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ANALYSIS OF BUPRENORPHINE AND ITS N-DEALKYLATED METABOLITE IN PLASMA AND URINE BY SELECTED-ION MONITORING

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SUMMARY

A selected-ion monitoring method was developed for determination of buprenorphine and its N-dealkylated metabolite (norbuprenorphine) in human plasma and urine. N-Propylnorbuprenorphine was added as internal standard to 2—3 ml of sample and the alkaloids were extracted with toluene—2-butanol at pH 9.4. After back-extraction in dilute sulphuric acid, the compounds were heated at 110°C. This procedure led to quantitative loss of methanol followed by ring formation between the 6-methoxy group and the branched side-chain of all compounds. The derivatives were extracted into dichloromethane—2-butanol and treated with pentafluoropropionic anhydride. The resulting derivatives were suitable for selected-ion monitoring analysis. The coefficient of variation was found to be 4.5% at 5 ng/ml and 8.9% at 50 ng/ml in urine. The corresponding values for plasma were 6.2% and 5.3%, respectively.

The lower limit of detection in plasma was 150 pg/ml, permitting analysis of plasma levels of buprenorphine for 24 h and urine levels of buprenorphine and norbuprenorphine for more than seven days after a therapeutic dose of buprenorphine. This method is the first with sufficient specificity and sensitivity for characterization of the clinical pharmacokinetics of buprenorphine.

INTRODUCTION

Buprenorphine, N-cyclopropylmethyl-7α-[1-(*S*)-hydroxy-1,2,2-trimethyl-

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propyl]-6,14-*endo*-ethano-6,7,8,14-tetrahydronororipavine, is a long-acting opiate analgesic of the oripavine series [1]. It is highly potent with a typical dose range of 0.3—0.6 mg (0.005—0.01 mg/kg) for parenteral administration. Studies on its pharmacokinetics have been rendered difficult by the low blood concentrations attained and lack of sufficiently specific and sensitive methods. Lloyd-Jones et al. [2] used a selected-ion monitoring method and were able to measure levels above 20 ng/ml attained by the injection of 5 mg/kg in baboons. For use in humans a sensitive radioimmunoassay has been developed [3]. Using this technique Bullingham and co-workers [4—7] have studied the single-dose kinetics and bioavailability of intravenous, intramuscular and sublingual buprenorphine. The sensitivity of the method was high, down to 50 pg/ml, but with possibilities of cross-reaction either with the glucuronide conjugate (serum L30) or with the N-dealkylated metabolite (serum L31). It is therefore uncertain to what extent metabolites of buprenorphine can have contributed to the measured blood levels.

The present communication describes a new selected-ion monitoring procedure for the determination of both buprenorphine and its N-dealkylated metabolite. The method which is based on a crucial chemical degradation step has a sensitivity with a lower limit in plasma and urine of about 150 pg/ml.

EXPERIMENTAL

Materials

Buprenorphine hydrochloride, norbuprenorphine hydrochloride, N-propyl-norbuprenorphine hydrochloride (internal standard) and the acid rearrangement product, RX 2001 M, were gifts from Reckitt and Colman, Pharmaceutical Division, Hull, U.K., through the courtesy of Dr. G. Lloyd-Jones. Pentafluoropropionic anhydride (PFPA) was obtained from Reagenta, Uppsala, Sweden. All other chemicals and solvents were of analytical grade, available through ordinary commercial channels.

Carbonate buffer (pH 9.4) was prepared by titrating a saturated solution of sodium bicarbonate with a saturated solution of sodium carbonate to pH 9.4.

Thin-layer chromatography (TLC) was conducted on silica gel F_{254} plates (E. Merck, Darmstadt, F.R.G.). The solvent system used was chloroform—diethyl ether—ammonia (75:25:1).

Analysis in plasma

The internal standard (20 or 40 ng) and 1 ml of carbonate buffer (pH 9.4) were added to 2.0 ml of plasma in a 15-ml test tube. After addition of 6 ml of toluene containing 20% (v/v) of 2-butanol, the tube was extracted for 15 min using a mechanical shaker. The two phases were separated by centrifugation; the upper organic layer was transferred to a new tube containing 1 ml of 0.05 *M* sulphuric acid. After extraction and centrifugation the organic phase was discarded and the aqueous phase was heated in a 110°C oil-bath for 1 h. The aqueous phase was then made alkaline with 0.5 ml of carbonate buffer, and extracted with 4 ml of dichloromethane containing 20% (v/v) of 2-butanol. The sample was centrifuged, the aqueous phase discarded and the organic phase

poured into a clean tube and evaporated to dryness under a stream of nitrogen at 60°C.

The pentafluoropropionyl (PFP) derivatives were prepared by addition of 100 μl of PFPA. The tubes were then allowed to stand for 15 min at 70°C before the excess PFPA was evaporated by a stream of nitrogen at 60°C. The residue was dissolved in 50 μl of chloroform and the contents of the tube were thoroughly mixed. Then 1—5 μl of this mixture was injected for gas chromatography—mass spectrometry (GC—MS).

The concentrations of buprenorphine and norbuprenorphine were evaluated using a calibration graph established by the use of five standard samples carried through the same procedure.

Analysis in urine

For the determination of free buprenorphine and norbuprenorphine, 20 or 40 ng of the internal standard were added to 3.0 ml of urine, and the sample was alkalinized by the addition of 1 ml of carbonate buffer (pH 9.4) and extracted with 8 ml of toluene—2-butanol (8:2). The organic layer was transferred to a tube containing 1 ml of sulphuric acid (0.05 *M*) and the subsequent extraction steps were then carried out as described above for plasma.

When the total (free and conjugated) amount of buprenorphine and norbuprenorphine was determined, the internal standard was added to 3.0 ml of urine, followed by addition of concentrated hydrochloric acid to a final concentration of 2 *M*. The tube was then heated to 110°C for 1 h, alkalinized with 300 μl of ammonia and 1 ml of carbonate buffer (pH 9.4) and the drugs were extracted with 8 ml of the toluene—2-butanol mixture. The same extraction steps were then performed as described above with the exception that the heating was omitted after the back-extraction step.

Mass spectrometric conditions

The GC—MS analysis was performed on a Finnigan 4000 gas chromatograph—mass spectrometer equipped with a fused-silica OV-1701 capillary column (Orion, 25 m × 0.3 mm I.D.). Helium was used as carrier gas, and column temperature was 280°C.

The injection system used was of the solventless type. A droplet (1—5 μl) of the sample was transferred to the tip of a movable glass needle, and after evaporation of the solvent the needle with the sample was moved down to the column.

The mass spectrometer was operated in the electron-impact mode with the electron emission current at 0.3 A and the energy of the electrons at 40 eV. The electron multiplier voltage was set at 2.5 kV. All GC—MS data were collected, stored and processed using an Incos 2300 Data System.

RESULTS AND DISCUSSION

Recovery

The recovery of buprenorphine was determined using ^{3}H-labelled buprenorphine. The yield of this compound through the extraction procedure was 30%. Silanization of all glassware did not improve the recovery.

Chemical degradation

When we first considered the development of a gas chromatographic assay we decided that a chemical modification of the molecule would be desirable to reduce problems with adsorption and resultant low sensitivity. A mild acid hydrolysis of buprenorphine, norbuprenorphine and N-propylnorbuprenorphine gave a quantitative loss of methanol followed by ring formation between the side-chain and the methoxy group as shown for buprenorphine in Fig. 1. That the degradation gave a single product was shown by TLC (Fig. 2).

Gas chromatography of pentafluoroacylated derivatives of the degradation products gave for each compound a single peak. The structure of these compounds could be deduced from the mass spectra (Fig. 3A—C). For the buprenorphine derivative, the molecular ion at *m/z* 581 indicates that loss of 32 corresponding to methanol had occurred from the parent compound. Major fragments of *m/z* 540 (loss of cyclopropyl) and 497 (loss of part of the furano ring) supported the structure shown in Fig. 3A.

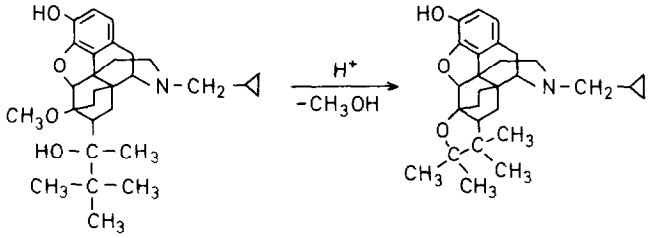

Fig. 1. Chemical degradation of the buprenorphine molecule following mild acid hydrolysis. The result is quantitative loss of methanol and ring formation between the 6-methoxy group and the side-chain. The same degradation also occurs for norbuprenorphine and N-propylnorbuprenorphine upon treatment with dilute acid.

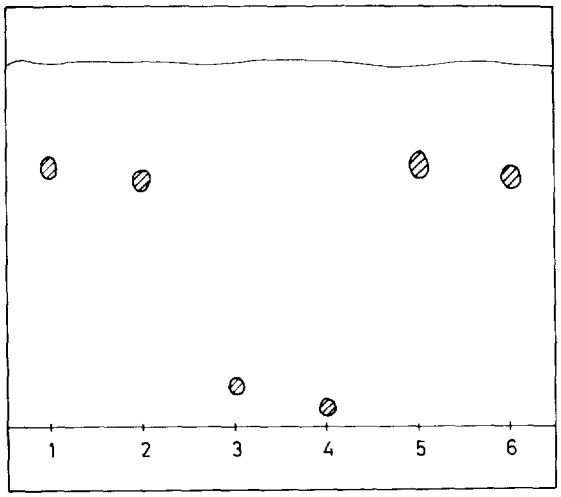

Fig. 2. Thin-layer chromatogram showing (1) buprenorphine, (2) degradation product of buprenorphine, (3) norbuprenorphine, (4) degradation product of norbuprenorphine, (5) internal standard, and (6) degradation product of internal standard. Each compound gives a single spot, indicating that the degradation step is quantitative.

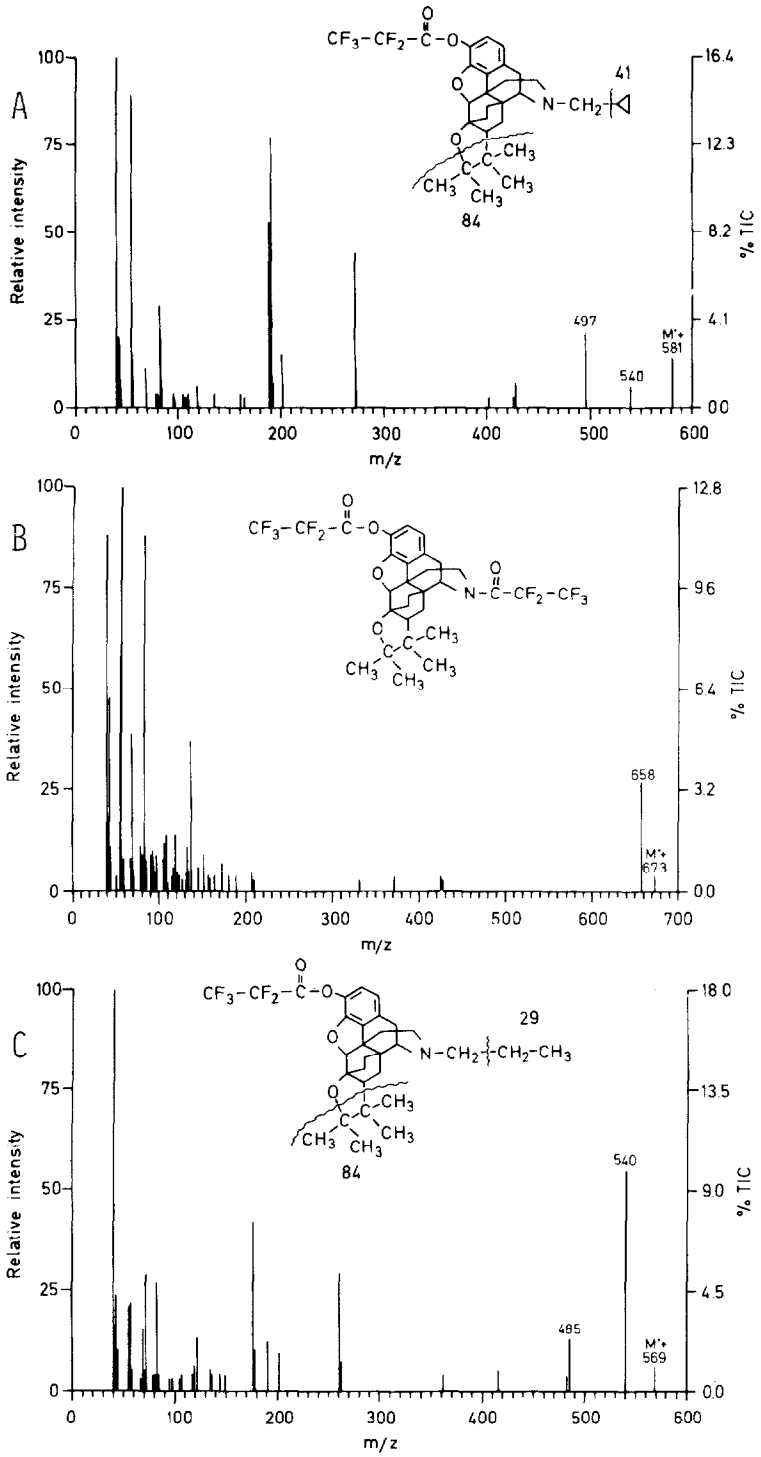

Fig. 3. Mass spectra of the pentafluoroacylated derivatives of the degradation products of (A) buprenorphine, (B) norbuprenorphine and (C) N-propylnorbuprenorphine (internal standard).

Comparison between the buprenorphine acid rearrangement product, RX 2001 M (supplied by Reckitt and Colman), and the degradation product obtained in our laboratory upon treatment of buprenorphine with dilute acid, showed coincident retention times for the two peaks with gas chromatography, as well as identical mass spectra and identical R_F values upon TLC analysis. Thus the structure of the degradation products of buprenorphine was identical to that of RX 2001 M.

For norbuprenorphine a similar structure could be proposed (Fig. 3B). Usefully a considerable portion of the ion yield was found at *m/z* 658, presumably appearing through loss of one methyl group from the molecular ion.

N-Propylnorbuprenorphine was converted to a similar derivative by the acid treatment. The mass spectrum of the PFP derivative is shown in Fig. 3C. The base peak at *m/z* 540 is presumably formed by loss of ethyl (29) by cleavage β to the nitrogen.

These mass spectra provide strong evidence for quantitative removal of methanol in all three compounds with the formation of a furano ring between the oxygen function at carbon-6 and the side-chain. Furthermore, the mass spectra of the PFP derivatives revealed strong peaks in the high mass region suitable for focusing with selected-ion monitoring (mass fragmentography). Accordingly, the fragments chosen for the further analytical work were *m/z* 581 for buprenorphine, *m/z* 658 for norbuprenorphine and *m/z* 569 for N-propyl-norbuprenorphine.

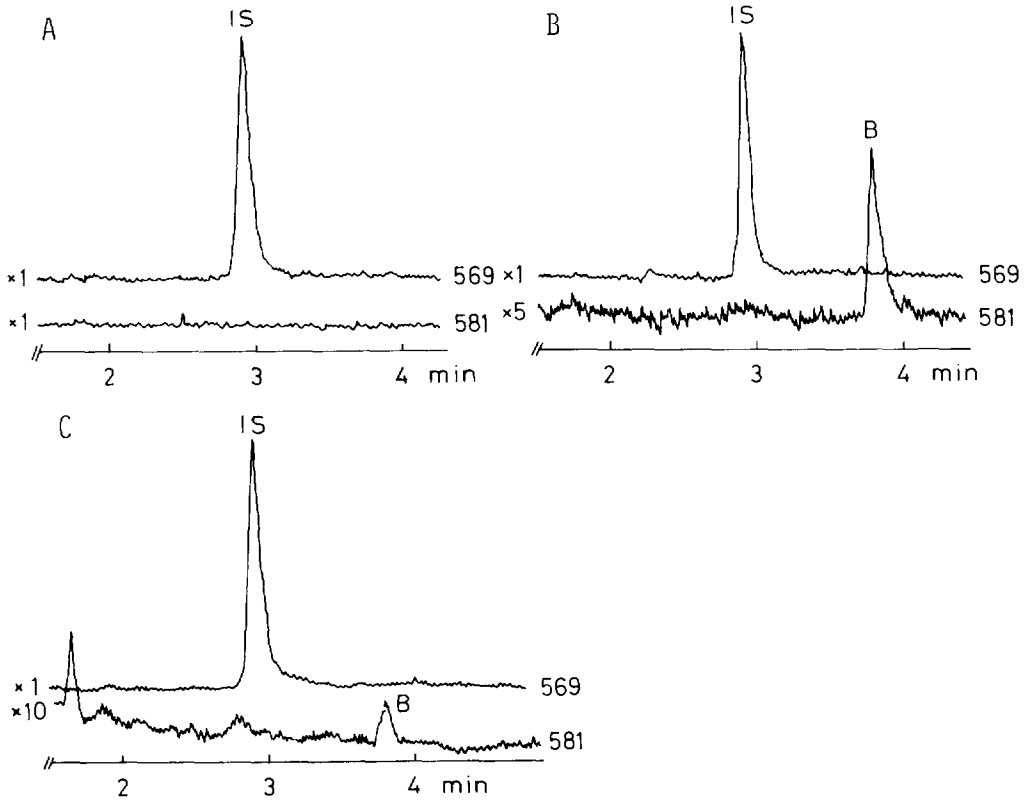

Fig. 4.

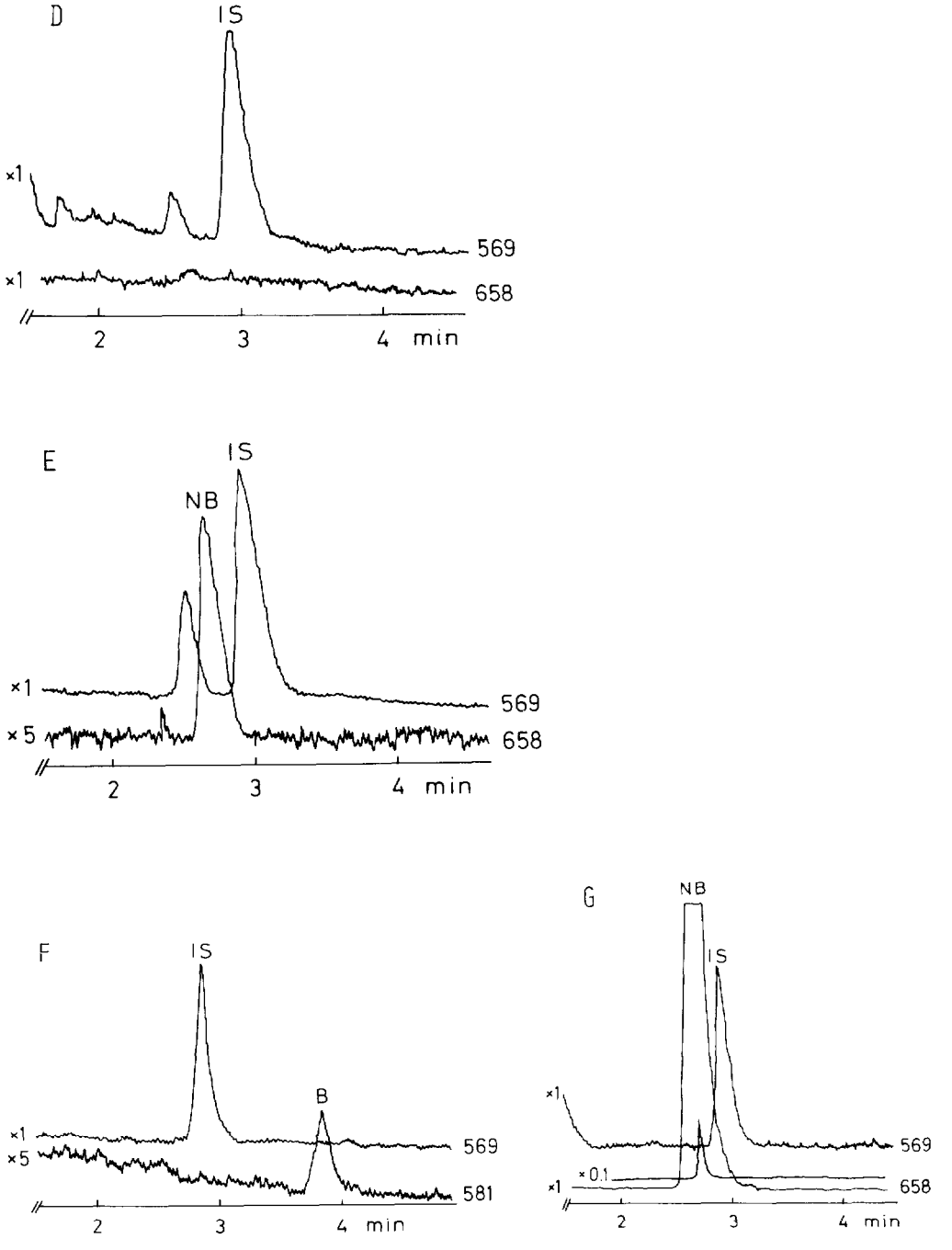

Fig. 4. Selected-ion monitoring profiles obtained from the assay of buprenorphine (B) and norbuprenorphine (NB) after intravenous administration of 0.6 mg of buprenorphine to a healthy volunteer. IS = internal standard. (A) Plasma blank, buprenorphine; (B) buprenorphine in plasma, 1 h; (C) buprenorphine in plasma, 10 h; (D) plasma blank, norbuprenorphine; (E) norbuprenorphine in plasma, 1 h; (F) buprenorphine in urine, four days; (G) norbuprenorphine in urine, four days.

Analysis in plasma

The mass fragmentograms of blank plasma revealed no major background at *m/z* 581 (Fig. 4A). One hour after intravenous injection of 0.6 mg of buprenorphine to a healthy volunteer, a strong peak was observed with a retention time corresponding to buprenorphine (Fig. 4B). Significant peaks of buprenorphine were observed even after 10 h (Fig. 4C) and 24 h. A plasma elimination curve for buprenorphine is shown in Fig. 5.

The buprenorphine derivative gave a very sensitive signal permitting analysis with a lower limit of 150 pg/ml. Norbuprenorphine was found to be present in plasma for a few hours after the injection of buprenorphine (Fig. 4E).

To determine the precision of the assay, buprenorphine was added to normal plasma, which was then divided into six equal samples and analysed separately. When the added buprenorphine was 50 ng/ml, the coefficient of variation was 5.3%, while at the 5 ng/ml level it was 6.2%.

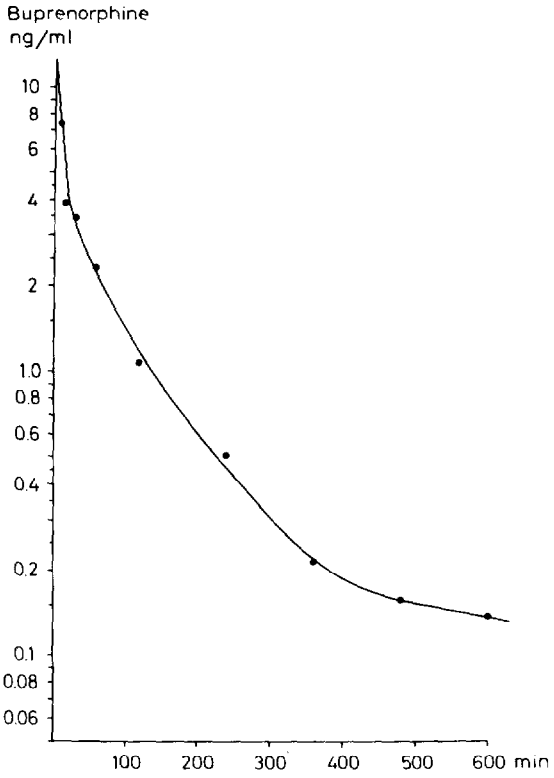

Fig. 5. Plasma elimination curve for buprenorphine after administration of 0.6 mg intravenously to a healthy volunteer.

Analysis in urine

In previous studies in rats, the urinary excretion products of buprenorphine were dominated by conjugated material, mainly the glucuronide conjugate [8]. We therefore decided to analyse both free and conjugated drug in urine. The total amount of buprenorphine in urine was determined after hydrolysis of the conjugates with hydrochloric acid, while the amount of free drug was

determined without prior treatment with the acid. The amount of conjugated buprenorphine in urine was calculated as the difference between total and free drug.

After injection of 0.6 mg of buprenorphine, both norbuprenorphine and buprenorphine were excreted in urine for at least seven days (data not shown). The selected-ion monitoring profiles of buprenorphine and norbuprenorphine four days after injection are shown in Fig. 4F and G, respectively.

The cumulative amounts of both buprenorphine and norbuprenorphine excreted over four days in one subject are shown in Fig. 6.

The precision of the assay was determined by adding buprenorphine to normal human urine, which was divided into six equal samples and analysed separately. The concentration of buprenorphine in each sample was 50 or 5 ng/ml. The coefficients of variation at these levels were 8.9% and 4.5%, respectively.

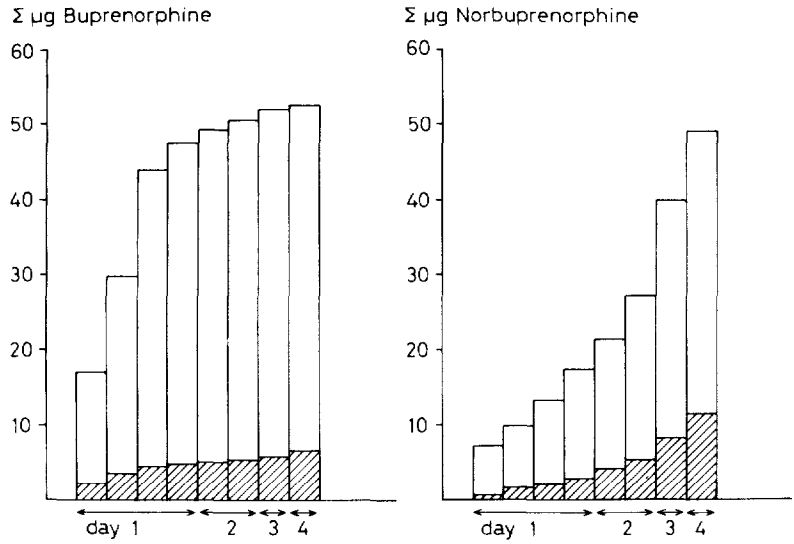

Fig. 6. Cumulative amount of buprenorphine and norbuprenorphine excreted in urine after intravenous administration of 0.6 mg of buprenorphine. □, Conjugated drug or metabolite; ▨, free drug or metabolite.

Comments on usefulness

Buprenorphine is one of the more interesting newer opiate drugs. It combines a high degree of analgesic efficacy with a low degree of opiate-like side-effects such as respiratory depression and physical dependence [1]. Its development in clinical medicine has, however, been hampered by lack of knowledge of its pharmacokinetics in man. Thus neither the bioavailability nor the single- or multiple-dose pharmacokinetics have been adequately determined. The present analytical method can hopefully help rectify the situation.

The analytical method is based on a crucial chemical degradation step leading to a loss of the secondary alcohol group in the form of methanol and the formation of a furane ring. The product has as the PFP ester improved

properties for GC—MS analysis in comparison to the parent compound due to decreased adsorption and the presence of a suitable ion for focusing in the high mass region (*m/z* 581). The same degradation patterns were found for norbuprenorphine and N-propylnorbuprenorphine used as internal standard. Thus the major metabolite of buprenorphine together with buprenorphine itself could be determined simultaneously by the basic procedure. The same chemical degradation was recently reported by Cone et al. [9] but was not used for analytical purposes. The application of the present method in the characterization of the clinical pharmacokinetics of buprenorphine is currently under way.

ACKNOWLEDGEMENT

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REFERENCES

1 R.C. Heel, R.N. Brogden, T.M. Speight and G.S. Avery, Drugs, 17 (1979) 81—110.
2 J.G. Lloyd-Jones, P. Robinsson, R. Henson, S.R. Biggs and T. Taylor, Eur. J. Drug Metab. Pharmacokin., 5 (1980) 233—239.
3 A.J. Bartlett, J.G. Lloyd-Jones, M.J. Rance, I.R. Flockhart, G.J. Dockray, M.R.O. Bennett and R.A. Moore, Eur. J. Clin. Pharmacol., 18 (1980) 339—345.
4 R.E.S. Bullingham, H.J. McQuay, A. Moore and M.R.D. Bennett, Clin. Pharmacol. Ther., 28 (1980) 667—672.
5 R.E.S. Bullingham, H.J. McQuay, D. Dwyer, M.C. Allen and R.A. Moore, Brit. J. Clin. Pharmacol., 12 (1981) 117—122.
6 P.J.Q. Watson, H.J. McQuay, R.E.S. Bullingham, M.C. Allen and R.A. Moore, Brit. J. Anaesth., 54 (1982) 37—43.
7 R.E.S. Bullingham, H.J. McQuay, E.J.B. Porter, M.C. Allen and R.A. Moore, Brit. J. Clin. Pharmacol., 13 (1982) 665—673.
8 D. Brewster, M.J. Humphrey and M.A. McLeavy, Xenobiotica, 11 (1981) 189—196.
9 E.J. Cone, C.W. Gorodetzky, W.D. Darwin and W.F. Buchwald, J. Pharm. Sci., 73 (1984) 243—246.