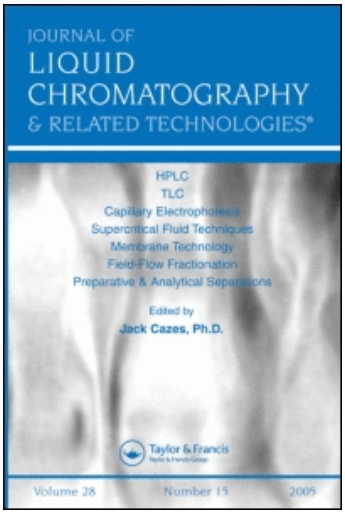

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DETERMINATION OF BENZIODARONE IN HUMAN PLASMA AND TABLETS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

A rapid HPLC method is described for benziodarone assay in human plasma using a LiChrosorb RP-18 column and UV detection at 240 nm. Protein precipitation is followed by extraction of benziodarone and khellin (internal standard). Extraction of the active substances with diethyl ether at pH ca. 4.8 ensures fairly good recovery (91.04%, mean). Detection limit for this method of determination is 20 ng using a 1 ml sample. The method is specific and can also be used for the determination of benziodarone in pharmaceuticals (tablets).

INTRODUCTION

Benziodarone, 2-ethyl-3-benzofuranyl 4-hydroxy--3,5-diiodophenyl ketone is a drug effective in the treatment of cardiovascular disorders. It has also been shown to have uricosuric activity and is proposed for treatment of primary hyperuricemia; in this case benziodarone can be administered in combination with allopu-

rinol. The both substances have different mechanisms of action but they act in synergism. Several studies on the properties of benziodarone in UV and visible spectral regions have been published (1-3) but there are no publications about the assay of benziodarone in pharmaceuticals and biological fluids.

MATERIALS

Reagents

Benziodarone was obtained from Sanofi-Labaz (France), "Uricodue" - tablets (50 mg of benziodarone and 100 mg of allopurinol) were obtained from Istituto Farmacoterapico Italiano S.p.A. (Italy), khellin was purchased from Fluka AG, Buchs SG (Switzerland). Heparinized human whole blood was obtained from the District Blood Centre in Lublin. All other reagents and solvents were of analytical grade.

Apparatus

A type LC-730 liquid chromatograph (Laboratorni Přistroje Praha, Czechoslovakia) with a variable - wavelength UV detector and a 250 x 4 mm steel column packed with LiChrosorb RP-18 (dp = 5 µm) was used. A reciprocating shaker, type 327 (Premed, Poland) and a high - speed centrifuge, type T 52.1 (Zentrifugenbau Engelsdorf, Germany) were applied.

METHODS

The mobile phase was acetonitril-phosphate buffer pH 2.6 (0.067 M potassium dihydrogen phosphate adjusted to pH 2.6 with phosphoric acid, pH tolerance $\pm$ 0.1), 9:1 (v/v).

The flow rate was 1 $ml \cdot min^{-1}$. Detection was by UV absorption at 240 nm, detector output range was 0.04

AUFS for assay in plasma, 0.08 AUFS for assay in tablets, recorder chart speed was 0.3 $cm \cdot min^{-1}$.

Solutions

Stock solutions (1 $mg \cdot ml^{-1}$ and 10 $mg \cdot ml^{-1}$) of benziodarone and khellin were prepared by dissolving appropriate amounts of the substances in methanol. Working dilutions of 0.01 $mg \cdot ml^{-1}$ and 0.1 $mg \cdot ml^{-1}$ of benziodarone and 0.005 $mg \cdot ml^{-1}$ and 0.1 $mg \cdot ml^{-1}$ of khellin were prepared from the stock solutions.

Linearity Test for the Plasma Samples

From the working solution of benziodarone (0.01 $mg \cdot ml^{-1}$) following volumes were pipetted: 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 ml and 0.2 ml of the solution of khellin (0.005 $mg \cdot ml^{-1}$) was then added to each sample and made with mobile phase up to an equal volume of 1.0 ml. 20 µl of each sample was injected into the column. All measurements were repeated three times at each concentration. The calibration curve based on the peak height ratios of benziodarone to that of internal standard was constructed. It was then used to calculate the results.

Extraction Procedure

To the glass centrifuge tubes containing 1.0 ml of blood plasma (obtained from heparinized human blood) 0.2 - 1.2 ml of working solution of benziodarone (0.01 $mg \cdot ml^{-1}$) and 0.4 ml of working solution of khellin (0.005 $mg \cdot ml^{-1}$) were added. Then, acetonitril was added to a final volume of 3.0 ml and the mixture was centrifuged for 20 min at 1100 g. Afterwards 1.5 ml of supernatant plasma from each sample was pipetted to the funnels and 4.0 ml of phosphate buffer pH 4.8 (0.067 M

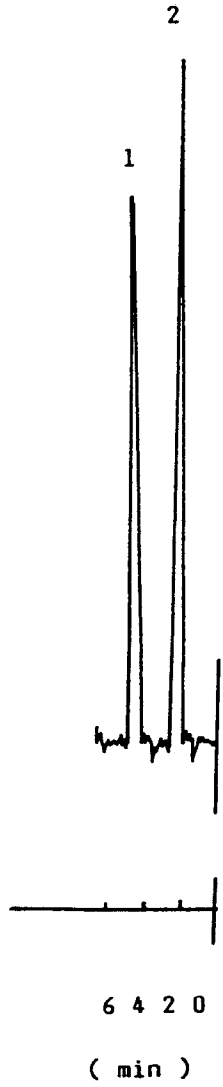

FIGURE 1. Typical chromatogram of benziodarone (1) and internal standard - khellin (2). Peaks correspond to 3.0 and 1.0 µg per ml of benziodarone and khellin, respectively.

potassium dihydrogen phosphate - 0.067 M disodium hydrogen phosphate, 9.75:0.25 v/v adjusted to pH 4.8 with phosphoric acid) and 3.0 ml of freshly distilled diethyl ether were added and then extracted by shaking for 10 min. The ether extract was separated and the aqueous phase was extracted once more with 3.0 ml of diethyl ether. The mixed ether extracts were evaporated to dryness in a stream of nitrogen. After dissolving each sample in 1.0 ml of mobile phase, 20 µl was injected into the column.
Fig. 2 shows typical chromatograms of extracted plasma.

Linearity Test for Tablets

From the solution containing 0.1 $mg \cdot ml^{-1}$ of benziodarone 0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 ml were pipetted into 10-ml measuring flasks. Then, 0.2 ml of the solution of khellin (0.1 $mg \cdot ml^{-1}$) was added to each sample and made with acetonitril up to 10.0 ml. 20 µl of each sample was then injected into the column. The calibration was calculated in the same way as described above for plasma.

Determination of Benziodarone in Tablets

Tablets of benziodarone 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 khellin solution (0.1 $mg \cdot ml^{-1}$) was added and made with acetonitril up to 10.0 ml.
Then, 20 µl of each sample was injected into the column.

RESULTS AND DISCUSSION

Khellin, proposed here as an internal standard (i.s.), is a model compound for cardiovascular drugs of benzofuran and benzopyran type.

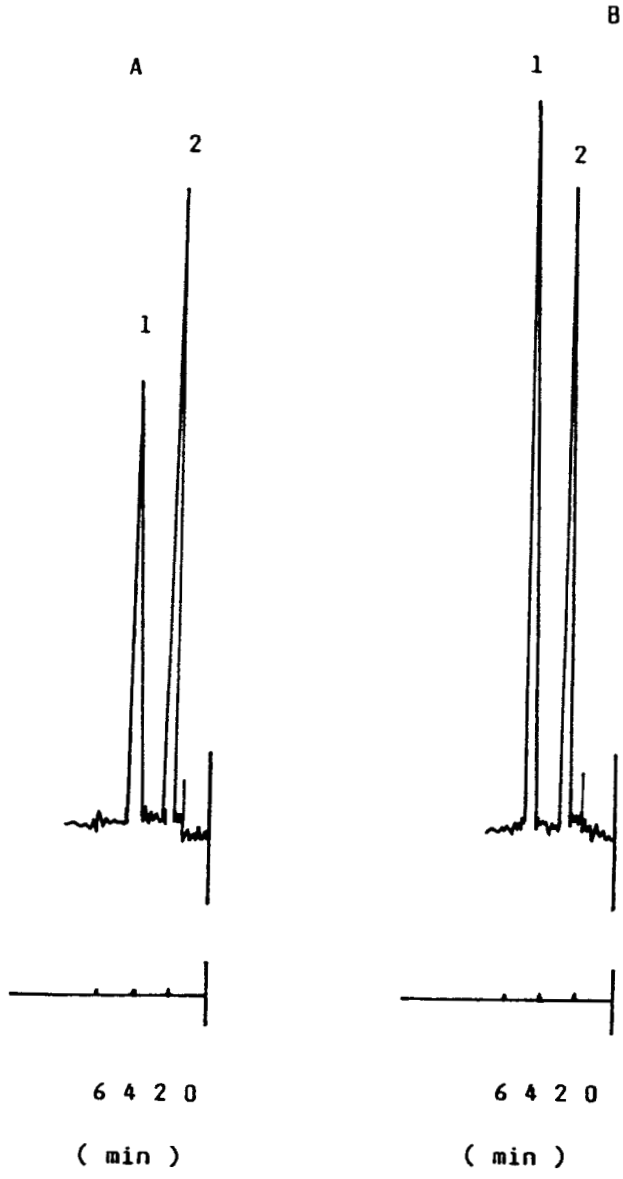

FIGURE 2. Typical chromatograms of extracted plasma treated as in the text containing:
A - 3.0, B - 5.0 µg per ml of benziodarone (1) and both 1.0 µg per ml of khellin (2).

TABLE 1

Concentration of Benziodarone (added) in Plasma (n = 3, at each level of addition)

No.	Benziodarone content (ng) in 20 µl of mobile phase	h/h'		Recovery (%)
		in working solutions of benziodarone and khellin	after extraction from plasma	
1	20	0.25	0.27	93.36
2	40	0.54	0.45	86.03
3	60	0.75	0.68	90.77
4	80	1.0	0.91	93.14
5	100	1.20	1.10	91.07
6	120	1.41	1.32	91.88

h = height of benziodarone peak, h' = height of khellin peak

In a preliminary study different pH conditions: 2.3 - 7.0 and various organic solvents were used for the extraction of benziodarone and i.s. from plasma. Diethyl ether at pH ca 4.8 gave the best reproducibility and a fairly good recovery (91.04%, mean). The data about concentration of benziodarone in plasma of patients treated with therapeutic doses of the drug have not been published yet. In the quantitative HPLC procedure described here benziodarone was reproducibly determined in plasma at concentration 1.0 - 6.0 $\mu g \cdot ml^{-1}$. Comparatively in plasma taken from patients treated with structurally similar drug - benziodarone (2-ethyl-3-benzofuranyl 4-hydroxy-3,5-dibromophenyl ketone) peak plasma concentrations of 1.4 - 2.9 $\mu g \cdot ml^{-1}$

were attained in about 3 hours following a single oral dose of 100 mg to 7 subjects (4); benziodarone is administered in intial dose of 600 mg daily and maintenance dose of 300 - 400 mg daily (4). In the range 1.0 - 6.0 $\mu g \cdot ml^{-1}$ the calibration curve for benziodarone assay in plasma was linear ($r = 0.9983$) and a regression line through the data points was $y = 0.01147x + 0.0553$ (x = concentration of benziodarone in ng per 20 µl of the mobile phase, $y = h/h'$). The precision of analysis was determined at six different concentrations of the drug.

The method described above makes it possible to determine of benziodarone also in pharmaceuticals. The calibration curve for benziodarone assay in tablets was linear ($r = 0.9982$) in the range 2.0 - 12.0 $\mu g \cdot ml^{-1}$ (regression line was $y = 0.00764 x - 0.03$). Recovery of benziodarone after extraction of tablets with methanol was found to be 98.30%, mean. Allopurinol (a component of "Uricodue" tablets) was found not to interfere in the assay (its retention time was 1.83 min).

Under the conditions used for the HPLC system, benziodarone and khellin had retention times 4.75 and 2.66 min, respectively. The method is simple, reliable and fairly sensitive and it is thought to be used in clinical and pharmaceutical analysis.

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REFERENCES

1. J.Kracmarova, A.Kovarova, Z.Stejskal, Pharmazie, 43: 173-6 (1988).

2. J.Kracmar, J.Kracmarova, A.Kovarova, Cesk.Farm., 37: 53-60 (1988).

3. G.Heinisch, H.Matous, W.Rank, R.Wunderlich, Sci. Pharm., 49: 472-82 (1981).

4. E.G.C.Clarke, ed., Isolation and Identification of Drugs, The Pharmaceutical Press, London, 1988.

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