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Short Communication

Sensitive determination of piritramide in human plasma by gas chromatography

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ABSTRACT

A selective and sensitive method for the determination of piritramide in human plasma is described. A 1-ml aliquot of plasma was extracted with 10 ml of hexane–isoamyl alcohol (99.5:0.5, v/v) (extraction efficiency 86%) after addition of 50 μl of 2 *M* ammonia and 20 μl of aqueous strychnine solution (100 ng per 10 μl) as internal standard. Gas chromatography was performed with a J&W DB-1, 30 m × 0.53 mm I.D. separation column, film thickness 1.5 μm, using an nitrogen–phosphorus-sensitive detector. The assay was linear in the concentration range 3.75–2250 ng/ml ($r = 0.999$), with a lower limit of detection of 1–2 ng/ml. The precision was determined using spiked plasma samples (10 and 50 ng/ml), with coefficients of variation of 3.5 and 3.1% (intra-day: $n = 5$) and 4.6 and 4.1% (inter-day: $n = 4$). In the range 3.75–150 ng/ml, the accuracy of the assay was 3.36%. The method was used for the determination of piritramide plasma concentrations in patients receiving intra- or post-operative analgesia.

INTRODUCTION

Piritramide [piritramid, pirinitramide, 1'-(3-cyano-3,3-diphenylpropyl)-(1,4'-bipiperidine)-4'-carboxamide] is a synthetic opioid [1] which is used for premedication as well as for intra- and post-operative analgesia [2–4]. The determination of plasma piritramide concentrations for the evaluation of pharmacokinetic parameters in patients receiving post-operative analgesia requires a sensitive assay, especially in the later phase of elimination. Thin-layer chromatographic (TLC)

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[5], high-performance liquid chromatographic (HPLC) [6–8] and gas chromatographic–mass spectrometric (GC–MS) [9] procedures suitable for separation or identification of piritramide in standard solutions have been reported, but no data were given about plasma extraction procedures and quantification. Therefore, we developed a gas chromatographic assay for the determination of piritramide plasma concentrations which is linear down to about 4 ng/ml.

EXPERIMENTAL

Reagents and chemicals

Aqueous piritramide injection solution (7.5 mg/ml) was obtained from Janssen (Neuss, Germany). Standard solutions (750 ng/ml) were prepared by addition of 10 μl of the injection solution to 100 ml of doubly distilled water or methanol. Strychnine was purchased from Sigma (Deisenhofen, Germany). All other chemicals were obtained from Merck (Darmstadt, Germany) and were of the highest purity available.

Apparatus and GC conditions

The plasma concentrations were determined by gas chromatography (Hewlett Packard GC 5890 Series II) using a nitrogen–phosphorus (NP)-sensitive detector equipped with a J&W DB-1, 30 m × 0.53 mm I.D. separation column, film thickness 1.5 μm. The conditions for the gas chromatograph were: injector temperature 300°C; detector temperature, 300°C; oven temperature gradient, 290°C for 9 min, 50°C/min, 310°C for 10 min; helium was used as carrier gas (19 ml/min), with 3.2 ml/min hydrogen gas flow to the detector and 100 ml/min air flow to the detector. Injector parameters: on-column mode, septum purge 15 ml/min. A time-programmable Hewlett Packard HP 3394A integrator was used for printing chromatograms and for data calculation. For best graphic results, the integrator attenuation was programmed: ATT 2^2 or ATT 2^3 for the strychnine peak, ATT 2^0 for the piritramide peak and ATT 2^8 for the background signal.

Plasma sample preparation

A 1-ml sample of plasma, spiked with 200 ng of strychnine in 20 μl of doubly distilled water as internal standard, was alkalinized with 50 μl of 2 *M* ammonia. Afterwards, piritramide and strychnine were extracted with 10 ml of hexane–isoamyl alcohol (99.5:0.5, v/v) by vortex-mixing (30 s). After centrifugation (3000 *g*, 5 min), the organic layer was evaporated under vacuum and the residue was redissolved in 100 μl of toluene–ethanol (1:1, v/v). A 1-μl aliquot of this solution was injected into the separation column.

Calibration curves, precision and recovery

For calibration, plasma standards spiked with fixed amounts of the internal standard and with piritramide concentrations ranging between 3.75 and 2250

ng/ml were prepared and assayed as described above. Calibration curves were plotted by correlating the peak-height ratio of piritramide to strychnine against the corresponding piritramide concentrations. The accuracy of the method was evaluated by assaying plasma samples spiked with known piritramide concentrations. The intra- and inter-day precision data of the assay were estimated by measuring plasma standards with 10 and 50 ng/ml piritramide on day 1 ($n = 5$) and on the three following days. The extraction efficiencies were determined by comparison of the detector signals obtained from methanolic piritramide solutions without extraction and from spiked piritramide plasma samples extracted as described above at a concentration of 75 ng/ml (the internal standard, dissolved in methanol, was added to the organic phase after the extraction procedure).

Application of the method

The assay was used to determine piritramide plasma concentrations in patients receiving intra- and post-operative analgesia. Piritramide had been applied via a cubital vein catheter as a bolus, whereas heparinized blood samples were withdrawn via a superior caval vein catheter (placed for medical indications) within a period of 37 h. After centrifugation (3000 *g*, 5 min) the plasma samples were assayed as described.

RESULTS AND DISCUSSION

Piritramide (for structure see Fig. 1) is a lipophilic synthetic opioid which is easily extracted from aqueous solutions by organic solvents under alkaline conditions. The best extraction results were obtained using hexane–isoamyl alcohol (99.5:0.5, v/v) (extraction efficiency 86%), whereas *n*-heptane–isoamyl alcohol (99.5:0.5, v/v) (extraction efficiency 67%), diethyl ether (23%) and hexane (0%) were less useful or not useful. Toluene extracted piritramide well (96%), but shaking or vortex-mixing resulted in emulsions. With ethyl acetate, plasma peaks interfered with the strychnine peak in the chromatogram. Since strychnine showed comparable extraction properties and an appropriate retention time, and since this substance is usually not present in human plasma, it was used as an internal standard.

At a signal-to-noise ratio of 3:1, the lower limit of detection for spiked piritra-

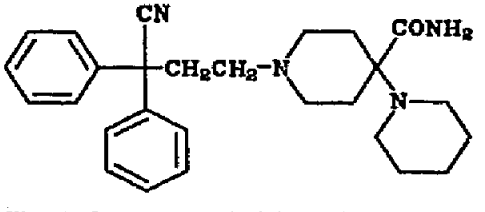

Fig. 1. Structure of piritramide.

TABLE I

ACCURACY OF THE ASSAY

Concentration of piritramide (ng/ml)		Accuracy[a] (%)
Spiked	Found	
3.75	3.84	2.35
7.50	7.68	2.39
10.00	11.33	13.35
15.00	14.56	2.92
25.00	24.18	3.29
37.50	38.35	2.26
50.00	49.88	0.24
150.00	149.93	0.04
Mean		3.36

[a] Calculated according to ref. 10.

mide plasma samples (1 μl injected) was about 1–2 ng/ml using the NP detector. The assay was linear in the concentration range studied: 3.75–2250 ng/ml (r = 0.999). In the low concentration range, 3.75–150 ng/ml, the least squares regression line had a slope of 0.005947 and a y-intercept of -0.00203 (where y is the piritramide/strychnine peak-height ratio and x is the piritramide concentration in ng/ml) with a coefficient of correlation r = 0.9999. The accuracy of the method is shown in Table I; the theoretical concentrations (spiked concentration) agreed well with the assayed concentrations (found concentration) with a mean accuracy of 3.36%. For intra- and inter-day precision studies, the mean concentration, standard deviation and coefficient of variation are shown in Table II.

The assay was used for the determination of piritramide concentrations in plasma samples drawn from the superior caval vein in patients receiving intra- or

TABLE II

INTRA- AND INTER-DAY PRECISION FOR PIRITRAMIDE

Spiked concentration (ng/ml)	Found concentration (mean ± S.D.) (ng/ml)	Coefficient of variation (%)
Intra-day (n = 5)		
10	10.0 ± 0.35	3.5
50	48.4 ± 1.52	3.1
Inter-day (n = 4)		
10	9.1 ± 0.42	4.6
50	52.7 ± 2.18	4.1

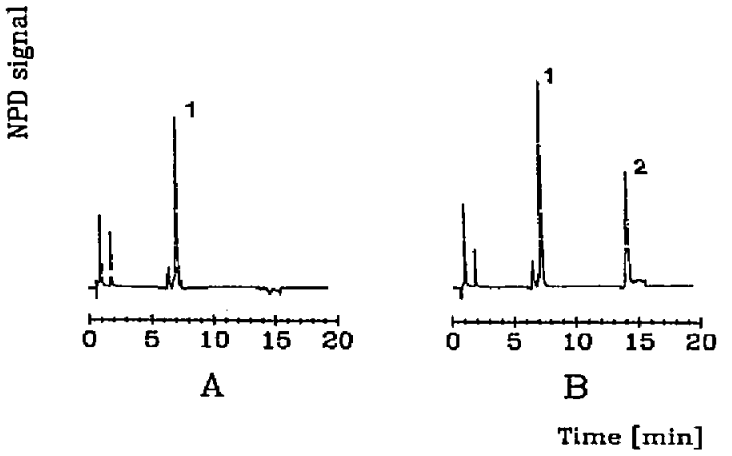

Fig. 2. Chromatograms of plasma samples withdrawn from a patient before (A) and 30 min after (B) intravenous application of 3 mg of piritramide (peak 1 = strychnine; peak 2 = piritramide). The piritramide concentration calculated from chromatogram B was 15 ng/ml. The integrator attenuation was set as follows: ATT 2^3 for the strychnine peak, ATT 2^0 for the piritramide peak and ATT 2^8 for the background signal.

post-operative analgesia. Fig. 2 shows chromatograms of plasma samples withdrawn from a patient before (A) and 30 min after (B) intravenous application of 3 mg of piritramide. The piritramide concentration calculated from chromatogram B was 15 ng/ml. Fig. 3, as an example, depicts a piritramide concentration *versus* time curve from another patient after intravenous application of 15 mg.

Compared with the previously published methods which allow separation or identification of piritramide [5–9], our method offers the possibility of determining piritramide concentrations in human plasma. The assay is linear over the wide concentration range which is observed after intravenous application of piritramide and it is sensitive enough for the determination of the low concentrations in

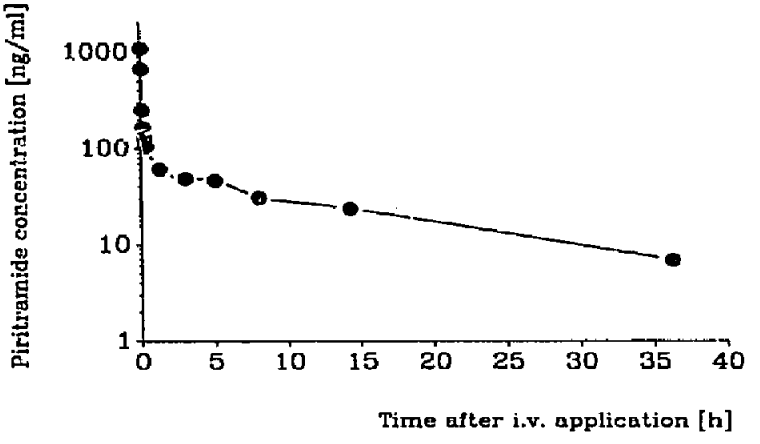

Fig. 3. Concentration *versus* time curve obtained from a patient after application of 15 mg of piritramide. The plasma samples were withdrawn from the superior caval vein.

the later elimination phase. No interfering peaks were observed in plasma samples from patients receiving various supplementary drugs.

In conclusion, our method should be useful for the estimation of pharmacokinetic data of piritramide in man.

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