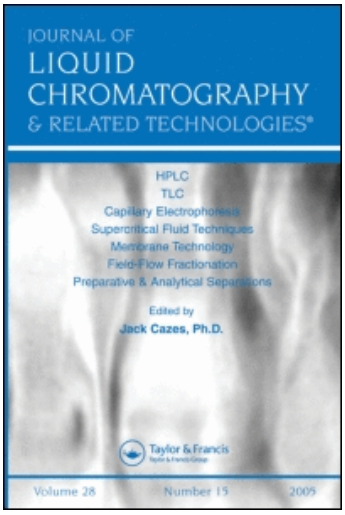

Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Determination of Benzbromarone and Benzarone in Pharmaceuticals by High-Performance Liquid Chromatography

A. Gumieniczek[a]; L. Przyborowski[a]
[a] Department of Medicinal Chemistry, Medical Academy in Lublin, Lublin, Poland

To cite this Article Gumieniczek, A. and Przyborowski, L.(1994) 'Determination of Benzbromarone and Benzarone in Pharmaceuticals by High-Performance Liquid Chromatography', Journal of Liquid Chromatography & Related Technologies, 17: 16, 3411 — 3419
To link to this Article: DOI: 10.1080/10826079408013521
URL: http://dx.doi.org/10.1080/10826079408013521

JOURNAL OF LIQUID CHROMATOGRAPHY, 17(16), 3411-3419 (1994)

DETERMINATION OF BENZBROMARONE AND BENZARONE IN PHARMACEUTICALS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

A. GUMIENICZEK AND L. PRZYBOROWSKI

Department of Medicinal Chemistry
Medical Academy in Lublin
Chodźki Str. 6
20-093 Lublin, Poland

ABSTRACT

Assay procedure based on high-performance liquid chromatography has been developed for the specific determination of benzbromarone and benzarone in pharmaceutical dosage forms, using a LiChrosorb RP-18 column, a mobile phase acetonitrile-phosphate buffer pH 2,6 (9:1 v/v) and UV detection at 240 nm. Benzbromarone and benzarone have been extracted from tablets with methanol and determined in the range 2-12 µg/ml with fairly good recovery (mean 99,63% and 99,78% for benzbromarone and benzarone, respectively) and reproducibility.

INTRODUCTION

Benzbromarone, 2-ethyl-3-benzofuranyl 4-hydroxy-3,5-dibromophenyl ketone is a widely used uricosuric drug which increases urinary urate excretion.

Its debrominated metabolite - benzarone, 2-ethyl-3-benzofuranyl 4-hydroxyphenyl ketone, is also pharmacologically active. It has been shown to have uricosuric and more powerful fibrinolytic activity and is proposed for treatment of vascular disorders. The following methods have been used for estimation of benzbromarone in pure substance: non-aqueous titration[1,2], voltammetry and controlled potential coulometry[3]. A few analytical procedures, almost exlusively based on gas chromatography-mass spectrometry and high-performance liquid chromatography, have been described for determination of benzbromarone and/or benzarone in biological samples (plasma, urine)[4-11]. However, there are no publications about the analysis of these drugs in their dosage forms which are needed as analytical tools for stability studies of drugs and for their determination in commercial formulations. That is why it was considered to develop assay procedure which would serve as a rapid and reliable method for quality control of benzbromarone and benzarone pharmaceutical formulations.

MATERIALS

Reagents

Benzbromarone and benzarone were obtained from Sanofi-Labaz (France). Desuric® (100 mg of benzbromarone) and Fragivix® (100 mg of benzarone) tablets (Laboratiores Labaz, France) were used. Phosphate buffer: 0,067 M potassium dihydrogen phosphate adjusted to pH 2,6 with phosphoric acid; pH tolerance ± 0,05, acetonitrile and methanol LiChrosolv® for chromatography (E.Merck, Germany) were applied. All other solvents and reagents were of analytical grade.

Apparatus

A liquid chromatograph , type LC-730 (Laboratorni Pristroje Praha, Czechoslovakia) with a variable - wavelength UV detector and 250 x 4 mm steel column packed with LiChrosorb RP-18 (dp = 7 µm) was used. A reciprocating shaker, type 327 (Premed, Poland) was applied.

METHODS

The mobile phase was acetonitrile-phosphate buffer pH 2,6 (9:1, v/v). The flow rate was 1 ml/min. Detection was by UV absorption at 240 nm, detector output range was 0.08 AUFS, recorder chart speed was 0,3 cm/min.

Solutions

Stock solutions (1 mg/ml) of benzbromarone and benzarone were prepared by dissolving appropriate amounts of the substances in methanol. These solutions were stable for at least six months at 4°C. Working dilutions of 0,1 mg/ml of benzbromarone and benzarone were prepared from the stock solutions.

Calibration curve for benzbromarone assay

From the working solution of benzbromarone 0,2, 0,4, 0,6, 0,8, 1,0 and 1,2 ml volumes were pipetted into 10-ml measuring flasks.
Then, 0,2 ml of internal standard solution (0,1 mg/ml of benzarone) was added and made with acetonitrile up to 10,0 ml. 20 µl of each sample was then injected into the column. All measurements were repeated three times for each concentration. The peak heights were measured and the peak heights ratios of analyte to internal standard were then plotted against the res-

pective concentration of benzbromarone, in order to obtain the calibration curve.

Calibration curve for benzarone assay

The calibration for assay of benzarone was prepared in the same way as it is described above for benzbromarone, using benzbromarone as an internal standard (the same solutions and volumes pipetted). The calibration curve was based on the peak heights ratios of benzarone to that of internal standard against the respective concentration of benzarone.

Sample preparation

Tablets of benzbromarone

Tablets of benzbromarone were ground to a fine powder and amounts equivalent to 2-12 mg (after a declaration) of the compound were extracted with methanol in 100-ml volumetric flasks.
Filtered 1,0 ml volumes of the extracts were transfered into 10-ml flasks, 0,2 ml of internal standard solution (0,1 mg/ml) was added and made with acetonitrile up to 10,0 ml. Then, 20 µl of each sample was injected into the column.

Tablets of benzarone

Estimation of benzarone in tablets was developed in the same way as in the case of benzbromarone, using benzbromarone as an internal standard.

RESULTS AND DISCUSSION

A reversed-phase HPLC procedure was developed as a suitable method for the analysis of benzbromarone and benzarone dosage forms.

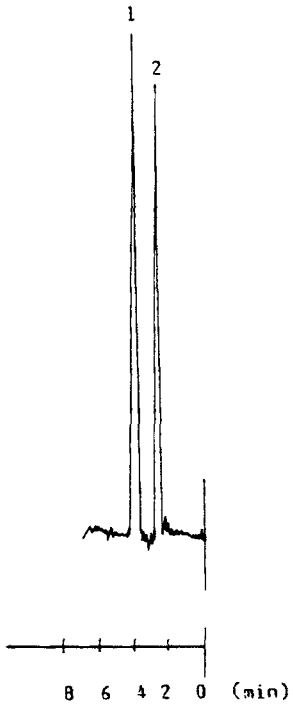

FIGURE 1. Typical chromatogram of benzbromarone (1) and benzarone (2). Peaks correspond to 6 µg/ml of benzbromarone and 4 µg/ml of benzarone.

The chromatographic conditions were adjusted in order to provide a versatile HPLC procedure capable of separating benzbromarone and benzarone and therefore also potentially useful for pharmacokinetic studies. A mixture of acetonitrile-phosphate buffer pH 2,6 (9:1 v/v), at flow rate of 1,0 ml/min was found to be an appropriate mobile phase, allowing for adequate and rapid separation of benzbromarone and benzarone (retention times 4,08 and 2,75 min, respectively). Typical chromatogram of benzbromarone and benzarone under described HPLC conditions is presented in Figure 1.

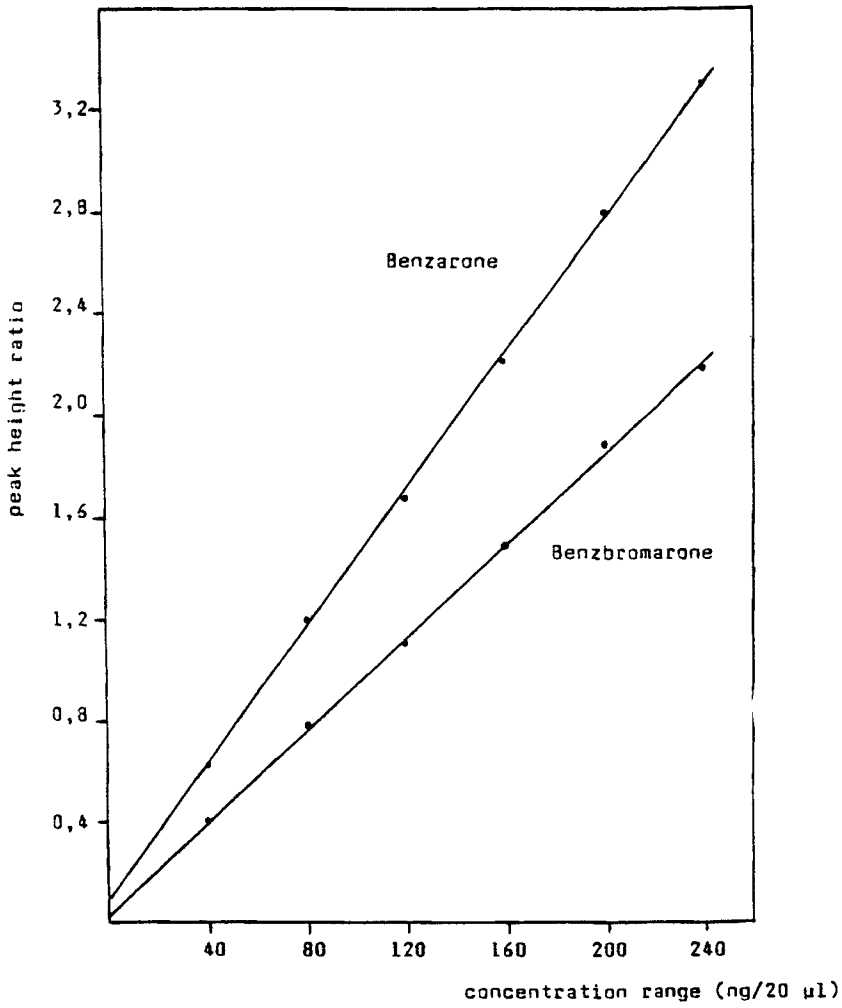

FIGURE 2. Standard curves for benzbromarone and benzarone assay (each point is the mean of three determinations).

TABLE 1

Results of the Determinations of Benzbromarone and Benzarone in Dosage Forms (n = 5, at each concentration)

No.	Amount expected (ng/20 µl)	Amount found (mean ± SD)	Coefficient of variation (%)
	BENZBROMARONE		
1	40	39,45 ± 0,54	1,37
2	80	79,72 ± 0,54	0,68
3	120	119,77 ± 0,54	0,45
4	160	158,92 ± 0,83	0,52
5	200	201,85 ± 0,54	0,27
6	240	238,79 ± 0,54	0,23
	BENZARONE		
1	40	39,67 ± 0,37	0,93
2	80	79,94 ± 0,37	0,46
3	120	120,06 ± 0,30	0,25
4	160	158,99 ± 0,36	0,22
5	200	201,79 ± 0,30	0,15
6	240	238,33 ± 0,30	0,13

SD - standard deviation

For quantitative applications linear calibration curves were obtained over the working concentration range 2-12 µg/ml. For assay of benzbromarone $y = 0,00899x + 0,04933$, $r = 0,9995$, for assay of benzarone $y = 0,01341x + 0,092$, $r = 0,9997$ where y - peak height ratio of benzbromarone or benzarone to that of internal standard and x - concentration of drugs in ng per 20 µl of the mobile phase.

Methanol was chosen as the extraction organic solvent because of solubility properties of benzbromarone and benzarone and a reversed-phase mode of chromatography. Recovery of benzbromarone after extraction from tablets was found to be 99,63% for benzbromarone and 99,78% for benzarone.

The precision of the chromatographic analysis in tablets was determined at six concentrations of both drugs. The coefficients of variation were obtained by repeating the procedure five times for each sample (Table 1).

The described method is simple and fairly reliable for pharmaceutical analysis.

ACKNOWLEDGEMENTS

We wish to thank the company Sanofi-Labaz for supplying benzbromarone and benzarone standards.

REFERENCES

1. J. Schnekenburger, M. Quade-Henkel, Dtsch. Apoth.-Ztg., 124: 1167-70 (1984)
2. W. Messerschmidt, Dtsch. Apoth.-Ztg., 28: 11-12 (1978)
3. H.B. Gallo, J.M. Kauffman, G.J. Patriarche, G.G. Guilbault, Anal. Lett., 19: 2011-21 (1986)
4. P.W. Luecker, K. Wetzelsberger, Arzneim.-Forsch., 29: 842-6 (1979)
5. H. Vergin, G. Bishop, J. Chromatogr., 183: 383-6 (1980)
6. V. Nitsche, H. Mascher, Arzneim.-Forsch., 31: 510-12 (1981)
7. W. Stueber, H. Moeller, J. Chromatogr., 224: 327-31 (1981)
8. J.X. De Vries, J. Walter-Stack, A. Van de Loo, J. Kocher, J. Chromatogr., 382: 167-74 (1986)
9. J.X. De Vries, J. Walter-Sack, A. Ittensohn, J.Chromatogr., 417: 420-7 (1987)

10. H. Maurer, P. Wollenberg, Arzneim.-Forsch., 40: 460-2 (1990)

11. P.J. Arnold, R. Guserle, V. Luckow, R. Hemmer, H. Grote, J. Chromatogr., 554: 267-80 (1991)

Received: April 2, 1994
Accepted: May 5, 1994