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Analytical Method Validation for Related Substances of Benzbromarone Drug of Uricosuric Agent Category

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ABSTRACT: A simple, cost effective, sensitive, accurate and precise reverse phase high performance liquid chromatographic method is developed for the determination of impurities in Benzbromarone tablets. Eclipsed Plus C18 column (100 × 4.6mm, 5µ) µ i.d in isocratic mode, with mobile phase containing Glacial acetic acid: Acetonitrile: water: Methanol in the ration 2.5:12.5:195:450 was used. A flow rate of 1.0mL/min and detection was carried out with 231nm.The retention times of Benzbromarone was the 15.4min. The method is validated by determining its sensitivity, precision, linearity, accuracy. The proposed method is simple, rapid, sensitive, accurate and precise and so that it can be applied for routine quality control analysis of Benzbromarone Tablets forms.

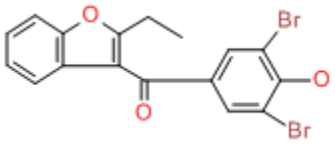
Keywords: Benzbromarone, Related Substances, Uricosuric agent, RP-HPLC.

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: C17-H12-Br2-O3
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.

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. Experimental details

A HPLC instrument (Agilent HPLC with PDA detector) was used. HPLC grade acetonitrile, Methanol (Rankem Ltd., Ranbaxy India) and HPLC grade water (Milli-Q water) were used in this study. AR grade Glacial acetic acid was obtained from Merck, India.

The fixed dose of Benzbromarone tablets 50mg was procured from local market. Mobile phase comprised of Glacial acetic acid: Acetonitrile: water: Methanol in the ration 2.5:12.5:195:450. Chromatographic separations were achieved by using Eclipsed Plus C18 column (100 × 4.6mm, 5µ) µ i.d analytical column with flow rate of 1.0 mL/min with detection at 231 nm as per the isocratic mode.The injection volume was kept as 20µL. The column temperature was kept at 25ºC.Diluent used for the sample and standard preparation was mobile phase.

B. Impurity Stock Solution

Weigh accurately and transfer 1 mg of each Benzbromarone Impurity A and Benzbromarone Impurity C into 10 ml volumetric flask. Add 5 ml of Methanol and sonicate to dissolve. Dilute to volume with Diluent and mix.

C. Standard preparation

Weigh and transfer about 20 mg of Benzbromarone working standard into 20 ml volumetric flask. Add 5 ml of Methanol into it and sonicate to dissolve. Dilute to volume with diluent and mix. Filter through 0.45 µm nylon membrane filter. Collect the filtrate after discarding first few ml of the filtrate. Pipette out 2.0 ml of this solution into a 20 ml volumetric flask. Dilute to volume with diluent and mix. Further, pipette out 1.0 ml of this solution into a 100 ml volumetric flask. Dilute to volume with diluent and mix. (This solution contains 0.001 mg/ml of Benzbromarone).

D. Sample preparation

Weigh accurately and transfer equivalent to 50 mg of Benzbromarone into 50 ml volumetric flask. Add 5 ml of methanol and sonicate for 5 minutes with intermittent shaking. Dilute to volume with diluent. Mix well. Centrifuge the solution at 3000 RPM for 10 minutes. Filter through 0.45 µm nylon syringe filter. Collect the filtrate after discarding first few ml of the filtrate. (This solution contains about 1.0 mg/ml of Benzbromarone).

III. RESULTS AND DISCUSSION

A. Method validation

Specificity. pecificity 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.

Benzbromarone Impurity C :

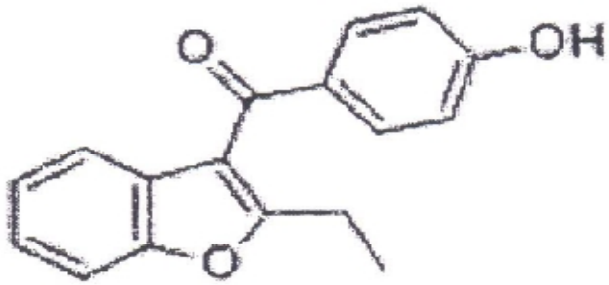
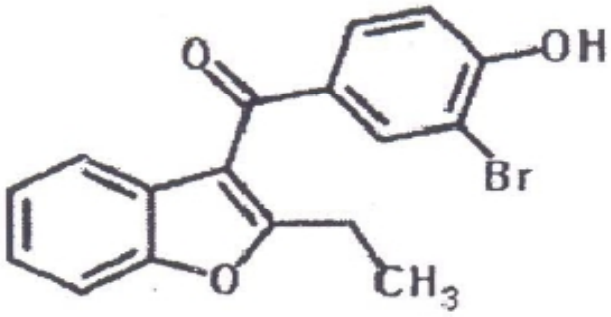


Fig. 1. Chemical structure of Benzbromarone related Impurities.

Benzbromarone Impurity A :



Benzbromarone :

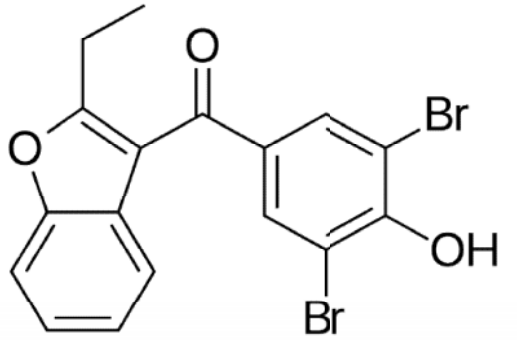


Fig. 2. Standard chromatogram of Benzbromarone.

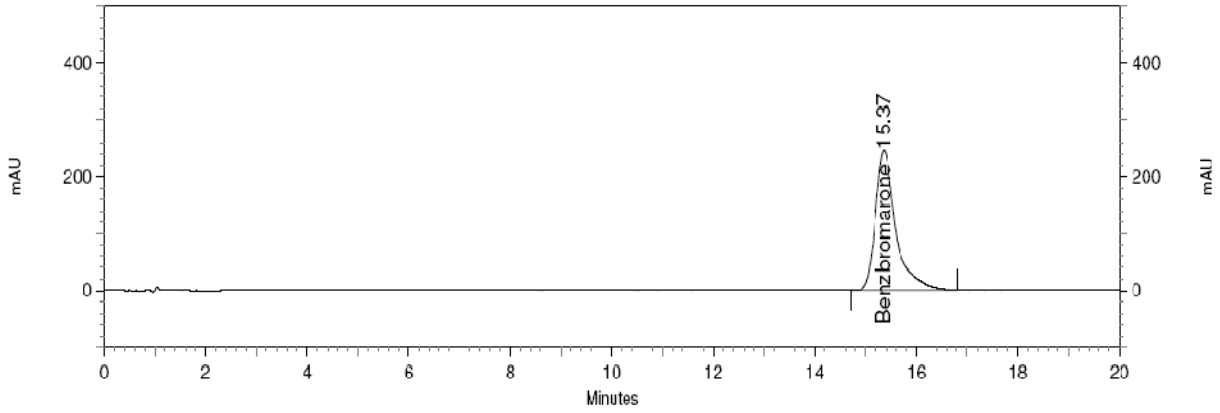


Fig. 3. Typical Sample chromatogram of Benzbromarone.

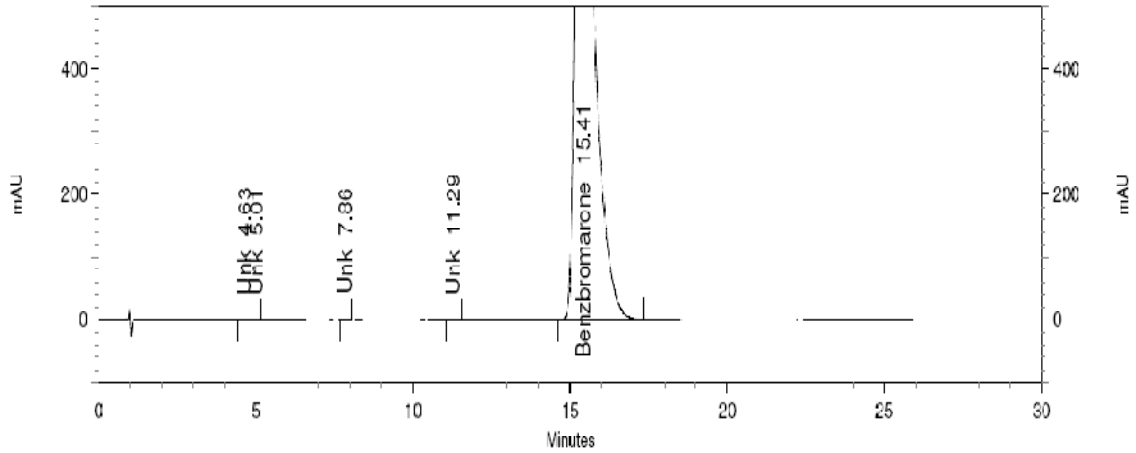
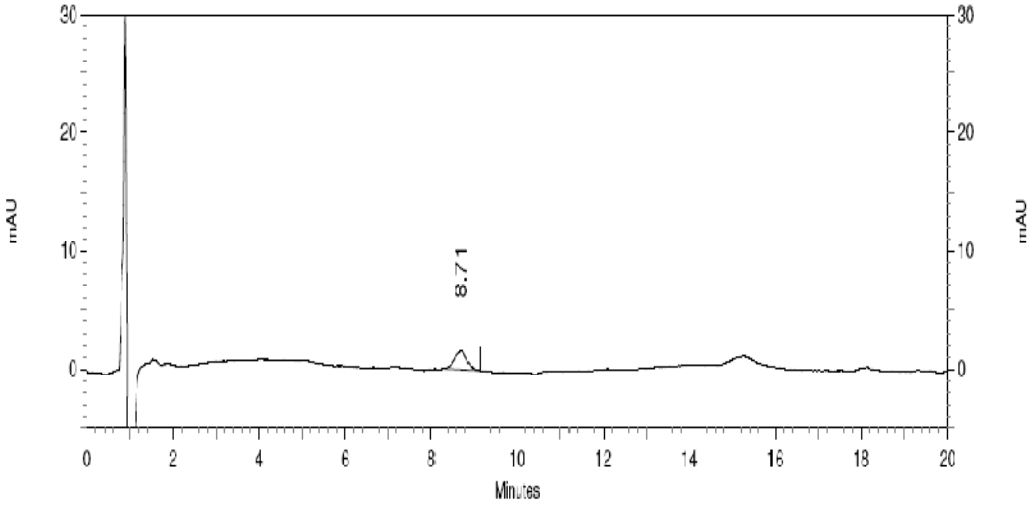


Fig. 4. Typical Benzbromarone Impurity A chromatogram.



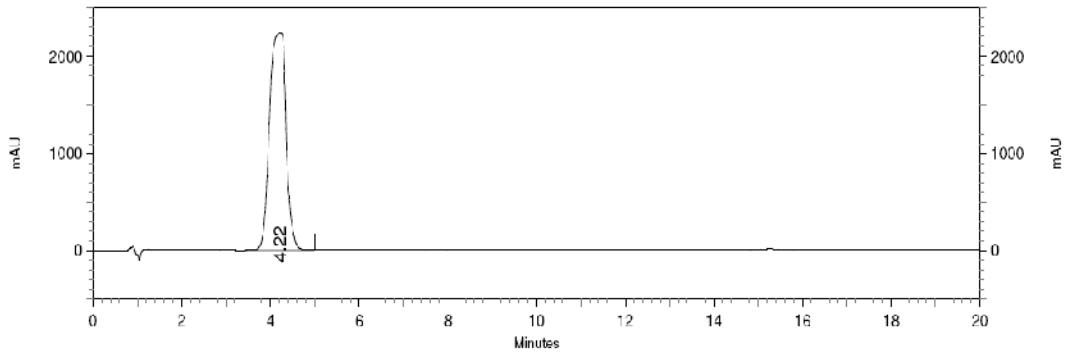


Fig. 5. Typical Benzbromarone Impurity C chromatogram.

Linearity. Linearity of the method was checked by preparing solutions at seven concentration levels of LOQ (Level 1) to 150% (Level 7) for Benzbromarone, Benzbromarone Impurity A and Benzbromarone Impurity C. 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, Benzbromarone Impurity A and Benzbromarone Impurity C was found to be 0.998, 0.9992 and 0.9994 respectively. which indicates good linearity. (Fig. 6 to 8).

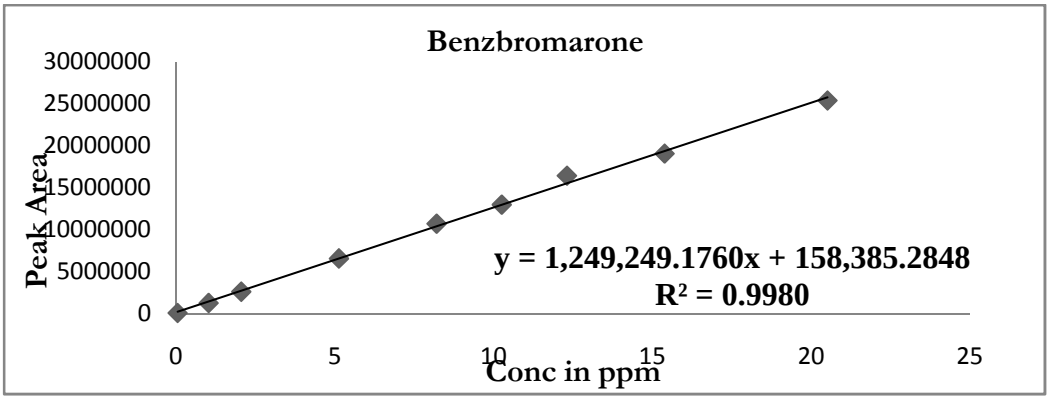


Fig. 6. Typical Linearity Graph of Benzbromarone.

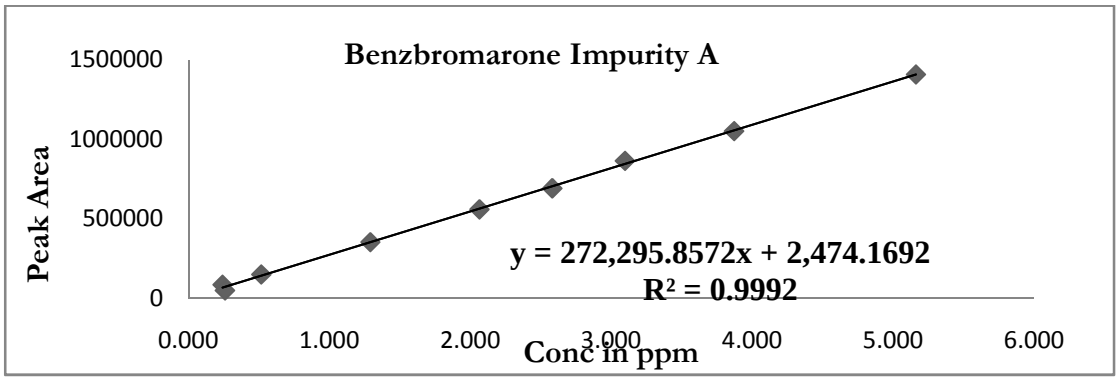


Fig. 7. Typical Linearity Graph of Benzbromarone Impurity A.

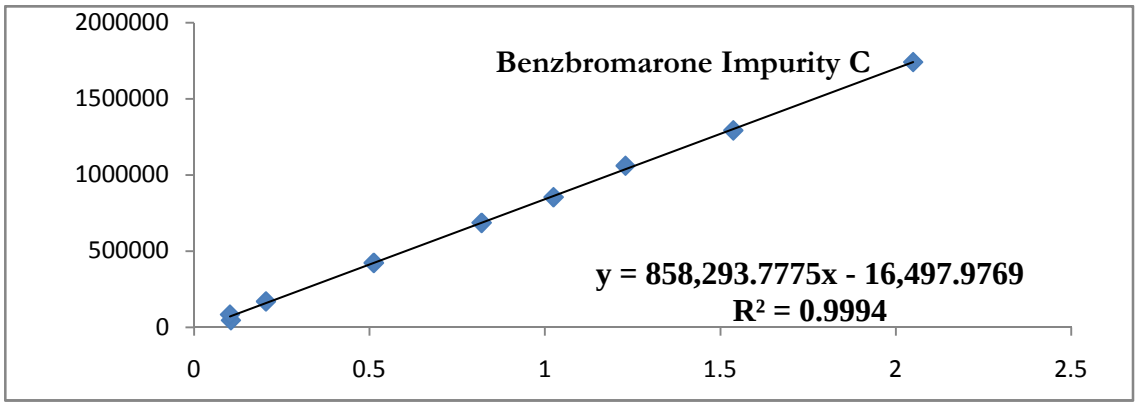


Fig. 8. Typical Linearity Graph of Benzbromarone Impurity C.

Accuracy. Benzbromarone, Benzbromarone Impurity A and Benzbromarone Impurity Cs were spiked in placebo solution at LOQ, 25%, 50%, 100%, 150% and 200%. Each spiked solution was prepared in triplicate and injected. The recovery percentage and %RSD were calculated for each analyte. Results are tabulated in table 1 to table no. 3.

Table 1: Recovery for Benzbromarone Content.

Accuracy Level	Sample No.	Actual Amount added in mg	Recovered Amount in mg	% Recovery	Mean % Recovery	Std. Dev.	% RSD
LOQ Level I	1	0.005	0.005	113.7	109.0	4.56	4.18
	2	0.005	0.005	104.6			
	3	0.005	0.005	108.6			
Level II (25%)	1	0.247	0.259	104.9	102.5	3.33	3.25
	2	0.247	0.257	103.9			
	3	0.247	0.244	98.7			
Level III (50%)	1	0.495	0.516	104.3	104.9	1.40	1.33
	2	0.495	0.527	106.5			
	3	0.495	0.514	103.9			
Level IV (100%)	1	0.989	0.932	94.2	96.1	2.46	2.56
	2	0.989	0.943	95.3			
	3	0.989	0.979	98.9			
Level V (150%)	1	1.484	1.450	97.7	98.8	1.47	1.49
	2	1.484	1.492	100.5			
	3	1.484	1.459	98.3			
Level VI (200%)	1	1.979	1.932	97.7	98.2	1.01	1.03
	2	1.979	1.932	97.6			
	3	1.979	1.967	99.4			
			Overall Mean Recovery	**101.6**			
			Std. Dev.	**5.03**			
			% RSD	**4.95**			

Table 2: Recovery for Benzbromarone Impurity A.

Accuracy Level	Sample No.	Actual Amount added in mg	Recovered Amount in mg	% Recovery	Mean % Recovery	Std. Dev.	% RSD
LOQ Level I	1	0.026	0.025	93.9	94.7	1.19	1.26
	2	0.026	0.025	96.1			
	3	0.026	0.025	94.2			
Level II (25%)	1	0.063	0.063	99.4	104.1	4.16	4.00
	2	0.063	0.068	107.4			
	3	0.063	0.067	105.4			
Level III (50%)	1	0.126	0.122	96.3	97.4	2.50	2.57
	2	0.126	0.121	95.7			
	3	0.126	0.127	100.3			
Level IV (100%)	1	0.253	0.248	98.2	98.9	0.91	0.92
	2	0.253	0.252	99.9			
	3	0.253	0.249	98.5			
Level V (150%)	1	0.379	0.383	101.1	102.8	1.53	1.49
	2	0.379	0.394	104.1			
	3	0.379	0.390	103.1			
Level VI (200%)	1	0.505	0.502	99.5	104.7	4.50	4.30
	2	0.505	0.542	107.2			
	3	0.505	0.542	107.4			
			Overall Mean Recovery	**100.4**			
			Std. Dev.	**4.47**			
			% RSD	**4.45**			

System and method precision. The system for two impurities in Benzbromarone was checked. The sample was prepared by dissolving tablets in diluent of target analyte concentration and injected six times. The %RSD was found to be less than 5.0% 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 5.0% RSD for analytes, indicating satisfactory method precision.

Stability in analytical solution. A sample solution of Benzbromarone with impurity Spiked were 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 impurities were nearly identical to that obtained at 0h and additional peaks were not observed which indicate solution stability.

Table 3: Recovery for Benzbromarone Impurity C.

Accuracy Level	Sample No.	Actual Amount added in mg	Recovered Amount in mg	% Recovery	Mean % Recovery	Std. Dev.	% RSD
LOQ Level I	1	0.010	0.010	98.6	100.1	1.79	1.79
	2	0.010	0.010	102.1			
	3	0.010	0.010	99.7			
Level II (25%)	1	0.245	0.233	95.1	95.4	1.18	1.24
	2	0.245	0.237	96.7			
	3	0.245	0.231	94.4			
Level III (50%)	1	0.490	0.469	95.8	96.0	0.38	0.40
	2	0.490	0.472	96.4			
	3	0.490	0.468	95.7			
Level IV (100%)	1	0.979	0.962	98.3	98.6	0.26	0.26
	2	0.979	0.968	98.8			
	3	0.979	0.967	98.7			
Level V (150%)	1	1.469	1.421	96.7	97.2	0.50	0.51
	2	1.469	1.434	97.7			
	3	1.469	1.428	97.3			
Level VI (200%)	1	1.958	1.909	97.5	97.9	0.51	0.52
	2	1.958	1.916	97.8			
	3	1.958	1.929	98.5			
			Overall Mean Recovery	**97.5**			
			Std. Dev.	**1.82**			
			% RSD	**1.87**			

V. CONCLUSION

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

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