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Analytical Method Development and Validation for Assay of Benzbromarone Drug of Uricosuric Agent Category

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ABSTRACT: An isocratic reversed-phase HPLC method with PDA detector has been developed for the assay of Benzbromarone in bulk drug. The assay of Benzbromarone was analysed. The analysis was performed using Eclipsed Plus C18 column (100 × 4.6mm, 5µ) as a stationary phase with column oven temperature 25°c and UV detection at 231nm. The separation was achieved using isocratic program of Glacial acetic acid: Acetonitrile: water: Methanol in the ration 2.5:12.5:195:450. The method was optimized based on the peak shapes and resolution of Benzbromarone and its impurity. The method was validated as per International Conference of Harmonization (ICH) guidelines in terms of linearity, precision, accuracy, specificity, robustness and solution stability. The sample concentration were injected was 0.1 mg/ml. The method is linear within the range of 50 to 150 µg/ml for Benzbromarone.

Keywords: Benzbromarone, Assay, 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).

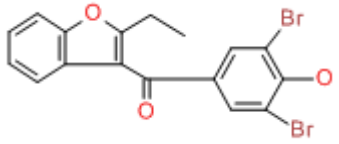
Uricosurics are often used in the treatment of gout, a disease in which uric acid crystals form deposits in the joints [2]. By decreasing plasma uric acid levels, uricosurics help dissolve these crystals, while limiting the formation of new ones. However, the increased uric acid levels in urine can contribute 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[7-9], 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.

The overall aim of this paper is to determine if the withdrawal of benzbromarone was in the best interests of gouty patients and to present a benefit-risk assessment of benzbromarone.

II. MATERIAL AND METHODS

A. Drug and reagents

Pure Benzbromarone and its impurities was obtained as gratis sample from IPCA laboratories (Mumbai, India). Analytical reagent (AR) grade Methanol, Glacial acetic 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), precision analytical balance (Mettler Toledo, Schwerzenbach, Switzerland).

C. Chromatographic conditions

The numbers of column such as waters symmetry C18 (250 × 4.6mm, 5.0µm), YMC packpro C18 (250 × 4.6mm, 5.0µm), Inertsil ODS 3V (250 × 4.6mm, 5.0µm) were used during method development. 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 sample during method development and Validation

The diluent was selected for dissolving Benzbromarone was mixture of mobile phase. Standard solution of Benzbromarone were prepared in Mobile phase having concentration of 0.1 mg/ml. Benzbromarone sample solution were prepared in the concentration of 0.1 mg/ml and injected.

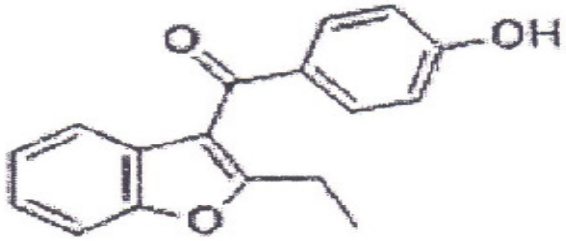
Table 1.

Sr. No.	Mobile phase	Stationary Phase	Result
1.	Phosphate buffer, acetonitrile and methanol (50:25:25 v/v/v)	waters symmetry C18 (150 × 4.6mm, 5.0µm) .	Benzbromarone and impurity C co-eluted
2.	Phosphate buffer, acetonitrile and methanol (50:30:20 v/v/v)	waters symmetry C18 (150 × 4.6mm, 5.0µm) .	Benzbromarone and impurity C co-eluted
3.	0.1% Phosphate buffer in water: Acetonitrile and Methanol(80:10:10 v/v/v)	phenomenex luna C18 (250 × 4.6mm, 5.0µm)	Benzbromarone and impurity C co-eluted
4.	0.1% Phosphate buffer in water: Acetonitrile and Methanol(80:10:10 v/v/v)	Inertsil ODS (250 × 4.6mm, 5.0µm)	Benzbromarone and impurity C co-eluted
5.	Acetonitrile:Water:Methanol (100:550:350)	Inertsil ODS (250 × 4.6mm, 5.0µm)	Benzbromarone and impurity C co-eluted
6.	Acetonitrile:Water:Methanol (100:350:550)	Inertsil ODS (250 × 4.6mm, 5.0µm)	Benzbromarone and impurity C co-eluted
7.	Acetonitrile:Water:Methanol (200:350:450)	Zorbax Eclips (100 × 4.6mm, 5.0µm)	Benzbromarone and impurity C co-eluted
8.	Acetonitrile:Water:Methanol (100:300:450)	Zorbax Eclips (100 × 4.6mm, 5.0µm)	Benzbromarone and impurity C co-eluted
9.	Acetonitrile:Water:Methanol (50:195:450)	Zorbax Eclips (100 × 4.6mm, 5.0µm)	Benzbromarone and impurity C co-eluted
10.	GAA:Acetonitrile:Water:Methanol (2.5:12.5:195:450)	Zorbax Eclips (100 × 4.6mm, 5.0µm)	Benzbromarone and impurity C Separately eluted

IV. METHOD DEVELOPMENT AND COLUMN SELECTION

Chemical structure of Benzbromarone and Its impurities are shown in (Fig. 1). The sample of Benzbromarone procured from market which was selected for validation studies. Different mobile phase and stationary phases were employed to developed a suitable LC method for the quantitative determination of Benzbromarone in their respective formulations. A number of column containing various packing materials of ODS supplied by different manufacturers and different mobile phase composition were tried to get good peak shapes and selectivity for the impurities present in Benzbromarone.

Benzbromarone Impurity C:



Benzbromarone Impurity A:

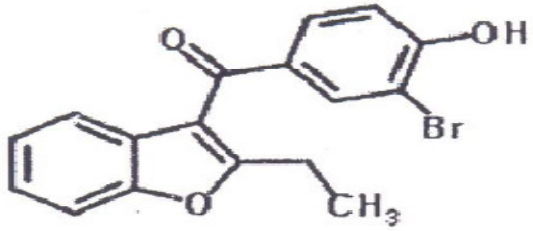


Fig. 1. Chemical structure of Benzbromarone related Impurities.

The separation was achieved using isocratic program of Glacial Acetic Acid : Acetonitrile : Water : Methanol (2.5 : 12.5 :195 : 450).

V. RESULTS AND DISCUSSION

A. Method validation

Specificity. Specificity of the method is its ability to detect and separate all the impurities 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 50 ppm (Level 1) to 150 ppm (Level 7) for Benzbromarone. 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 was found to be 1.00. which indicates good linearity (for Benzbromarone).

Accuracy. Benzbromarone analytes were spiked in placebo solution at 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 ranged from **98.65-100.77%** .

System and method precision. The system for two impurities in Benzbromarone was checked. The sample was prepared by dissolving tablets in diluents of target analyte concentration and injected six times. 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 were 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 2.

Table 2 : Method Precision Precision Results of Benzbromarone.

Sr.No.	% Assay of Benzbromarone
Sample-1	100.26
Sample-2	101.35
Sample-3	99.86
Sample-4	100.85
Sample-5	100.09
Sample-6	101.62
Mean	100.67
SD	0.72
%RSD	0.71

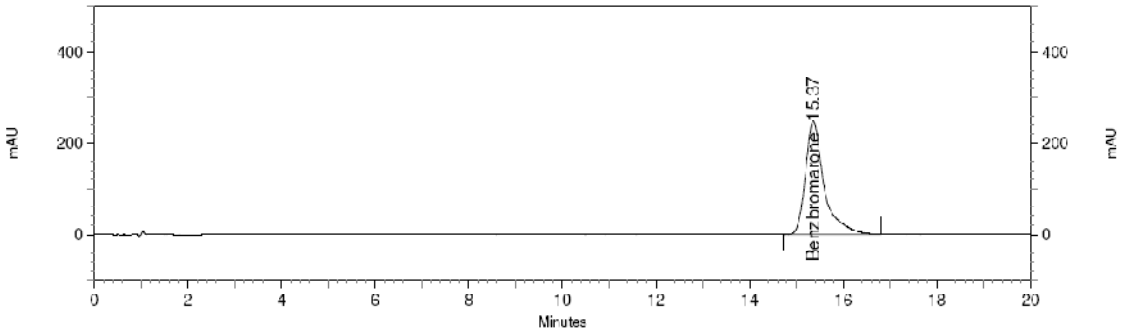


Fig. 2. Standard chromatogram of Benzbromarone.

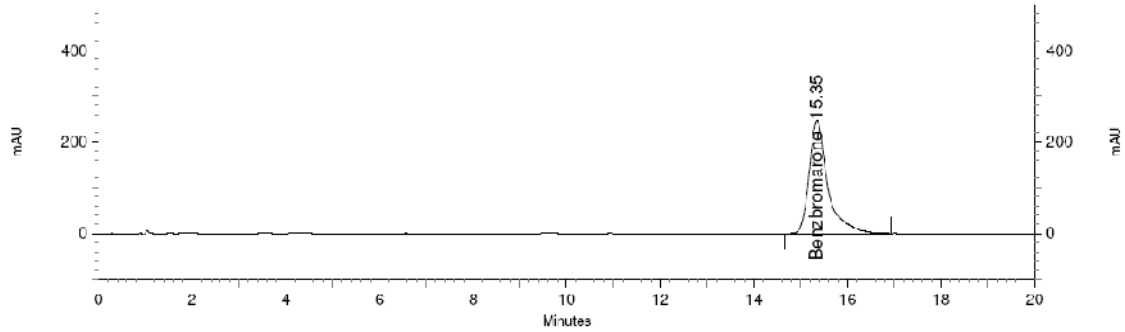


Fig. 3. Typical Sample chromatogram of Benzbromarone.

Stability in analytical solution. A solution of Benzbromarone was prepared and kept at room temperature. This solution was injected at intervals of 0, 2, 4, 8, 12, 16, 20 and 24hr. Area of all the Analytes were nearly identical to that obtained at 0h and additional peaks were not observed which indicate solution stability.

Sample preparation of Benzbromarone for routine analysis. Weighed sample equivalent to 100 mf of benzbromarone sample in 100 ml volumetric flask, dissolved in diluents and dilute up the volume with diluents. Injected this solution into HPLC to determine the amount of analyze present in the sample.

VI. CONCLUSION

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

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