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Short Communication

Determination of bencyclane in human plasma by means of capillary gas chromatography and nitrogen–phosphorus selective detection

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ABSTRACT

A sensitive and specific method for the determination of bencyclane in human plasma is presented. Bencyclane was extracted from human plasma with two 3-ml volumes of isooctane and was shaken for 10 min. The organic phase was separated and evaporated to dryness at 40°C under a nitrogen stream. The residue was dissolved and an aliquot was injected into the gas chromatograph. The separation was performed with a DB-17 column with helium as the carrier gas. Nitrogen-selective detection was performed. The quantification was performed with the signal output. The limit of detection was 1 ng/ml.

INTRODUCTION

Bencyclane, N,N-dimethyl-3-{[1-(phenylmethyl)cycloheptyl]oxy}-1-propanamin, is a cycloalkane ether with a molecular mass of 289.45. It has been used for many years as a spasmolytic, muscle-relaxing and vasodilatory therapeutic agent for the treatment of peripheral and cerebral vascular circulatory disorders. The action is thought to rely on an inhibition of the intracellular calcium inflow and the resulting electromechanical decoupling in smooth muscles.

In 1984, Mohri *et al.* [1] published a radioimmunoassay and a gas chromatographic–mass spectrometric (GC–MS) method for the analysis of bencyclane, with a limit of detection of 1 ng/ml. Independently, Gielsdorf *et al.* [2] published in the same year a quantitative assay method for bencyclane by means of GC–MS

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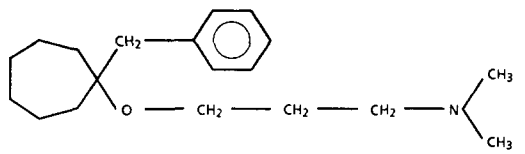
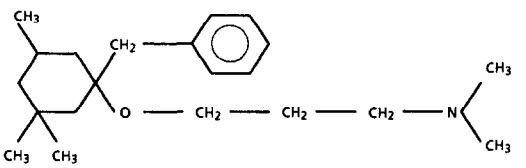
and chemical ionization. In 1985, Eckard and Weyhenmeyer [3] published another GC–MS assay method, and in the same year Marzo *et al.* [4] described a GC method with flame ionization detection (FID) and thermionic-specific detection. The GC–FID method developed by Marzo *et al.* [4] yields a limit of detection of 0.5–1 μg/ml, and thus cannot be used for pharmacokinetic investigations because of its poor sensitivity. The thermionic-specific assay method described by the same authors yields a sensitivity of 10 ng/ml. The chromatograms presented in those reports clearly show that the chromatographic quality required today is far from achieved.

This paper describes the development of a new assay method. The molecular structures of bencyclane and the internal standard used, N,N-dimethyl-3-{[1-(phenylmethyl)cycloheptyl]oxy}-1-propanamin (TH 837, I.S.), are shown in Fig. 1. Furthermore, the results of validation experiments, as described here, proved that this method can be used in pharmacokinetic studies.



Bencyclane



TH 837

Fig. 1. Molecular structures of bencyclane and internal standard TH 837.

EXPERIMENTAL

Instruments and instrumental conditions

The gas chromatograph used was a Hewlett Packard 5890 (Hewlett Packard, Palo Alto, CA, U.S.A.). The carrier gas was helium 6.0 of 99.9999% purity (Messer Griessheim, Duisburg, Germany). A Hewlett Packard split–splitless injector was used at 250°C, with a purge delay of 30 s. The column was a DB-17 fused-silica capillary (30 m × 0.25 mm, film thickness 0.25 μm) (J&W Scientific, Folsom, CA, U.S.A.). The nitrogen–phosphorus detector (rubidium bead) was also from Hewlett Packard, and was operated at 290°C and a current strength of 50 pA.

The helium internal pressure-reducing station was operated at 160 kPa, the column head pressure was 100 kPa, and the helium flow-rate on the column was

0.80 ml/min [the flow-rate (plus auxiliary gas) was 30 ml/min]. For hydrogen, the internal pressure-reducing station was operated at 120 kPa, and the hydrogen flow-rate at the detector was 4.0 ml/min. For synthetic air, the internal pressure-reducing station was operated at 210 kPa, and the air flow-rate at the detector was 70 ml/min. The autosampler, a Hewlett Packard 7673A, had an injection volume of 1 μl, and was rinsed ten times with 8 μl of ethanol–isooctane (9:1). A Hewlett Packard work station 300/9000 was used for integrations. The temperature was held at 100°C, for 1.0 min initially, then increased at 40°C/min to 250°C, then held for 7.5 min. The equilibration time was 3.0 min. The injection amplitude in the automatic process was 19 min 15 s.

Chemicals and reagents

Bencyclane was obtained from Iropharm (Aclo, Eire), the I.S. (TH 837) from Thiemann Arzneimittel (Waltrop, Germany) and human pool plasma from the blood bank of the University Hospital Düsseldorf (Düsseldorf, Germany). Absolute ethanol, isooctane, dipotassium hydrogenphosphate and methanol, all of reagent purity were from Merck (Darmstadt, Germany) and double-distilled water was prepared in our laboratory.

To make the bencyclane stock solution, 5 mg of bencyclane base were weighed into a 100-ml volumetric flask and dissolved in 100.0 ml of methanol. This corresponded to a concentration of 50 μg/ml. The solution was stored at 20°C and protected from light.

To make the I.S. stock solution, 5 mg I.S. base were weighed into a 100-ml volumetric flask and dissolved in 100.0 ml of methanol. This corresponded to a concentration of 50 μg/ml (solution A). The solution was stored at 20°C and protected from light.

To make the I.S. working solution, 2.5 ml of solution A were diluted with methanol to 50 ml (2.5 μg/ml). Of this solution, 50 μl (125 ng) were used.

Analytical procedure

For each calibration curve and quality control measurement, independent stock solutions were prepared. For the determination of the recovery, intra-assay and inter-assay reproducibility, the samples were spiked with a stock solution: 1 ml of stock solution with 1 ml of methanol yielded a concentration of 500 ng per 20 μl or 25 μg/ml. This solution was diluted to produce the following concentrations: 5, 10, 25, 50, 100 and 250 ng per 20 μl.

A 20-μl volume of the stock solution and 50 μl of the I.S. solution were transferred to a 15-ml AR-Glas universal vial, and evaporated to dryness under a nitrogen stream. After this, 1 ml of blank plasma was added to the dry residue and mixed on a vortex mixer for 15 s. Next, 300 μl of 1 *M* dipotassium hydrogenphosphate solution were pipetted into the vial and mixed on a vortex mixer for 15 s. The samples were extracted twice by vertical rotation after the addition of 3 ml isooctane per sample (10 min). The extracted plasma samples were centrifuged at

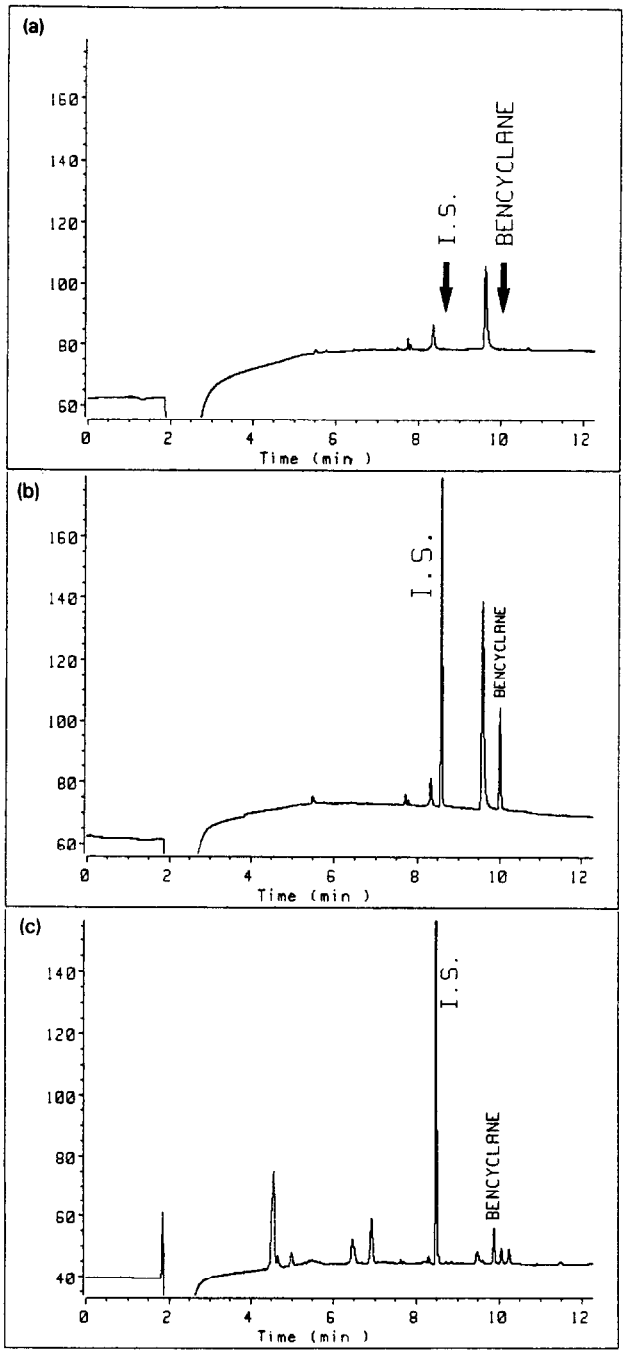


Fig. 2. Chromatograms of a blank plasma (a), a spiked plasma sample (b) and a real sample, containing 5 ng/ml bencyclane and 125 ng/ml I.S. (c).

4100 *g* for 10 min. The organic phase was removed an evaporated to dryness in a 4-ml AR-Glas vial. The walls of the vial was rinsed with 150 μl of ethanol–isooctane (9:1). The samples were evaporated to dryness again, and the residue was dissolved in 20 μl of ethanol–isooctane (9:1) then shaken on a vortex mixer and pipetted into a 100-μl microvial, which was sealed tightly. Of this solution, 1 μl was injected into the gas chromatograph.

The calibration samples were obtained by pipetting 50 μl of the I.S. and 20 μl of each bencyclane dilution into human blank plasma as described above.

The recovery rate was determined as follows: 1 ml of a spiked plasma samples was analysed as described above, and the analytical recovery was determined five times at three different calibration marks. For comparison, injections of the same concentrations without plasma were made.

RESULTS AND DISCUSSION

The selectivity of the assay can be verified by comparing the chromatograms of a spiked plasma sample and human blank plasma. As can be seen quite clearly, there are no interfering peaks (Fig. 2).

The detection limit (defined as three times the baseline noise) for the plasma samples examined was 1 ng/ml of human plasma. As the practical level of determination we chose the 5 ng/ml human plasma value. The linearity of the calibration curve was examined and shown to be good over the concentration range 5–500 ng/ml. In general, the *r* values obtained were above 0.999.

The values of the absolute analytical recovery of bencyclane were in general greater than 80% at all concentrations. The recovery of the internal standard, determined at the concentration examined, was 100%.

The intra-assay reproducibility was determined by assaying five different spiked plasma pools. A calibration curve established beforehand was used as reference. All analyses were performed in randomized order. Over the concentration range the intra-assay coefficient of variation (C.V.) was better than 6.5% at 1 ng/ml and 5.8% at 500 ng/ml.

The inter-assay reproducibility was determined by comparing the ratios of the calibration cuves on fourteen different days. Over the concentration range the inter-assay C.V. was better than 8.7% at 1 ng/ml and 6.0% at 500 ng/ml.

Over more than eight months, neither the plasma samples nor the calibration samples showed any sign of deterioration. Plasma samples were stored at −20°C until use and protected from light. The stock solutions of bencyclane and of the I.S. standard were stable for at least fourteen days.

Neither pool plasma nor plasma samples from more than 100 different volunteers and patients showed any interferences in the chromatograms.

The method described is suitable for the analysis of bencyclane in human plasma owing to its selectivity, its sensitivity and its reproducibility. It is therefore useful for routine analyses, as required for pharmacokinetic studies.

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