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Note

High-performance liquid chromatographic determination of benzbromarone and the main metabolite benzarone in serum

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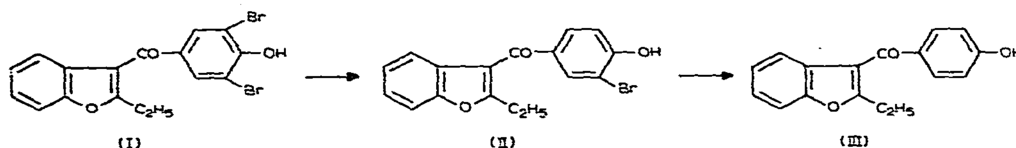
Benzbromarone, 2-ethyl-3-(4-hydroxy-3,5-dibromobenzoyl)-benzofuran, a well-known uricosuric agent, reduces serum uric acid concentrations by increasing renal clearance thereof in both healthy volunteers and gouty patients [1—3]. After absorption from the gastrointestinal tract benzbromarone is successively dehalogenated in the liver to its main metabolite, benzarone.



Bromobenzarone (II), the monohalogen metabolite, accounts for approximately 2% of the benzbromarone administered and is excreted exclusively in the faeces (radiochemical investigations) [4]; benzbromarone (I) and benzarone (III), on the other hand, are excreted almost entirely via the liver and the bile. Using radioactive-labelled material it has been shown that, for gouty patients, approximately 0.1% of the total dose appears as conjugated benzbromarone and 1% as conjugated benzarone in the urine [4]. In addition to the radiochemical methods, benzbromarone and benzarone serum concentrations can be determined using both spectrofluorimetry [5] and gas chromatography (GC) [6, 7].

This paper is aimed at describing a specific, sensitive and rapid procedure for simultaneously determining benzbromarone and benzarone serum concentrations using high-performance liquid chromatography (HPLC). This method is

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also suitable for measuring urine levels, but as renal excretion of the mother substance is negligible, urine level determination plays a subordinate role in benzbromarone therapy.

EXPERIMENTAL

Chemicals

All chemicals and solvents were p.a. standard, water bidistilled; all were prefiltered using a GV 100/1 glass filtration apparatus (ref. No. 392700) and filterdiscs, RC 58, 0.2 μm (ref. No. 371628) both from Schleicher and Schüll (Dassel, G.F.R.). Methanol, acetic acid, ethyl acetate were obtained from E. Merck (Darmstadt, G.F.R.) and acetonitrile HPLC Grade S from Rathburn Chemicals (Walkerburn, Great Britain). For calibrating the system benzbromarone charge No. Wi302782 and benzarone charge No. Wi30577 were used. Benzbromarone is manufactured as Narcaricin[R] (Heumann-Pharma, Nürnberg, G.F.R.) and as Uricovac[R] (Labaz, Erkrath, G.F.R.). Benzarone is manufactured as Fragivix[R] (Sanol, Monheim, G.F.R.).

Instruments

All investigations described in this paper were carried out on an ALC/GPC-204 system (Waters, Königstein/Taunus, G.F.R.) which was equipped with an injector, Model U6K and UV absorbance detector (254 nm), Model 440. The peaks obtained were quantitatively integrated by a Waters Data Module, Model 730.

Sample preparation

Serum (3 ml) was mixed with methanol (4 ml) and shaken for 1 min (Vibro-Fix, Jahnke and Kunkel, Staufen, G.F.R.). After centrifuging for 15 min at 3270 *g* (Hettich EBA 3S) the aqeuous methanolic phase was extracted and filtered through 0.2-μm filterholders of the type FP 030/3 (Schleicher and Schüll). Finally 10—30 μl of the filtrate were injected into the HPLC system.

HPLC system

Stationary phase. A reversed-phase material, μBondapak C_{18}, 10 μm (Waters) was packed into a column of 30 cm × 4 mm I.D.

Mobile phase. A mixture was employed consisting of methanol—water—acetonitrile—ethyl acetate—acetic acid (72:23:3:1:1). The system was, in addition, flow programmed to rise linearly from 1 to 2 ml/min, 3 min after sample injection, and thereafter remain constant.

RESULTS AND DISCUSSION

Both benzbromarone and benzarone are eluted as well-defined peaks with slight tailing, when the mobile phase methanol—water—acetonitrile—ethyl acetate—acetic acid is employed and the flow-rate is programmed as previously described. Under these conditions all retention times are reproducible. Ten aliquots, each 10 μl, of a spiked aqueous—methanolic serum extract (2.84 μg/ml benzbromarone and 1.25 μg/ml benzarone) were injected successively

into the reversed-phase system. Even over a space of several days reproducible mean retention times of 4.88 (coefficient of variation 0.95%) and 9.47 min (coefficient of variation 0.55%) were obtained for benzarone and benzbromarone respectively (Table I).

TABLE I

RECOVERY VALUES FOR BENZBROMARONE AND BENZARONE FROM SPIKED SERUM SAMPLES

Benzbromarone		Benzarone	
Concentration (μg/ml)	Recovery (%), mean ± S.E.	Concentration (μg/ml)	Recovery (%), mean ± S.E.
0	—	0	—
1.29	78.4 ± 1.10	0.91	89.1 ± 1.1
2.58	79.0 ± 0.61	1.83	91.2 ± 0.8
5.16	81.4 ± 2.96	3.66	89.2 ± 0.7
10.32	80.9 ± 3.12	7.32	90.8 ± 1.3
$\bar{x}$ ± S.D.	80.0 ± 1.45		90.02 ± 1.04

The relationship peak area to concentration is linear in the range 0.25—22.5 μg/ml for benzbromarone and for benzarone in the range 0.15—15 μg/ml. The detection limit for this particular method and for benzbromarone lies in the region of 140 ng/ml; benzarone has a detection limit of about 90 ng/ml.

Table I shows extraction reproducibility for the mother substance and the main metabolite. Recoveries of 80 ± 1.45% were obtained for benzbromarone over a range of 1.29—10.30 μg/ml and of 90 ± 1% for benzarone over a range of 0.91—7.30 μg/ml. Due to the high degree of reproducibility for both substances, standardisation for routine analysis can be carried out externally. Fig. 1 shows typical chromatograms of spiked and patients' sera (Fig. 1B and C, respectively). Peaks from the blanks (Fig. 1A) can be seen to interfere in no way whatever with those of either benzbromarone or benzarone.

The HPLC method described in this paper allows simultaneous determination of the uricosuric agent benzbromarone and its main metabolite benzarone from both volunteer and patient sera. In comparison to the GC method already published [7] an extremely rapid extraction and quantification of the two substances can be achieved, without forgoing the high reproducibility. The method is thus suitable for bioavailability studies on preparations containing benzbromarone. After administration of a single oral dose (100 mg) to seven healthy volunteers, maximum serum levels of 1.84 ± 0.87 mg/l occur [8]. The elimination half-life is 2.77 ± 1.07 h. Benzarone, the major metabolite, can be detected in the serum 3 h after dosing of benzbromarone. The maximum benzarone serum levels of 0.79 ± 0.21 mg/l occur 6 h after dosing. Benzarone is eliminated from the volunteers serum with an elimination half-life of 13.52 ± 2.18 h.

Both substances are excreted mainly via the liver and bile. In urine, only benzarone can be detected (1.55% of the benzbromarone dose) conjugated as a β-glucuronide.

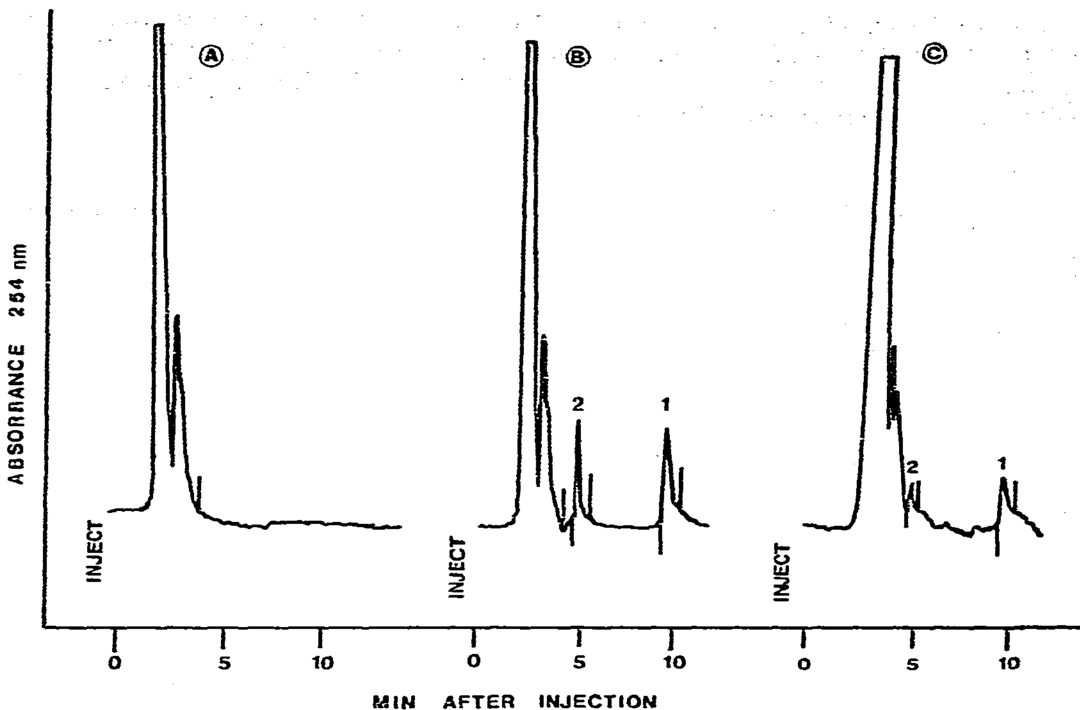

Fig. 1. Chromatograms of (A) serum blank; (B) serum spiked with 2.85 μg/ml benzbromarone and 1.83 μg/ml benzarone and (C) serum sample of a volunteer taken 6 h after administration of 100 mg benzbromarone where peaks 1 and 2 are benzbromarone and benzarone, respectively.

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