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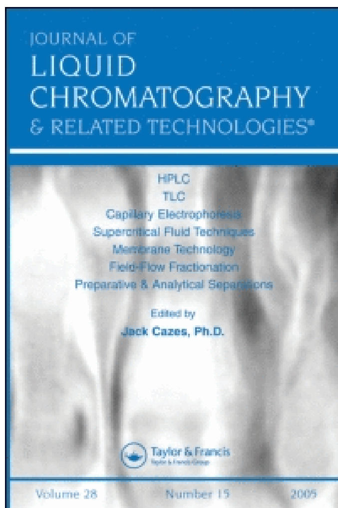

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SENSITIVE DETERMINATION OF PIRITRAMIDE IN HUMAN PLASMA BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

A selective and sensitive method for the determination of piritramide in human plasma is described. After addition of 50 µl of 2 M ammonia and 20 µl of aqueous promethazine solution (100 ng/10 µl) as an internal standard, 1 ml of plasma was extracted with 5 ml of toluene (extraction efficiency: 93.9 ± 2.6%; mean ± S.D.; n=5). HPLC was performed with a phenyl hypersil NC-04 column, particle size 5 µm, 250 x 4 mm I.D.; mobile phase: 8 parts of acetonitrile and 2 parts of 10 mM potassium phosphate buffer (pH 3.3). The flow rate was set to 2 ml/min and the column temperature was 22°C. The assay was linear in a concentration range of 3.75 - 3000 ng/ml (r = 0.999), with a lower limit of detection of 3 ng/ml. The precision was determined using spiked plasma samples (15 ng/ml; 300 ng/ml), with coefficients of variation of 6.1 and 5.9% (intraday; n=5) and 6.5 and 0.2% (interday; n=3). In the range of 5.6 - 1500 ng/ml, the accuracy of the assay was 2.82%. The method was used for the determination of piritramide plasma concentrations in patients receiving intra- or postoperative analgesia.

INTRODUCTION

Piritramide (piritramid, pirinitramide, 1'-(3-cyano-3,3-diphenylpropyl)-[1,4'-bipiperidine]-4'-carboxamide) is a synthetic opioid [1] used for premedication as well as for intra- and postoperative analgesia [2 - 4]. The evaluation of pharmacokinetic parameters requires a sensitive assay for the determination of

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piritramide in plasma. TLC [5], HPLC [6 - 8] and GC/MS [9] procedures suitable for separation or identification of piritramide in standard solutions have been reported, but except of a GC method we have previously published [10], no data are given about plasma extraction procedures and quantification. Therefore, we developed a sensitive and selective HPLC assay for the determination of piritramide in human plasma which is linear in a concentration range of 3.75 - 3000 ng/ml.

EXPERIMENTAL

Reagents and Chemicals

Aqueous piritramide injection solution (7.5 mg/ml) was obtained from Janssen (Neuss, F.R.G.). 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. Promethazine was purchased from Sigma (Deisenhofen, F.R.G.). The aqueous promethazine standard solution (10 µg/ml) was freshly prepared every day. All other chemicals were obtained from Merck (Darmstadt, F.R.G.) and of the highest purity available.

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Apparatus and HPLC Conditions

The HPLC system consisted of a Merck/Hitachi L-6200 intelligent pump (Merck, Darmstadt, F.R.G.) coupled with a six-port injector (Rheodyne, Cotati, CA, U.S.A.), a phenyl hypersil NC-04 column, particle size 5 µm, 250 x 4 mm I.D. (Bischoff, Leonberg, F.R.G.) and a Merck/Hitachi 655A variable wavelength UV monitor (Merck, Darmstadt, F.R.G.) fitted with a 2.2 µl flow-cell, wavelength: 219 nm. A Merck/Hitachi D-2000 chromato-integrator was used for printing chromatograms and for data calculation.
The mobile phase for the HPLC assay consisted of 8 parts of acetonitrile and 2 parts of 10 mM potassium phosphate buffer (adjusted to pH 3.3 with phosphoric acid). The flow rate was 2 ml/min and the column temperature was 22°C.

Plasma Sample Preparation

One milliliter of plasma, spiked with 200 ng of promethazine in 20 µl of doubly distilled water as an internal standard, was alkalinized with 50 µl of 2 M ammonia. Piritramide and promethazine were extracted with 5 ml of toluene by vortex mixing (30 s). Afterwards, 3 ml of toluene were added and the extraction tube was gently shaken. After centrifuging (3000 g; 5 min) the organic layer was

evaporated under vacuum and the residue was redissolved in 100 μl of methanol. 80 - 100 μl of this solution was injected into the separation column.

Calibration Curves, Precision and Recovery

For calibration, plasma standards were spiked with fixed amounts of the internal standard and with piritramide concentrations ranging between 3.75 and 3000 ng/ml. Calibration curves were plotted by correlating the peak-height ratio of piritramide to promethazine against the corresponding piritramide concentrations. The accuracy of the method was evaluated by assaying plasma samples spiked with known piritramide concentrations. The intra- and interday precision of the assay was estimated by measuring plasma standards with 15 and 300 ng/ml piritramide at day one (n = 5) and on the 2 following days. The extraction efficiency was determined by comparison of the detector signals obtained from methanolic piritramide solutions without extraction and from spiked piritramide plasma samples extracted as described above (concentration: 75 ng/ml). The selectivity was determined by injection of methanolic standard solutions of various drugs.

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RESULTS AND DISCUSSION

Piritramide could be extracted from plasma with toluene under alkaline conditions (extraction efficiency: 93.9 ± 2.6%; mean ± S.D.; n=5), but vortex mixing in some cases resulted in emulsions. To improve the separation of the two phases, 3 ml of toluene were added to the emulsions and the sample was gently shaken prior to centrifuging. Since promethazine showed comparable extraction properties and an appropriate retention time, it was used as an internal standard. Unfortunately, promethazine decomposed in aqueous as well as in methanolic solutions within 2 days, so that the standard solution had to be freshly prepared every day. At a signal-to-noise ratio of 2:1, the lower limit of detection for spiked piritramide plasma samples (100 μl injected) was about 3 ng/ml. The assay was linear in the concentration range studied; 3.75 - 3000 ng/ml (r = 0.999). In the low concentration range; 3.75 - 300 ng/ml, the least squares regression line had a slope of 0.005 and an y-intercept of -0.0103 (y: piritramide/promethazine peak height ratio, x: piritramide concentration; ng/ml); with a coefficient of correlation: r = 0.999. The accuracy of the method is shown in Table 1; the theoretical concentrations (spiked conc.) agreed well with the assayed concentrations (found conc.) with an accuracy mean value of 2.82%. For intra- and interday precision studies, the mean concentration, standard deviation and coefficient of variation are shown in Table 2. In Table 3, the retention times of various drugs commonly used during anaesthesia are

TABLE 1

Accuracy of the Assay

Concentration of piritramide (ng/ml)		Accuracy* (%)
Spiked conc.	Found conc.	
5.6	5.61	0.18
7.5	7.47	0.40
15.0	15.16	1.07
60.0	56.87	5.22
150.0	157.63	5.09
300.0	299.87	0.04
750.0	789.33	5.24
1500.0	1420.20	5.32
	Mean:	2.82

*Calculated according to ref. 11

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TABLE 2

Intra- and Interday Precision for Piritramide

	Spiked concentration (ng/ml)	Found concentration (mean ± S.D.) (ng/ml)	C.V. (%)
Intraday	15	14.2 ± 0.86	6.1
(n=5)	300	299.1 ± 17.50	5.9
Interday	15	15.2 ± 0.99	6.5
(n=3	300	299.5 ± 0.44	0.2

TABLE 3

Selectivity of the Assay

Drug	Retention time (min)
Piritramide	5.58
Promethazine	6.62
Diazepam	1.26
Droperidol	1.22
Etomidate	3.74
Fentanyl	4.90
Flunitrazepam	1.18
Ketamine	3.98
Methohexital	1.16
Midazolam	3.16
Pancuronium bromide	1.05
Suxamethonium chloride	1.12
Temazepam	1.20

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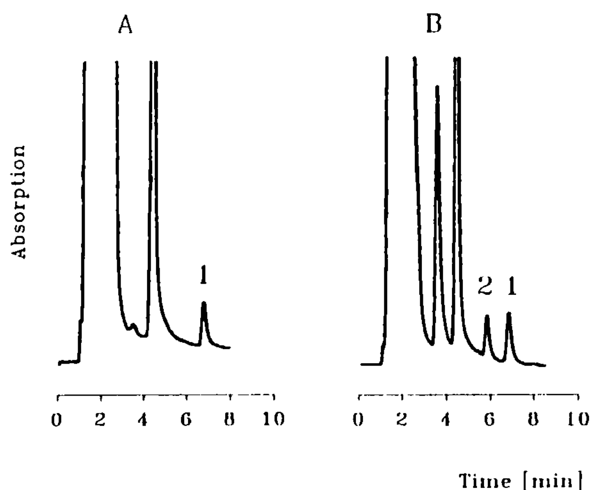

Figure 1

Chromatograms of peripheral venous plasma samples withdrawn from a patient before (A) and 10 min after (B) i.v. application of 3 mg of piritramide (peak 1: promethazine, peak 2: piritramide). The piritramide concentration calculated from chromatogram (B) was 180 ng/ml.

listed. As it is depicted, interferences did occur neither with the piritramide nor with the promethazine peak. The assay was used for the determination of piritramide plasma concentrations in patients receiving intra- or postoperative analgesia. Fig. 1 shows chromatograms of peripheral venous plasma samples withdrawn from a patient before (A) and 10 min after (B) i.v. application of 3 mg of piritramide. The piritramide concentration calculated from chromatogram (B) was 180 ng/ml.

This assay - compared to the previously published methods which allow separation or identification of piritramide [5 - 9] - offers the possibility to determine piritramide concentrations in human plasma. In comparison to our GC assay [10], it is linear over the same concentration range, and it yields a similar limit of detection. As an advantage of the GC method, it allows repeated injections per sample (only 1 % of the extract is injected once), whereas HPLC requires the injection of the whole extract.

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

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