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Note

Quantitative determination of benzilonium bromide in plasma by gas chromatography—mass spectrometry after oxidation to benzophenone

HANS DAHLSTRÖM

Parke-Davis, Anderstorpsvägen 16, S-171 06 Solna (Sweden)

KURT LEANDER

Produktkontroll AB, Sandkilsvägen 11, S-184 00 Åkersberga (Sweden)

BJÖRN LINDSTRÖM*

National Board of Health and Welfare, Department of Drugs, Division of Clinical Drug Trials, S-751 25 Uppsala (Sweden)

and

MARIANNE MOLANDER

Royal Institute of Technology, Department of Analytical Chemistry, S-100 44 Stockholm (Sweden)

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Benzilonium bromide is a quaternary ammonium compound and an anticholinergic agent. While studying its pharmacological effects on urinary bladder function [1] we developed a method to quantify benzilonium bromide down to 5 ng/ml plasma; the method is described in this paper.

EXPERIMENTAL

Instrumental

A Finnigan 4000 gas chromatograph—mass spectrometer equipped with a Promim device was used (Finnigan, Sunnyvale, CA, U.S.A.). The column was a 3% OV-225 (1.4 m × 2 mm I.D.) operated at 175°C and directly interfaced with the ion source. Methane was used as the carrier gas (flow-rate 20 ml/min).

Under these conditions benzophenone had a retention time of 1.8 min. Injection temperature was 200°C and the ion-source temperature was 250°C. The electron energy was 70 eV. The mass spectrometer was focused to monitor the ions *m/e* 183 and 188.

Internal standard

The internal standard, benzilonium-d_5 bromide, was synthesised by a Grignard reaction of pentadeuterobromobenzene with methylphenylglyoxylate [2], and by reacting the obtained methyl pentadeuterobenzilate with 1-ethyl-3-hydroxypyrrolidine, followed by quaternisation with ethyl bromide [3].

Reagents

Carbonate buffer solution (pH 9.0, 0.1 *M*) containing 1 mg/ml sodium 2-hydroxy-3,5-di-*tert*-butylbenzene sulphonate (Bofors Nobel Kemi S-690 20, Bofors, Sweden); saturated potassium permanganate solution (ca. 0.4 *M*); potassium hydroxide solution (5.7 *M*); aqueous solution of benzilonium-d_5 bromide (200 ng/ml).

Sample preparation

To a screw-capped 15-ml tube containing a plasma sample (2 ml) were added internal standard solution (100 µl), carbonate buffer solution (2 ml) and dichloromethane (6 ml). The mixture was shaken for 15 min and then centrifuged (5 min, 500 *g*). The aqueous phase was removed and discarded. After addition of anhydrous sodium sulphate (0.5 g) the tube was shaken for a short while and then centrifuged. The dry dichloromethane phase was removed and filtered through a Pasteur pipette, stoppered with glass wool, into a new screw-capped tube. The solvent was evaporated in a stream of nitrogen. Potassium hydroxide solution (3 ml), potassium permanganate solution (2 ml) and hexane (2 ml) were then added to the tube. The mixture was shaken in a water bath at 70°C for 1 h. After cooling, the tube was centrifuged and the hexane phase was transferred to a conical tube (5 ml).

Removal of the solvent was done by evaporation in a stream of nitrogen. The residue was dissolved in ethyl acetate (20 µl) and an aliquot was injected on to the column.

RESULTS

Extraction

Isolation of the "benzilonium ion" from plasma was accomplished by the addition of sodium 2-hydroxy-3,5-di-*tert.*-butylbenzene sulphonate and extraction with dichloromethane at pH 9. The sulphonate ion used is strongly lipophilic but still water-soluble, which makes it a good counter-ion for ion-pair extraction of quaternary ammonium compounds [4]. The recovery of the "benzilonium ion" was determined gravimetrically and found to be at least 98%. The recovery of benzilonium (determined as benzophenone) through the method was estimated to be about 85% at the 50 ng/ml level. This was accomplished by comparing the molecular ion intensity of benzophenone, generated

from benzilonium by the method described, with that of a standard sample of benzophenone.

Oxidation

Oxidation of the "benzilonium ion" to benzophenone was performed according to a general procedure described by Hartvig et al. [5]. The benzophenone yield was 90%. When blank plasma samples were analysed, a peak appeared in the gas chromatograms corresponding to 0.5—1.5 ng/ml benzophenone. In plasma samples from the same patient, however, the background did not show any significant variation when the analyses were performed in one series using the same batches of reagents, solvents, pipettes, etc. In an attempt to minimize background disturbance, oxidation to benzophenone was performed by using barium peroxide in acidic solution. This procedure has been shown to give low reagent blanks [6]. However, on using this procedure, the yield of benzophenone from "benzilonium ion" was less than 10%.

Mass spectrometry

Chemical ionisation was preferred to electron impact since it was possible to achieve much higher sensitivity when analysing benzophenone with this mode of ionisation.

Sensitivity and precision of the method

The coefficient of variation obtained when analysing two series of plasma samples, nine samples in each series, to which had been added 5 and 50 ng of benzilonium bromide per ml, were 6% and 1.7%, respectively.

Application to biological samples

Five minutes after intravenous injection of 1 mg of benzilonium bromide in healthy adults the mean plasma concentration (five individuals) was 100 ng/ml. In the same individuals 10 mg taken orally two times daily for one week resulted in a plasma concentration of 1—4 ng/ml measured before taking the morning dose on the 8th day; and 2—3 h after the morning dose on that day the plasma concentration was 3—4 ng/ml. Since the obtained levels of benzilonium ion after the described oral administration were equal to or slightly above the detection limit of the method only a rough estimation of the plasma concentrations was possible in this case.

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