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Validated Stability Indicating RP-HPLC Method for Silmutanious Determination of Benzbromarone and Diclofenac Potassium in Vitro Dissolution Studies from Tablet Dosage Form

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ABSTRACT: A simple, rapid, and robust stability indicating RP-HPLC method has been developed and validated to measure Benzbromarone and diclofenac potassium in vitro drug release profile of drug from tablet formulation. An isocratic elution of samples performed on Zorbax Eclips plus C18 100 mm × 4.6 mm, 5 µm column with mobile phase consisting GAA: Acetonitrile : Water :Methanol (2.5: 12.5:195:450) v/v delivered at flow rate 1.0 mL/min. For dissolution study, the sink condition has been established from quantitative solubility of Benzbromarone and Diclofenac potassium API in different dissolution medium recommended by USP for immediate release formulation and the optimized dissolution condition was: Phosphate buffer solution with 0.25% SLS, USP Type II apparatus at 100rpm and 900 ml dissolution media for Benzbromarone content and SIF without pepsin, USP Type II apparatus at 50rpm and 900 ml dissolution media for Dislofenac potassium content The HPLC method and dissolution test condition were validated to meet requirement for regulatory filling and this validation inferred from specificity, precision, accuracy, linearity and robustness. In addition filter suitability, standard and sample solution stability was demonstrated. All results were acceptable and this confirmed that the method is suitable for its intended use in routine quality control dissolution analysis of drugs.

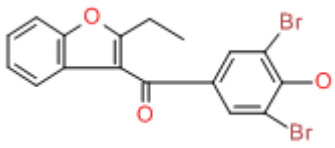
Keywords: Benzbromarone, Diclofenac potassium, dissolution, RP-HPLC.

I. INTRODUCTION

Antiretroviral drugs: Uricosuric medications (drugs) [1] are substances that increase the excretion of uric acid in the urine, thus reducing the concentration of uric acid in blood plasma. In general, this effect is achieved by action on the proximal tubule of the kidney. Drugs that reduce blood uric acid are not all uricosurics blood uric acid can be reduced by other mechanisms (see other Antigout medications).

Uricosurics are often used in the treatment of gout, a disease in which uric acid crystals form deposits in the joints [2]. By decreasing plasma uric acid levels, uricosurics help dissolve these crystals, while limiting the formation of new ones. However, the increased uric acid levels in urine can contribute to kidney stones. Thus, use of these drugs is contraindicated in persons already with a high urine concentration of uric acid (hyperuricosuria). In borderline cases, enough water to produce 2 liters of urine per day may be sufficient to permit use of an uricosuric drug.

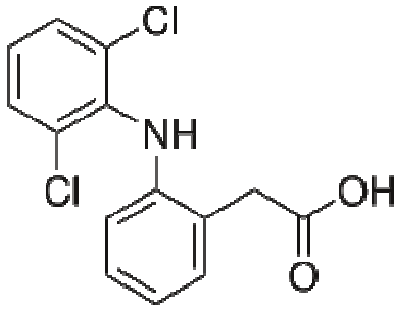
Benzbromarone [3]:-



IUPAC Name: Methanone, (3,5-dibromo-4-hydroxyphenyl)(2-ethyl-3-benzofuranyl)-
Molecular Formula: C_{17}-H_{12}-Br_2-O_3
Molecular Weight: 424,

Benzbromarone [4], a potent uricosuric drug, was introduced in the 1970s and was viewed as having few associated serious adverse reactions[5][6]. It was registered in about 20 countries throughout Asia, South America and Europe. In 2003, the drug was withdrawn by Sanofi-Synthelabo [7][8][9], after reports of serious hepatotoxicity [10], although it is still marketed in several countries by other drug companies [11]. The withdrawal has greatly limited its availability around the world [12], and increased difficulty in accessing it in other countries where it has never been available.

Diclofenac Potassium:



2-(2,6-Dichloroanilino)phenyl]acetic acid
$C_{14}H_{11}Cl_2NO_2$

Diclofenac potassium (DP) is 2-[(2,6-dichlorophenyl)amino] phenyl acetic acid which possesses anti-inflammatory and analgesic properties (Indian Pharmacopoeia, 2010, British Pharmacopoeia, 2004 and The United State Pharmacopoeia, 2005). The diclofenac potassium have synergetic action and is prescribed for symptomatic relief of low back pain, post-operative pain, and rheumatic arthritis osteoarthritis, musculoskeletal injuries and chronic pain associated with cancer.

However, the exhaustive literature survey revealed that none of the most recognized pharmacopoeias or any journals includes these drugs in combination for the simultaneous determination of Benzbromarone and Diclofenac potassium and the information regarding the stability of the drugs is not available. So it is felt essential to develop a liquid chromatographic procedure which will serve a reliable, accurate and stability indicating HPLC method for the simultaneous estimation and in vitro dissolution studies of benzbromarone and diclofenac potassium simultaneously in tablet dosage form

II. MATERIAL AND METHODS

A. Drug and reagents

Pure Benzbromarone and Diclofenac potassium was obtained as gratis sample from IPCA laboratories (Mumbai, India). Analytical reagent (AR) grade Methanol, Glacial acetic acid, potassium phosphate, Sodium laurel sulphate, Formic acid and Acetonitrile from sigma Aldrich (Mumbai, India). Water for HPLC studies was obtained from milipore water purifying system.

B. Apparatus and equipment

LC was carried out on Agilent HPLC system (Model no. 1100) with photodiode array detector. The output signal was monitored and processed using Chromeleon software . In all the studies, separations were achieved on a Eclipse plus C18 (100 mm × 4.6 mm i.d., particle size 5 µm) procured from LCGC (Banglore, INDIA). Other small equipment were PCI sonicator (22L500/CC/DTC made in), Dissolution apparatus (Electolab India) , precision analytical balance (Mettler Toledo, Schwerzenbach, Switzerland).

C. Chromatographic conditions

The separation was achieved using Isocratic program of Glacial Acetic Acid : Acetonitrile : Water : Methanol (2.5 : 12.5 :195 : 450) v/v. the flow rate was set at 1.0 ml/min and column was maintained at 25°C. The injection volume was set 20µl and detector was set at a wavelength of 231nm.

D. Preparation of dissolution medium and dissolution condition

For Benzbromarone Tablets:

Apparatus	:	USP type II (Paddle)
Medium	:	Deaerated Phosphate buffer solution with 0.25% SLS
Volume	:	900 mL
Speed	:	50 rpm
Temperature	:	37°C ± 0.5°C
Time	:	45 minutes

Preparation of Dissolution medium:

Phosphate buffer pH 6.8: Weigh 6.8 g potassium dihydrogen orthophosphate in 1000 mL of water, add 2.5 grams of sodium laurel sulphate to it and dissolved, adjust pH 6.8 ± 0.05 with dilute sodium hydroxide.

For Diclofenac Potassium:

Dissolution medium	:	SIF without pepsin
Volume	:	900 mL
Apparatus	:	USP Type 2 (Paddle)
Speed	:	50 rpm
Time	:	45 min
Temperature	:	37°C ±0.5°C
Sampling volume	:	10 mL

E. Preparation of standard during method Validation

The diluent was selected for dissolving Benzbromarone and diclofenac was mixture of mobile phase. Standard solution of Benzbromarone and Diclofenac were prepared in mobile phase having concentration of 0.055 mg/ml each. Benzbromarone sample solution were prepared in the concentration of 0.055 mg/ml each and injected.

F. Preparation of sample during method Validation

For Benzbromarone: Place the 900 mL of Phosphate buffer solution with 0.25% SLS in each dissolution vessel, assemble the apparatus, and equilibrate the Phosphate buffer solution with 0.25% SLS o 37 ± 0.5°C.

Place 1 tablet in each vessel and immediately operate the apparatus at 100 rpm. Within the time interval specified, withdraw 10.0 mL of specimen from a zone midway between the surface of the Phosphate buffer solution with 0.25% SLS and the top of the rotating paddle, not less than 1 cm from the vessel wall. Replace the aliquots withdrawn for analysis with equal volume of dissolution medium. Filter the aliquots.

For Diclofenac Potassium: Place the 900 mL of SIF without pepsin in each dissolution vessel, assemble the apparatus, and equilibrate the SIF without pepsin at 37 ± 0.5°C. Place 1 tablet in each vessel and immediately operate the apparatus at 50 rpm. Within the time interval specified, withdraw 10.0 mL of specimen from a zone midway between the surface of the SIF without pepsin and the top of the rotating paddle, not less than 1 cm from the vessel wall. Replace the aliquots withdrawn for analysis with equal volume of dissolution medium. Filter the aliquots.

III. RESULTS AND DISCUSSION

A. Method validation

Specificity. Specificity of the method is its ability to detect and separate analytes present in the drug. Specificity of the method is demonstrated in terms of spectral as well as peak purity data of the drug. Peak passed the peak purity test.

Linearity. Linearity of the method was checked by preparing solutions at seven concentration levels of 5 % (Level 1) to 150% (Level 7) for Benzbromarone and Diclofenac. Level 1 and level 7 was injected six times were as level 2, level 3, level 4, level 5 and level 6 was injected two times. The mean responses recorded for each analyte were plotted against concentration. The correlation coefficient for Benzbromaone and Diclofenac was found to be 1.00. which indicates good linearity (for Benzbromarone and Diclofenac).

Accuracy. Benzbromarone analytes were spiked in placebo solution at 5%, 50%, 100% and 150%. Each spiked solution was prepared in triplicate and injected. The recovery percentage and % RSD were calculated for each analyte. Recovery of Benzbromarone and Diclofenac ranged from 100.5% to 104.3% and 100.0% to 103.5% respectively.

System and method precision. The system for two analytes in Benzbromarone and diclofenac was checked.

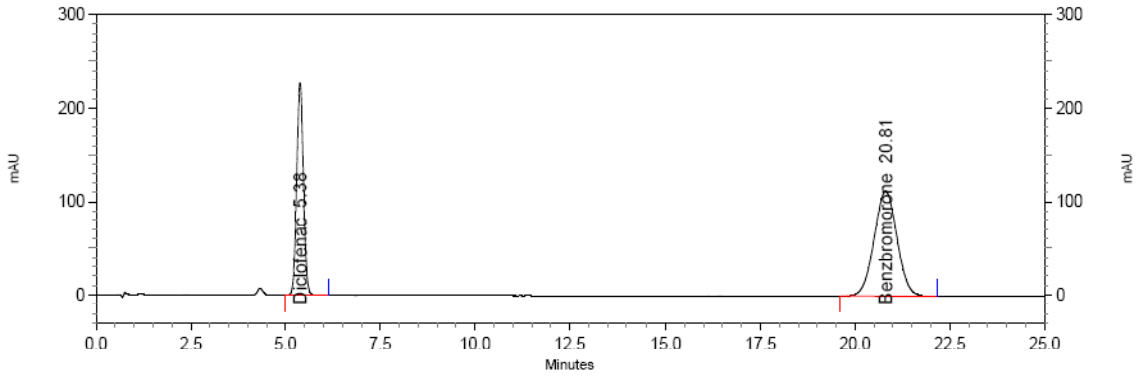


Fig. 1. Standard chromatogram of Benzbromarone.

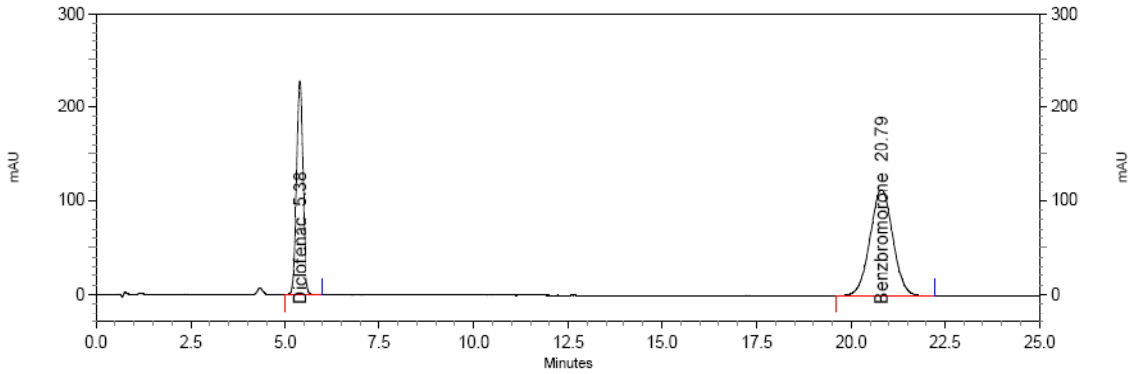


Fig. 2. Typical Sample chromatogram of Benzbromarone.

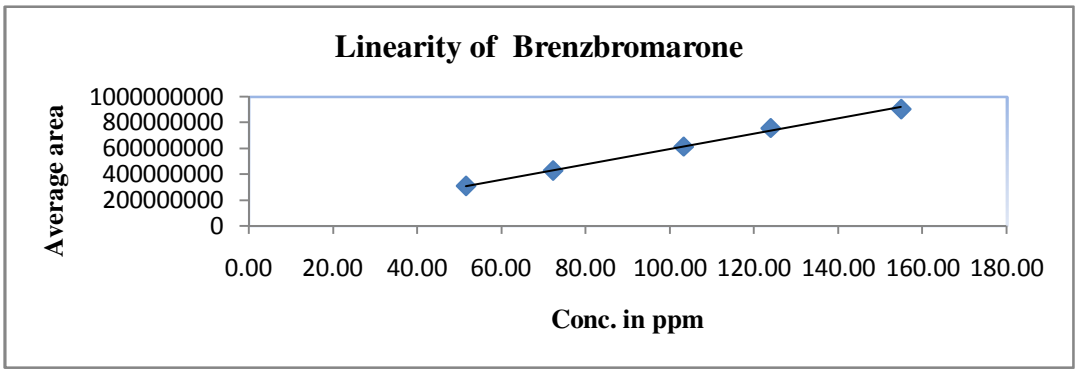


Fig. 3. Linearity graph of Benzbromarone.

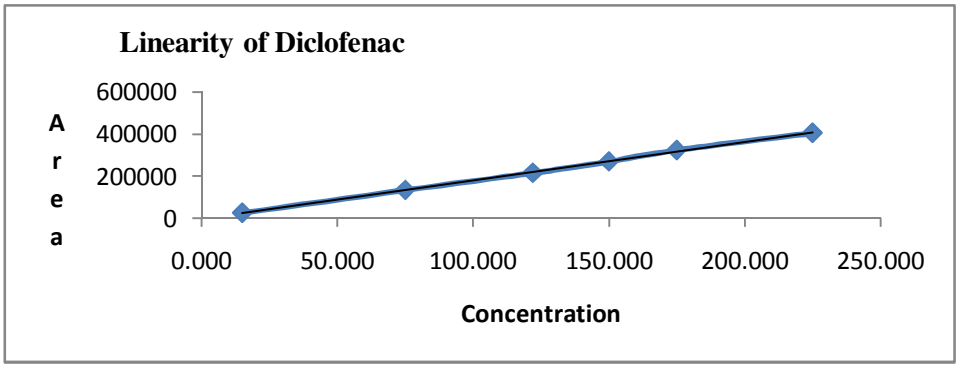


Fig. 4. Linearity graph of Diclofenac.

The sample was prepared by dissolving tablets in dissolution medium of target analyte concentration and injected. The %RSD was found to be less than 2.0% for system precision.

To determine the method precision six independent solutions were prepared with respect to target analyte concentration. Each solution was injected once. The variation in the results for the analytes was expressed in terms of % RSD. The values calculated were found to be below 2.0% RSD for analytes, indicating satisfactory method precision. The results are shown in Table.

Table 1: Benzbromarone and Diclofenac Precison Sample results.

Sr. No.	Dissolution of Benzbromarone %	Dissolution of Diclofenac %
1	101.2	88.0
2	100.9	88.0
3	100.7	87.9
4	100.1	87.8
5	100.7	87.8
6	100.0	87.7
Mean	**100.6**	**87.9**
Std. Dev.	**0.46**	**0.12**
% RSD	**0.46**	**0.14**

CONCLUSION

The proposed LC method is selective for the quantification of Benzbromarone and Diclofenac present in sample solution. Hence this method is useful for the detection Benzbromarone and Diclofenac in routine analysis.

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