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Note

Analysis of buprenorphine in plasma and urine by gas chromatography

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Buprenorphine, (6*R*,7*R*,14*S*)-17-cyclopropylmethyl-7,8-dihydro-7-[(1*S*)-1-hydroxy-1,2,2-trimethylpropyl]-6-O-methyl-6,14-ethano-17-normorphine, is a synthetic analgesic with narcotic agonist–antagonist properties. As an analgesic it is some 25–40 times more potent than morphine and has been used primarily in the post-operative period and in terminal cancer. Its molecular structure is similar to that of morphine, and it is administered intramuscularly or intravenously with a typical dose range of 0.3–0.6 mg (0.005–0.01 mg/kg), although recently the oral route has also been used. After parenteral or sublingual administration it is absorbed and metabolized, principally by N-dealkylation and conjugation.

Since analgesis is achieved using very low doses, the therapeutic levels found in plasma or serum are very low; therefore highly sensitive instrumental techniques are required in order to detect and confirm its presence. A radioimmunoassay technique has been developed [1,2]. The sensitivity of the method is high, but this technique is not always selective since cross-reaction can occur, either with the glucuronide conjugate or with the N-dealkylated metabolite. Blom et al. [3] have developed a method using gas chromatography–mass spectrometry (GC–MS) with selected-ion monitoring for the determination of buprenorphine and an N-dealkylated metabolite, by formation of derivatives with pentafluoropropionic anhydride. Hackett et al. [4] analysed buprenorphine in urine by high-performance liquid chromatography (HPLC), after hydrolysis with β-glucuronidase, extraction and purification by thin-layer

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chromatography (TLC). Cone et al. [5] have described the development of a ^{63}Ni electron-capture GC assay for buprenorphine, norbuprenorphine and the acid-catalysed rearrangement product, demethoxybuprenorphine, in human urine and faeces.

This paper describes a method for the identification and determination of buprenorphine in plasma and urine, based on GC with two detectors: a nitrogen–phosphorus detector for the acetyl derivative and electron-capture detector for the heptafluorobutyryl derivative.

EXPERIMENTAL

Materials

Buprenorphine hydrochloride was supplied by Laboratorios Dr. Esteve S.A. (Barcelona, Spain). Heptafluorobutyric anhydride was obtained from Supelco (Barcelona, Spain). Nalorphine hydrobromide was supplied by Instituto Nacional de Toxicologia (Madrid, Spain). Disposable Extrelut-20 columns were obtained from Merck (Darmstadt, F.R.G.). All other chemicals and solvents were of analytical-reagent grade.

Analysis in plasma

Plasma (2–4 ml) together with the internal standard (20–40 ng of nalorphine) was diluted to 18 ml with water, made alkaline with 2 ml of pH 9.2 buffer solution, 40% K_2HPO_4 (200 g in 500 ml of water) and left for 15 min. A 20-ml volume was introduced into an Extrelut-20 column and left for 10 min to achieve efficient diffusion. The column was eluted with two 20-ml portions of diethyl ether. The mixed eluates were dried over anhydrous sodium sulphate and evaporated to small volume at reduced pressure, with final evaporation to dryness under a stream of nitrogen.

Analysis in urine

In order to break the conjugation of buprenorphine with glucuronic acid, the urine was subjected to acid hydrolysis. To 10 ml of urine, the internal standard (20–40 ng of nalorphine) and 10 ml of 25% hydrochloric acid were added, and the mixture was maintained in a boiling water-bath for 1 h. After cooling, the pH was adjusted to ca. 7 with 10 *M* sodium hydroxide, followed by the addition of 4 ml of 40% K_2HPO_4 buffer (pH 9.2). Then 20 ml of this solution were introduced into an Extrelut-20 column and allowed to stand for 10 min. The column was eluted with two 20-ml portions of dichloromethane–2-propanol (85:15). The eluates were mixed and dried over anhydrous sodium sulphate, then evaporated at reduced pressure to a small volume, with final evaporation to dryness under a stream of nitrogen.

Acetyl derivative

The dry residues were dissolved by shaking in 100 μl of freshly mixed acetic anhydride–pyridine (1:1). The solution was kept in an 80°C water-bath for 30 min, after which it was evaporated to dryness under a stream of nitrogen. This residue was dissolved in 50 μl of chloroform for GC with nitrogen–phosphorus detection (NPD).

Heptafluorobutyryl derivative

A 200-μl volume of heptafluorobutyric anhydride was added to the dry residues, shaken to dissolve the residues and allowed to stand for 45 min. The solution was then evaporated to dryness under a stream of nitrogen, and the residue dissolved in 50 μl of ethyl acetate for GC with electron-capture detection (ECD).

Instrumental conditions

Acetyl derivative. A Hewlett-Packard Model 5710 A gas chromatograph, attached to a Hewlett-Packard 3388 A integrator and equipped with a fused-silica OV-1 column (HP-1, 5 m×0.530 mm I.D.) was used. The chromatographic conditions were: carrier gas, helium at a flow-rate of 25 ml/min; initial temperature, 210°C; initial time, 6 min; rate of temperature increase, 5°C/min; final temperature, 260°C; final time, 3 min; injector and detector temperatures, 300°C; injection volume, 1 μl. Under these conditions the relative retention time (RRT) of the buprenorphine acetyl derivative with respect to the nalorphine acetyl derivative is 2.26. The absolute retention time (t_R) is 13.81 min (Fig. 1).

Heptafluorobutyryl derivative. A Hewlett-Packard 5890 gas chromatograph, attached to a Hewlett-Packard 3392 A integrator and equipped with a fused-silica OV-1 column (HP-1, 5 m×0.530 mm I.D.) was used. The chromatographic conditions were: carrier gas, hydrogen at a flow-rate of 30 ml/min; initial temperature, 200°C; initial time, 2 min; rate of temperature increase, 10°C/min; final temperature, 270°C; final time, 5 min; detector and injector temperatures, 300°C; injection volume, 1 μl. Under these conditions the RRT of the buprenorphine heptafluorobutyryl derivative with respect to the nalorphine heptafluorobutyryl derivative is 2.23. The t_R is 7.38 min (Fig. 2).

RESULTS AND DISCUSSION

The precision of the method was determined by adding buprenorphine to normal human plasma and normal human urine, which were divided into six equal samples and analysed separately. For the heptafluorobutyryl derivative, when the amount of buprenorphine added to the samples of plasma was 50 μg/l, the coefficient of variation (C.V.) was 4.9%, whereas at the 10 μg/l level it was 5.9%. When the amounts of buprenorphine added to the urine samples

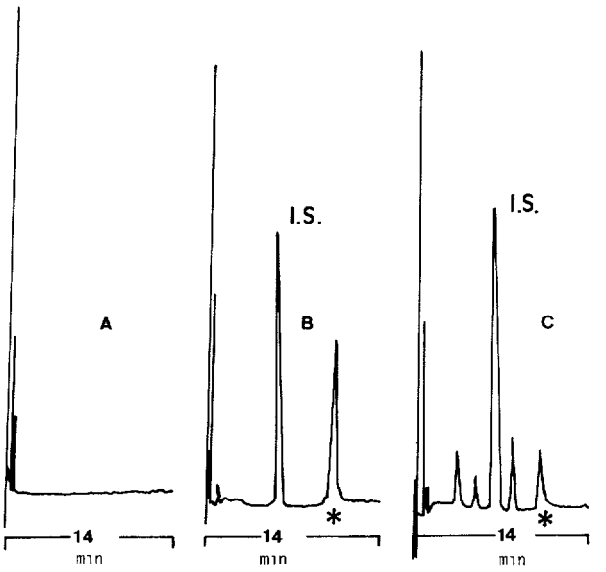

Fig. 1. Chromatograms from (A) an extract of a blank urine sample, (B) an extract from urine spiked with buprenorphine (100 μg/l) and (C) an extract of urine from a rat after intravenous administration of 0.6 mg of buprenorphine. The buprenorphine acetyl derivative peak is identified by an asterisk.

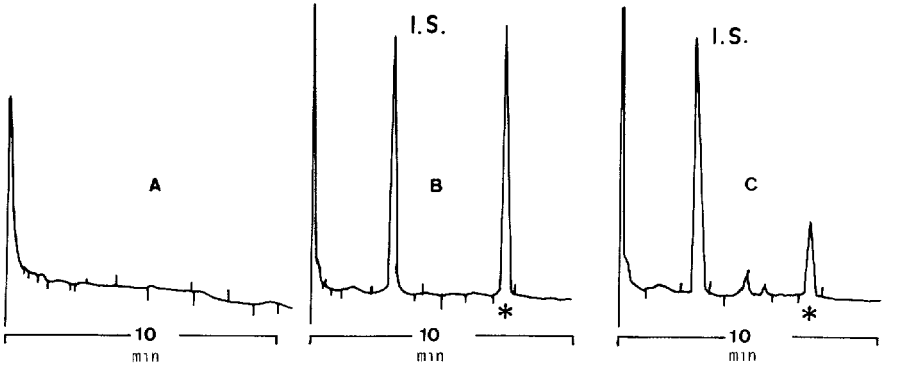

Fig. 2. Chromatograms from (A) an extract of a blank plasma sample, (B) an extract from plasma spiked with buprenorphine (10 μg/l) and (C) an extract of plasma from a rat after intravenous administration of 0.6 mg of buprenorphine. The buprenorphine heptafluorobutyryl derivative peak is identified by an asterisk.

were 50 and 10 μg/l, the C.V. were 7.2 and 6.4%, respectively. The limit of detection for the method was ca. 0.5 μg/l buprenorphine.

For the acetyl derivative, when the amount of buprenorphine added to normal plasma was 200 μg/l, the C.V. was 6.3%, whereas at the 100 μg/l level it was 8.4%. When the amounts of buprenorphine added to normal urine were

200 and 100 μg/l, the C.V. were 7.8 and 9.3%, respectively. The limit of detection was ca. 50 μg/l.

Other drugs of abuse did not interfere. In order to use the largest possible amount of sample, because of the low concentration of buprenorphine in biological fluids at therapeutic levels, we preferred to use Extrelut-20 columns. The dilutions were carried out according to the manufacturer's instructions for these columns. The extraction recoveries with the elution solvents were 98–105% in the case of diethyl ether for plasma and 95–100% with dichloromethane–2-propanol (85:15) for urine. These extraction recoveries were superior to those obtained with other elution solvents tested (heptane, diisopropyl ether, methyl acetate, ethyl acetate, dichlorometane, chloroform).

Given that amounts of buprenorphine present in blood and urine are so low at therapeutic doses, an extremely sensitive detector is required. The formation of the described derivatives noticeably increases the GC signal, detecting and confirming the presence of the substance. We could not obtain a standard for norbuprenorphine, the major metabolite of buprenorphine, which consequently would not be identified.

The comparative study of the detection limits of the method for the two derivatives clearly indicates that for determination of the drug at therapeutic or sub-therapeutic levels the preparation of the heptafluorobutyryl derivative is advisable, followed by GC–ECD analysis.

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