and add 10mL of mobile phase (1000µg/mL concentration). From this stock solution various aliquots are prepared and injected.

**Method Development**

**Table 3:** Optimized chromatographic conditions

| | |
|---|---|
| Mobile phase | Methanol: Ammonium Dihydrogen Ortho phosphate buffer (60:40 v/v) pH was adjusted to 6.0 using Ortho Phosphoric acid. |
| Column | C18 Phenomenex Luna (250x4.6 mm;5µ) |
| Flow rate | 1.0 mL/min |
| temperature | Ambient |
| Wavelength | 268 nm |
| Injection volume | 20 µl |
| Run time | 7.5 min |
| Retention time | About 3.64 min for Bromfenac Sodium |

**Optimized chromatogram**

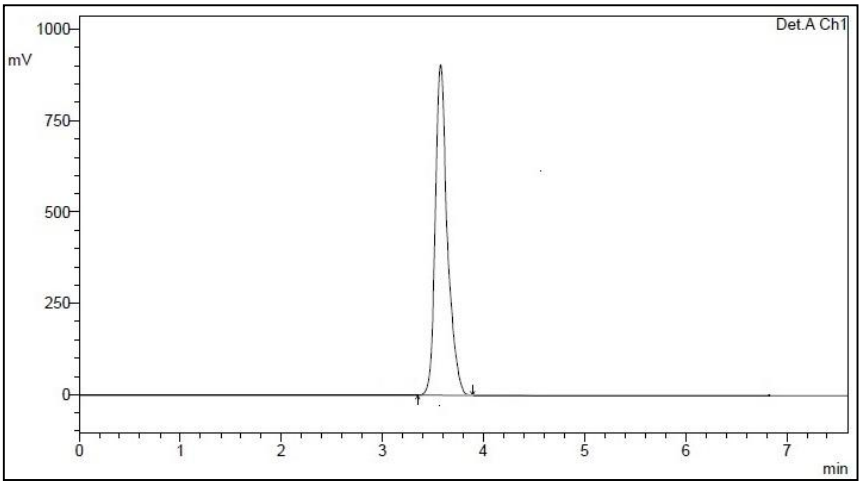


**Fig 2:** Optimized chromatogram of Bromfenac Sodium

**Method validation**

**1. Specificity**

**Specificity by Direct comparison method**

There is no interference of mobile phase, solvent and placebo with the analyte peak and also the peak purity of analyte peak which indicate that the method is specific for the analysis of analytes in their dosage form.

**Table 4:** Specificity Data

| S. No | Peak Name | Observation | |
|---|---|---|---|
| 1 | Blank | Nil | |
| 2 | Placebo | Nil | |
| 3 | Standard | Rt : 3.64min | Peak area : 731054 |

**2. System Suitability:** System suitability test was an integral part of method development and has been used to ensure adequate performance of the chromatographic system.

**Table 5:** Results of System Suitability

| Parameter | Result | Acceptance Limit |
|---|---|---|
| Retention time (Rt) | 3.64min | -- |
| Resolution factor | NA | -- |
| Number of theoretical plates (N) | 3912 | More than 2000 |
| Tailing factor (T) | 1.12 | Less than 2 |
| Number of injections: 6 replicates | | |

### 3. Precision

**Table 6:** Results for intraday and interday precision

| S. No. | Intraday Precision Area | Interday Precision Area |
|---|---|---|
| 1 | 731056 | 739665 |
| 2 | 725652 | 721879 |
| 3 | 740564 | 730565 |
| 4 | 738982 | 736412 |
| 5 | 751035 | 725895 |
| 6 | 740354 | 751654 |
| Mean | 737940.5 | 734345 |
| Std Dev | 7996.47 | 9774.69 |
| %RSD | 1.08 | 1.33 |

### 4. Linearity and Range

**Table 7:** Results for Linearity and range

| S. No | Concentration (µg/mL) | Peak Area |
|---|---|---|
| 1 | 6 | 183156 |
| 2 | 12 | 376598 |
| 3 | 18 | 551345 |
| 4 | 24 | 731054 |
| 5 | 30 | 914577 |
| 6 | 36 | 1112310 |

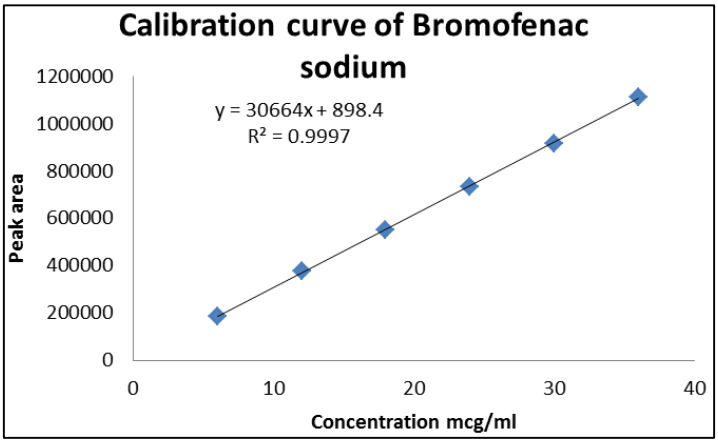


**Fig 3:** Calibration curve of Bromfenac Sodium

### 5. Accuracy

Accuracy of the method was determined by Recovery studies.

**Table 8:** Results for Accuracy

| Spiked Concentration (µg/mL) | Peak area | Amount added (µg/mL) | Amount Found (µg/mL) | Recovery | % Mean Recovery |
|---|---|---|---|---|---|
| 14 | 391345 | 12.42 | 12.63 | 101.69 | 100.71 |
| | 380312 | | 12.28 | 98.82 | |
| | 391065 | | 12.62 | 101.61 | |
| 24 | 770634 | 24.85 | 24.88 | 100.12 | 99.66 |
| | 766545 | | 24.75 | 99.59 | |
| | 764132 | | 24.67 | 99.28 | |
| 36 | 1135854 | 37.27 | 36.68 | 98.41 | 99.66 |
| | 1154031 | | 37.26 | 99.99 | |
| | | | | | |
| | 1160896 | | 37.49 | 100.58 | |

### 6. LOD & LOQ

**Table 9:** Results for LOD & LOQ

| S. No | Parameter | Slope | Standard Deviation | Value |
|---|---|---|---|---|
| 1 | Limit of Detection | 30664 | 7996.478 | **0.86** |
| 2 | Limit of Quantification | | | **2.60** |

**7. Robustness**

**Table 10:** Change of Flow rate (± 10%)

| S. No | Flow Rate | 0.9mL/min | 1mL/min | 1.1mL/min |
|---|---|---|---|---|
| 1 | | 725895 | 738982 | 731056 |
| 2 | | 751654 | 751035 | 725652 |
| 3 | | 738774.5 | 740354 | 740564 |
| 4 | Mean | 738774.5 | 743457 | 732424 |
| 5 | Std dev | 10516.06 | 5387.65 | 6164.17 |
| 6 | % RSD | 1.42 | 0.72 | 0.84 |

**Table 11:** Change in Temperature (± 5ºC)

| S. No | Temperature | 30 ºC | 35 ºC | 40 ºC |
|---|---|---|---|---|
| 1 | | 725652 | 721879 | 738982 |
| 2 | | 740564 | 730565 | 760165 |
| 3 | | 738982 | 736412 | 740354 |
| 4 | Mean | 735066 | 729618.7 | 746500.3 |
| 5 | Std dev | 6687.960726 | 5970.689 | 9678.599 |
| 6 | % RSD | 0.90 | 0.81 | 1.29 |

**Assay**

The assay of Bromfenac Sodium was found to be 98.21%.

**Table 12:** Overall summarized parameters of Bromfenac Sodium RP-HPLC

| Parameter | Bromfenac Sodium | Acceptable Limit |
|---|---|---|
| Linearity (µg/mL) | 6-36µg/mL | - |
| λ max (nm) | 268 nm | - |
| Correlation coefficient ($r^2$) | 0.999 | Not less than 0.999 |
| Slope (m) | 30664 | - |
| Intercept | 898.4 | - |
| Precision<br>(Intraday)(%RSD)<br>(Interday)(%RSD) | <br>1.08<br>1.33 | Less than 2% |
| Limit of detection (LOD) | 0.86 µg/mL | - |
| Limit of Quantification (LOQ) | 2.60 µg/mL | - |
| Accuracy (% mean recovery) | 100.01 | 98 – 102% |
| Assay (%) | 98.21 | 98 – 102% |
| Tailing factor | 1.12 | Less than 2 |
| Number of theoretical plates | 3912 | More than 2000 |
| Retention time(min) | 3.64 min | - |

**Conclusion**

A specific and selective LC method is described for the estimation of Bromfenac Sodium. Chromatographic separation was achieved on a c18 column using mobile phase consisting of a mixture of Methanol: Ammonium Dihydrogen Ortho phosphate buffer (60:40 v/v) pH was adjusted to 6.0 using Ortho Phosphoric acid with detection of 268 nm. Linearity was observed in the range 6-36 µg /mL for Bromfenac Sodium (r2 =0.999). The amount of drug estimated by the proposed methods was found to be 98.21%. The method can be used for the routine analysis of Bromfenac Sodium.

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