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Note

Analysis of buprenorphine by high-performance liquid chromatography

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Buprenorphine (temgesic) is a derivative of the opiate thebaine. It is a long-acting analgesic with both narcotic agonist and antagonist actions[1]. Buprenorphine is often prescribed for the treatment of chronic post-operative pain and for terminal cancer patients[2]. It has also been used recently for the treatment of heroin addicts[3]. Buprenorphine is available as an injection for intramuscular or intravenous administration, and as sublingual tablets. The usual recommended doses are 200–600 μg by slow intravenous or intramuscular injection repeated every 6–8 h or 400 μg sublingually also every 6–8 h.

However, there have been recent reports of buprenorphine being abused by opiate addicts and doses of 3 mg per day sublingually or by injection have been commonly encountered[4]. This drug has a similar effect to morphine and pethidine but is more readily obtainable since it is not subject to the stringent restrictions associated with controlled drugs. The problem of buprenorphine abuse is apparently on the increasse and this situation has necessitated the search for a rapid method for the analysis of buprenorphine both in the form of pharmaceutical preparations and in blood samples of addicts. This paper describes a high-performance liquid chromatographic (HPLC) method which goes some way towards fulfilling these requirements.

EXPERIMENTAL

HPLC conditions

A Perkin-Elmer Series 4 liquid chromatograph was used to deliver solvent at 1 ml/min. The eluent was monitored at 290 nm with a Perkin-Elmer LC-75 variable-wavelength ultraviolet detector. The column was 20 cm × 4.5 mm I.D. RP-18, 5 μm (Supelco) fitted with a Rheodyne injection system incorporating a 20-μl loop. Separation was achieved with a mobile phase of 0.05 *M* sodium pentanesulphonic acid–acetonitrile–methanol (30:15:55) to pH 2.0 with orthophosphoric acid.

Sample preparation

Human serum was prepared by centrifugation and kept frozen at −20°C until required for analysis. Serum (2 ml) was made alkaline with 1 *M* sodium hydroxide (0.1 ml) and extracted by shaking with 3-ml aliquots of diethyl ether. The serum

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sample was extracted three times and the extracts combined. The organic phase was evaporated to dryness under a stream of nitrogen and the residue redissolved in 100 μl of methanol. Samples of 20 μl were injected onto the column.

A straight line calibration graph was obtained for buprenorphine based on peak area measurements for concentrations of 2.5, 5.0, 10, 20, 50 and 100 ng/ml by addition of the drug to control serum and extraction by the procedure described above. Each point was taken as the average of two determinations.

Materials

Buprenorphine hydrochloride was supplied by Reckitt and Colman. The free base was prepared by dissolving the hydrochloride in water making alkaline with 1 *M* sodium hydroxide and extracting into diethyl ether. The ether extract evaporated to dryness under a stream of nitrogen and the residue redissolved in methanol and used for analysis. Tablets and injections of buprenorphine (Reckitt and Colman) were similarly subjected to a base/ether extraction prior to analysis by HPLC. Opiate standards were of pharmaceutical grade (MacFarlan Smith). All solvents were HPLC grade (Rathburn). Blood samples were obtained from hospitalised patients receiving high doses of buprenorphine by the sublingual route.

RESULTS AND DISCUSSION

Fig. 1 shows the chromatogram of buprenorphine together with the commonly abused opiates morphine, codeine and heroin. Thebaine and papaverine, whilst not showing good peak shape in this system, do not interfere with the analysis of buprenorphine. Basic ether extracts of serum samples containing buprenorphine yielded clean extracts with between 98 and 100% recovery. A straight line calibration graph was determined for buprenorphine and found as $y = 0.91x - 0.464$ with a correlation coefficient of 1.0. Limit of detection (signal-to-noise ratio >2) was approximately 2 ng on column.

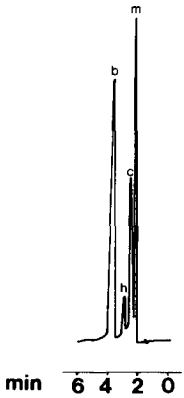

Fig. 1. Separation of commonly used opiates. Peaks: m = morphine; c = codeine; h = heroin; b = buprenorphine.

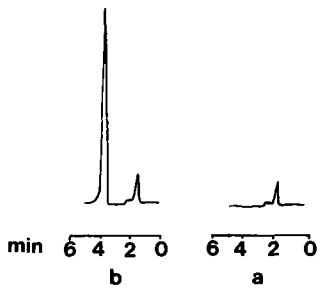

Fig. 2. (a) Chromatogram of a blank serum extract; (b) serum extract from an individual receiving buprenorphine.

The chromatogram of a blank serum extract (from a control subject who had not taken buprenorphine) is shown in Fig. 2a and the chromatogram of an extract of a serum sample from an individual taking high doses of buprenorphine is shown in Fig. 2b.

The method outlined is suitable for the analysis of buprenorphine in pharmaceutical preparations and for the detection of the drug in body fluids. Buprenorphine is rapidly metablished by the liver. Sublingual administration of 0.4–0.6 mg is reported to produce peak levels of 1–4 ng/ml after about 2 h as determined by radioimmunoassay[1,5]. These values are close the limit of detection of the described system (1 ng/ml) and sensitivity would need to be improved to enable the analysis of blood samples taken from patients receiving therapeutic doses of buprenorphine. However, sensitivity was found to be adequate for the detection of buprenorphine in patients receiving high doses of the drug. This method should therefore be suitable for the analysis of blood samples taken from abusers of buprenorphine.

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