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Note

# Determination of benzbromarone, bromobenzarone and benzarone in plasma by gas chromatography—mass spectrometry

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Benzbromarone, a benzofuran derivative, lowers serum urate and increases urinary urate excretion [1—4]. The drug undergoes extensive dehalogenation in the liver to form bromobenzarone and benzarone which also display hypouricaemic activity. Twelve hours after ingestion approximately 75% of the absorbed drug has been converted to benzarone [5].

A high-performance liquid chromatographic method for the determination of benzarone from human urine and serum was described by Lücker and Wetzelsberger [6]. The analytical procedure was based on a gradient elution on a reversed-phase column after chloroform extraction. Besides a study using tritium labelled compounds by Broekhuysen et al. [5], Yu [7] reported a gas chromatographic (GC) analysis of acetylated derivatives. This paper describes a gas chromatographic—mass spectrometric (GC—MS) method for the measurement of benzbromarone and its dehalogenated analogues after extraction from plasma and derivatization with trifluoroacetic anhydride. Accurate estimates of benzbromarone, bromobenzarone and benzarone plasma concentrations can be made following the ingestion of pharmacological amounts of the uricosuric agent.

## MATERIALS AND METHODS

### *Chemicals*

Benzarone, bromobenzarone and benzbromarone were obtained from Labaz (Düsseldorf, G.F.R.); trifluoroacetic anhydride was purchased from EGA (Steinheim, G.F.R.).

### *Apparatus*

A Varian 3700 gas chromatograph in combination with a MAT 44 mass

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spectrometer was used. The silanized glass column (2 m × 2 mm I.D.) was packed with 1% OV-17 on 80—100 mesh Chromosorb W (Perkin Elmer, Überlingen, G.F.R.). Prior to its initial use, it was conditioned overnight at 300°C with the carrier gas helium at a flow-rate of 30 ml/min. Operating temperatures were: column, 260°C; injection port, 290°C; separator, 280°C; transfer line, 280°C; ion source, 250°C. The electron energy was kept at 80 eV, the emission current at 0.8 μA.

*Analytical procedure*

Concentrations of benzarone, bromobenzarone and benzbromarone in the range between 10 and 2000 ng were added to 1 ml pooled male plasma samples. The addition of 1 ml 0.1 *N* hydrochloric acid and 5.0 ml chloroform was followed by an extraction for 1 h. After centrifugation (ca. 1950 *g*, 15 min) the water layer was removed by aspiration and 4.0 ml of the remaining chloroform phase were transferred with a Pasteur pipette to clean test tubes. Known concentrations of diazepam in chloroform solution were added for internal standardization. After evaporation to dryness trifluoroacetate (TFA) derivatives were formed by reacting the residue with 50 μl of a 20% solution of trifluoroacetic anhydride in acetonitrile. The samples were shaken ultrasonically for 30 sec. A 2—5-μl volume was analyzed by GC—MS. Benzarone/diazepam, bromobenzarone/diazepam and benzbromarone/diazepam peak height ratios were determined at least in triplicate for each benzarone, bromobenzarone and benzbromarone concentration to establish a standard curve.

## RESULTS AND DISCUSSION

The molecular ions after derivatization with TFA, *m/e* 520, *m/e* 440 and *m/e* 360, represented the base peaks and were used for sinlge ion monitoring (Figs. 1, 2 and 3). Therefore a rapid GC analysis without interference from endogenous substances was possible; retention times for benzarone, bromobenzarone and benzbromarone derivatives were 0.9, 1.2 and 1.5 min, respec-

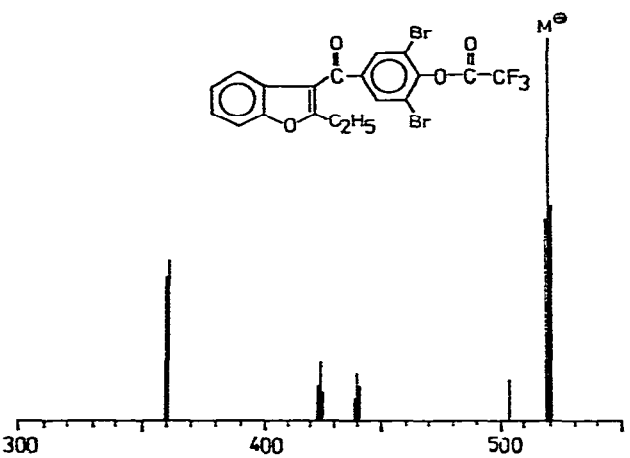


Fig. 1. Mass spectrum of derivatized benzbromarone.

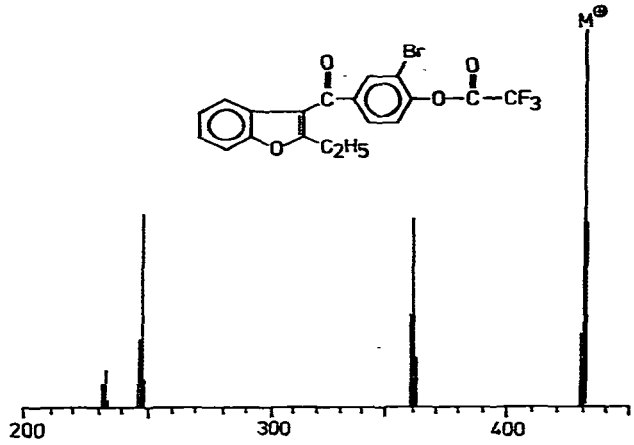


Fig. 2. Mass spectrum of derivatized bromobenzarone.

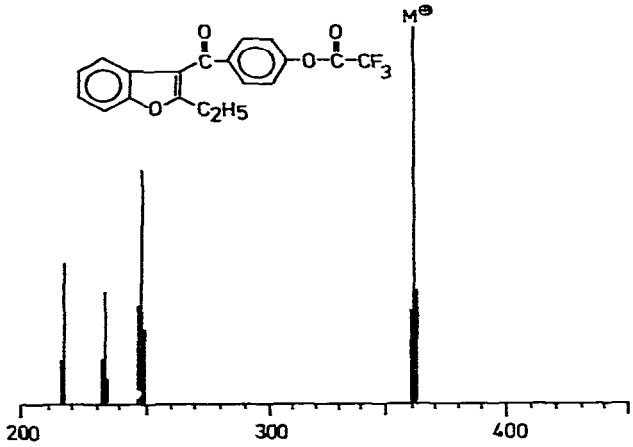


Fig. 3. Mass spectrum of derivatized benzarone.

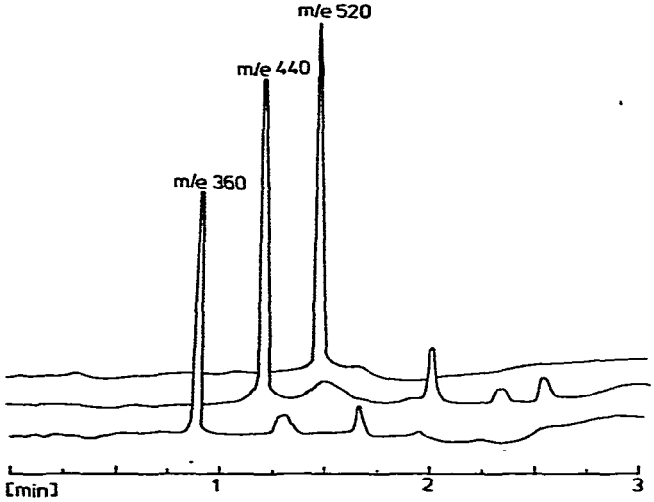


Fig. 4. Single ion monitoring after GC separation.

tively. Specificity was promoted by monitoring molecular ions. Fig. 4 shows a typical gas chromatogram of a spiked plasma sample. Derivatization with TFA was uncomplicated and rapid. A 20% solution of the reagent in acetonitrile was required to obtain complete derivatization (Fig. 5). Other acetylating and methylating techniques can be applied, but longer reaction times, fragmentation because of less stability of the molecular ions and less response have to be taken into account. Derivatization with silylating agents was unsuccessful because of the bulky bromide ions in the case of benzbromarone. Recovery from plasma by chloroform extraction was nearly quantitative: 91.7 ± 5.5% was found for benzarone, 92.0 ± 3.5% for bromobenzarone and 95.4 ± 3.2% for benzbromarone. Similar results were reported by Lücker and Wetzelsberger [4]. Linear and reproducible relationships between benzbromarone/diazepam, bromobenzarone/diazepam and benzarone/diazepam concentrations were obtained over the tested concentration range (Fig. 6).

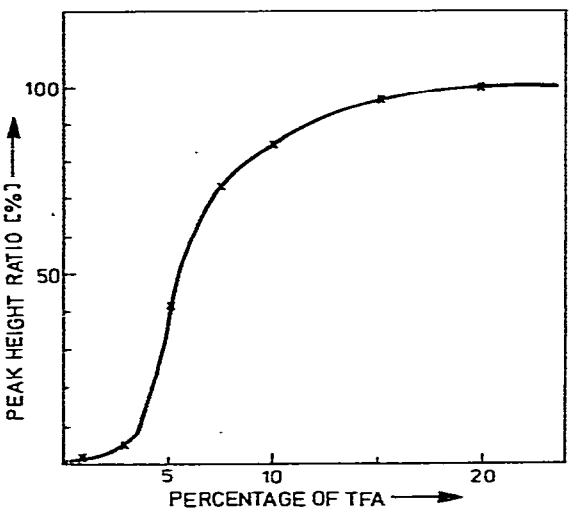


Fig. 5. Peak height ratio of benzbromarone/diazepam as a function of the percentage of TFA.

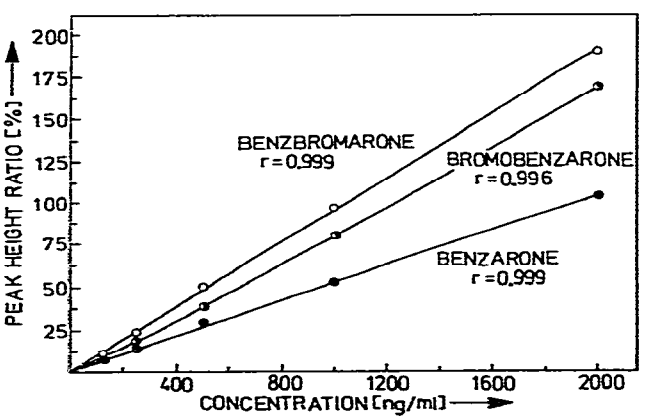


Fig. 6. Calibration curves of benzbromarone, bromobenzarone and benzarone.

The standard deviations at 100 ng benzarone, bromobenzarone and benzbromarone per ml plasma were 10.0, 5.3 and 3.7%, respectively, and at 2 μg per ml plasma were 2.5, 2.4 and 2.9%, respectively. The lowest concentrations that could be measured were 10 ng/ml plasma.

According to the literature data [7] plasma concentrations reach the maximum of 2—3 μg benzbromarone per ml plasma after the ingestion of a 80-mg dose, whilst even higher plasma levels were measured for benzarone. Therefore, the present method allows pharmacokinetic and metabolism studies of benzbromarone after therapeutic oral doses.

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