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Note

High-performance liquid chromatographic determination of pentazocine in plasma

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Pentazocine is a central-acting analgesic and a weak narcotic antagonist, which is structurally related to morphine. The intravenous administration is widely used in the management of patients with acute or chronic pain, such as cancer, colic fit or neuralgia.

For quantification of pentazocine in blood, plasma or serum, various techniques have been published including fluorometric [1–4], gas chromatographic (GC) [5–7], mass fragmentographic [8], radioimmunoassay [9] and radioreceptor assay [10] methods. Two high-performance liquid chromatographic (HPLC) methods have been reported: one requires chemical derivatization of pentazocine [11] and the other uses electrochemical detection [12]. Thus all available methods are time-consuming or expensive or not sensitive enough for application in human pharmacokinetic studies. We, therefore, developed a simple and rapid, but highly sensitive HPLC method using fluorometric detection for the quantification of pentazocine in plasma.

EXPERIMENTAL

Chemicals

Pentazocine [1,2,3,4,5,6-hexahydro-6,11-dimethyl-3-(3-methyl-2-butenyl)-2,6-methano-3-benzazocine-8-ol] as the pure compound (as HCl salt), as well as the intravenous formulation containing 30 mg of pentazocine per ampoule (50 mg pentazocine lactate; Fortral®) were gifts from Winthrop (Norderstedt, F.R.G.). The internal standard (I.S.), levallorphane tartrate (Lorphan®), was obtained from Hoffmann-La Roche (Grenzach, F.R.G.). All other chemicals and organic

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solvents were HPLC or reagent grade. The mobile phase was filtered and degassed under vacuum prior to use.

The stock standard solution of pentazocine was prepared by dissolving 113 mg of pentazocine · HCl (equivalent to 100 mg of pentazocine) in 100 ml of phosphate buffer (pH 7.4). Working standards were prepared in drug-free plasma from the stock solution standard to yield concentrations of 5–1000 ng/ml of plasma and were kept at 5°C. No degradation was detectable within 14 weeks.

Chromatographic system

The HPLC system consisted of a Model SP 8810 pump (Spectra Physics, Darmstadt, F.R.G.), a Model RF-535 fluorescence HPLC monitor (Shimadzu, Egling, F.R.G.) fitted with a Model 231 diluter–autosampler (Gilson/Abimed, Langenfeld, F.R.G.) and a D-2500 ChromatoIntegrator (Merck-Hitachi, Darmstadt, F.R.G.). Separation was achieved with a prepacked column (25 cm × 4.5 mm I.D., Nucleosil 5 μm RP 18, Macherey and Nagel, Düren, F.R.G.). The mobile phase was acetonitrile–0.05 *M* H_3PO_4 (33:67, v/v), at a flow-rate of 1.0 ml/min. Peaks were detected by means of a fluorescence monitor. The uncorrected wavelengths of the maxima were 278 nm for excitation and 324 nm for emission. The system was used at room temperature (20°C).

Analytical procedure

For the determination of pentazocine in plasma, a 1.00-ml aliquot was alkalinized by adding 0.20 ml of 1 *M* NaOH followed by extraction into 6.00 ml of ice-cooled diethyl ether containing *ca.* 120 ng of I.S. by agitating for 15 min at 4°C. After centrifugation for 5 min (1500 *g*, 4°C), 5.00 ml of the organic layer were removed and evaporated to dryness under a gentle stream of dry nitrogen. The residue was redissolved in 250–500 μl of the mobile phase prior to injection of 50 μl onto the HPLC column. The standard curve was prepared by injecting plasma extracts spiked with various amounts of pentazocine, simulating concentrations of 5, 10, 50, 100, 500 and 1000 ng/ml of plasma. For the quantification of unknown plasma samples, peak-area ratios for pentazocine relative to I.S. were used.

Precision of the assay

Five samples each of six plasma standards in the range 5–1000 ng/ml were analysed on four successive days. Peak area was plotted versus concentration of the standards and concentrations were back-calculated. The intra-day and inter-day variability of two different analysts were determined.

Recovery values

Recovery values were determined by comparing extracted spiked samples in the range 5–1000 ng/ml with unextracted standard solutions.

Application

The utility of the method was demonstrated after intravenous administration of 30 mg of pentazocine (Fortral) to healthy volunteers (five male, five female). After injection of pentazocine into the left cubital vein, blood was taken from an indwelling catheter of the right cubital vein. Blood samples were collected up to 6 h. The plasma was frozen immediately and stored at −30°C until analysis.

RESULTS

This method quantifies pentazocine in human plasma via a simple extraction procedure and an isocratic fluorimetric HPLC system. Chromatographic separation was completed within 8 min and no interfering peaks were observed when plasma extracts were chromatographed. Typical chromatograms of blank human plasma and a spiked plasma sample are shown in Fig. 1. The retention times were 4.9 min for levallorphane and 6.2 min for pentazocine. The limit of detection was found to be *ca.* 1 ng/ml of plasma. The limit of quantification was 4 ng/ml of plasma. The recovery values of pentazocine in human plasma are listed in Table I. The recovery of levallorphane used as I.S. was 103.2 ± 2.0% ($n=6$). The peak-area ratios of pentazocine were linearly related ($r>0.999$) to the amount of pentazocine added to blank human plasma in the range 5–1000 ng/ml. The inter-day and intra-day precisions in plasma over 4 days for two different analysts are given in Table II.

Characteristic plasma concentration *versus* time profiles of pentazocine following a single intravenous administration of 30 mg of pentazocine to two volunteers are shown in Fig. 2.

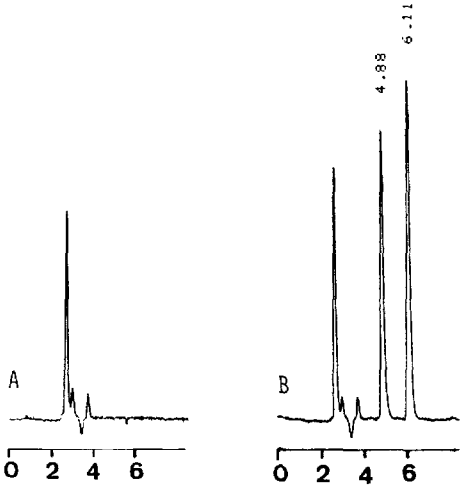

Fig. 1. Chromatograms of plasma extracts: (A) blank plasma; (B) plasma spiked with pentazocine · HCl (113 ng/ml; equivalent to 100 ng/ml pentazocine) and levallorphane tartrate (120 ng/ml; internal standard). The retention times were 4.9 min for levallorphane and 6.1 min for pentazocine.

TABLE I

ANALYTICAL RECOVERY OF PENTAZOCINE AT SIX DIFFERENT PLASMA CONCENTRATIONS

Amount added (ng/ml)	Recovery (mean ± S.D., $n=6$)(%)
5	99.1 ± 6.9
10	103.2 ± 9.5
50	102.7 ± 2.7
100	100.4 ± 1.1
500	99.9 ± 1.1
1000	100.6 ± 1.3

DISCUSSION

The sensitivity of this HPLC method is compared with those of previously reported methods in Table III. A major advantage of our method is that it requires minimal sample preparation compared with the extensive clean-up procedures and derivatization needed for GC analysis [5–7]. Other previous methods require large volumes of blood in order to determine small amounts of pentazo-

TABLE II

INTER- AND INTRA-DAY PRECISION OF PENTAZOCINE IN PLASMA OVER FOUR DAYS

Added (ng/ml)	Found (mean ± S.D.) (ng/ml)				
	Day 1 ($n=5$)	Day 2 ($n=5$)	Day 3 ($n=5$)	Day 4 ($n=5$)	Mean
Analyst 1					
5	4.9 ± 0.3	5.0 ± 0.2	4.6 ± 0.5	4.6 ± 0.5	4.7 ± 0.2
10	8.6 ± 1.0	9.3 ± 0.3	9.8 ± 0.6	9.5 ± 0.5	9.3 ± 0.5
50	46.5 ± 2.5	48.5 ± 1.0	47.2 ± 0.3	46.4 ± 3.2	47.2 ± 1.0
100	96.4 ± 0.7	96.8 ± 1.0	97.5 ± 1.0	97.5 ± 1.6	97.1 ± 0.5
500	496.0 ± 6.7	495.0 ± 3.2	498.0 ± 2.8	490.8 ± 4.2	495.0 ± 3.0
1000	999.0 ± 3.0	1000.0 ± 4.1	999.0 ± 5.4	1001.0 ± 10.1	999.8 ± 1.0
Analyst 2					
5	4.1 ± 0.7	4.9 ± 0.3	5.5 ± 0.5	4.8 ± 1.0	4.8 ± 0.6
10	9.8 ± 0.9	10.1 ± 0.4	10.1 ± 1.2	10.8 ± 0.7	10.2 ± 0.4
50	48.4 ± 0.9	48.9 ± 1.3	49.3 ± 0.6	50.6 ± 0.9	49.3 ± 0.9
100	97.3 ± 1.9	98.3 ± 1.1	98.8 ± 0.8	96.0 ± 1.3	97.6 ± 1.2
500	498.8 ± 12.2	492.3 ± 5.7	498.7 ± 2.3	496.8 ± 6.9	496.7 ± 3.0
1000	991.6 ± 19.6	1001.5 ± 12.6	1000.1 ± 4.6	1000.4 ± 1.7	998.4 ± 4.6

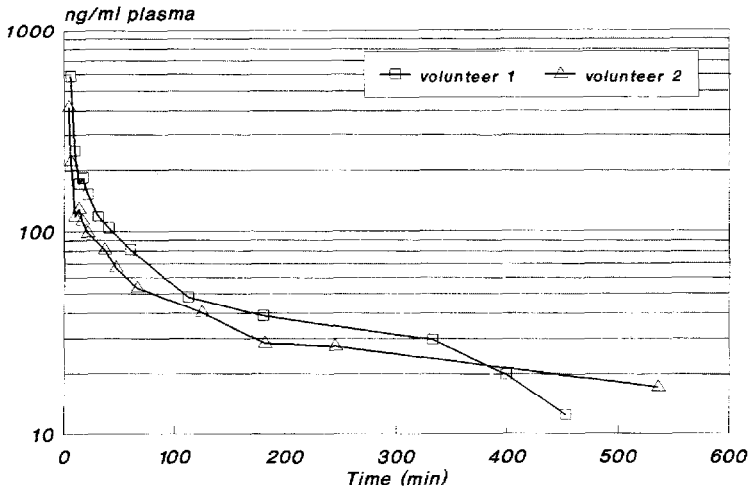

Fig. 2. Plasma concentration *versus* time profile of pentazocine following a single intravenous injection of 30 mg of pentazocine to two human volunteers.

cine [1–4,7]. The detection limit of our assay is lower than those of other fluorometric and some GC methods. The method described by Berkowitz *et al.* [3], for example, measures fluorescence in a quartz cuvette without chromatographic separation. Therefore it requires extensive cleaning of the glassware and great care during sample preparation to avoid interferences. Moreover, it was not possible to resolve pentazocine and one of its metabolites. The method reported by Borg and Mikaelson [4] is somewhat more selective owing to a chromatographic separation step using a glass column prior to fluorescence measurement in a quartz cuvette. However, in either case the sensitivity required to measure plasma

TABLE III

COMPARISON OF ANALYTICAL METHODS FOR DETERMINATION OF PENTAZOCINE IN PLASMA, BLOOD OR SERUM

Method	Sensitivity [ref.]
Fluorometric	30 ng/ml [1], 150 ng/ml [2], 30 ng/ml [3], 25 ng/ml [4]
Gas chromatographic	25 ng/ml [5], 5 ng/ml [6], 100 ng/ml [7]
Mass fragmentography	0.4–0.5 ng/ml [8]
Radioimmunoassay	1 ng/ml [9]
Radio-receptor assay	Not stated (< 1 ng/ml) [10]
HPLC with derivatization	10 ng/ml [11]
HPLC with electrochemical detection	1 ng/ml [12]
HPLC, present method	1 ng/ml

levels several hours after a therapeutic dose of pentazocine requires extraction of 3 ml and up to 5 ml of plasma, respectively.

Our method is somewhat less sensitive than mass fragmentography [8], radioimmunoassay [9] or radioreceptor assay [10], but these are all more tedious and more expensive procedures. The method proposed by Anderson *et al.* [11], HPLC after derivatization with 2-*p*-chlorosulphophenyl-3-phenylindone (DIS-CL) could not be reproduced in our laboratory. Moreover, we were unable to avoid interfering peaks, which probably represented reaction products of plasma constituents and DIS-CL.

Since plasma steady-state concentrations of pentazocine following injection of the clinically used 30 mg dose are in the range 10–100 ng/ml plasma, our method is applicable in the clinical situation. About 120–150 samples can be analysed by one person in the course of a working day using an automatic sampler running overnight.

In conclusion, the described HPLC method provides a simple approach for the determination of pentazocine in plasma after clinical doses for routine and investigative purposes.

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