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Determination of sameridine in blood plasma by nitrogen-selective gas chromatography

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Abstract

A gas chromatographic method was developed and validated for the determination of sameridine in human plasma. Sameridine is a new type of compound with both local anaesthetic and analgesic properties, when administered intrathecally. The method is based on liquid–liquid extraction of sameridine from 1.0 ml of plasma, followed by gas chromatography with nitrogen–phosphorus detection. Method validation results showed that this method is very sensitive, selective and robust. The limit of quantification was 1 n*M* for 1.0 ml of human plasma in the low-level range (1.00–75.0 n*M*) and the between-day accuracy and precision were measured at 99–104% of nominal values and 3.4–5.6% (RSD), respectively.

Keywords: Sameridine

1. Introduction

Sameridine hydrochloride, LPB-139, *N*-ethyl-1-hexyl-*N*-methyl-4-phenyl-4-piperidinecarboxamide hydrochloride (Fig. 1), is a new type of compound with both local anaesthetic and analgesic properties, when administered intrathecally [1,2]. In clinic studies it has been used to provide pain relief for arthroscopic knee surgery [1] and for inguinal hernia repair [2]. In order to perform pharmacokinetic studies of sameridine, a sensitive and selective bioanalytical method for its determination in plasma was required.

There are numerous methods available for the determination of basic drugs, e.g., local anaesthetics and analgesics in biological fluids, using gas chromatography with nitrogen–phosphorus detection (GC–NPD) [3–6].

The present work describes a robust, accurate and precise method based on the splitless sample injection on a fused-silica capillary column using NPD, which is highly selective to nitrogen-containing compounds such as sameridine. The ethyl analogue of sameridine, was used as an internal standard (Fig. 1). To study pharmacokinetic parameters after intrathecal administration [1], a limit of quantification (LOQ) of 1 n*M* was needed.

The free concentration of sameridine was determined by coupled-column liquid chromatography [7], and sameridine was also used as a model for the development of a molecular imprinting technique [8].

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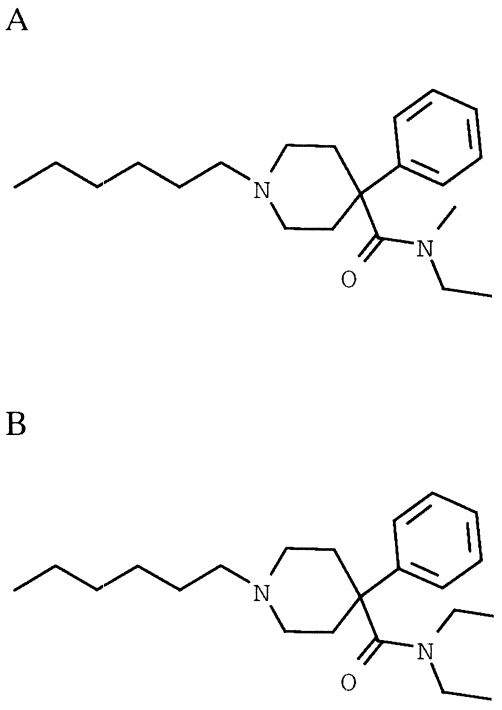

Fig. 1. Chemical structures of sameridine (A) and LPB-143, the internal standard (B).

2. Experimental

2.1. Chemicals and reagents

Sameridine hydrochloride, LPB-139, *N*-ethyl-1-hexyl-*N*-methyl-4-phenyl-4-piperidinecarboxamide hydrochloride (M_r 367.0), and the internal standard, LPB-143, *N*,*N*-diethyl-1-hexyl-4-phenyl-4-piperidinecarboxamide hydrochloride (M_r 381.0), were synthesized at the Department of Medicinal Chemistry, Astra Pain Control (Södertälje, Sweden), see Fig. 1. All the solvents used were of analytical purity grade and all other chemicals were of analytical-reagent grade or better.

2.2. Equipment

The gas chromatograph was an HP 5890 series II (Hewlett-Packard, Palo Alto, CA, USA), equipped with a split/splitless injector, an electronic pressure control and an NPD system. The splitless injections were performed with a HP 7673 automatic sampler. The chromatograms were recorded and integrated using PE-Nelson Access Chrom (Perkin-Elmer Nelson Systems, Cupertino, CA, USA) system. The samples were evaporated by a Turbo Vap (Zymark, Hopkington, MA, USA).

2.3. Analytical method

2.3.1. Handling of standards, quality control (QC) samples and test samples

Two standard stock solutions (about 10 mg/50 ml) of sameridine were prepared in deionized water and stored in a refrigerator. The standards and QC samples were prepared by adding diluted standard stock solution in drug-free plasma to give a suitable concentration.

Whole human blood samples were collected in heparinized Vacutainer tubes (Beckton Dickinson, USA). The samples were handled at room temperature and were centrifuged for separation of plasma within 60 min of collection. The plasma samples were then transferred to polypropylene tubes and stored at −20°C, until analysis.

2.3.2. Sample preparation procedure

To each sample tube containing 1.0 ml plasma, 50 μl of internal standard (LPB 143) solution was added, followed by 100 μl of 10% (w/v) sodium carbonate and 3 ml *n*-heptane. The tubes were slowly rotated in a rotary mixer for 20 min and centrifuged for 10 min at 3000 rpm and the organic phase was evaporated to dryness under a stream of nitrogen at 40°C. The residue was redissolved in 100–250 μl of *n*-heptane–ethanol (9:1, v/v), and an aliquot of 1–3 μl was injected into the GC system for analysis.

2.3.3. Gas chromatography

The columns used were fused-silica of 25 m×0.32 mm I.D., with a 5% diphenyl–95% dimethyl siloxane copolymer, and a film thickness of 0.17 μm (Ultra-2, Hewlett-Packard). The initial column temperature was set at 120°C for 1 min and was raised at a rate of 40°C/min to 280°C and then kept isothermal for 3 min. The temperatures of the splitless injection port and NPD system were 260°C and 300°C, respectively. Helium was used as carrier gas at an inlet pressure of 60 kPa (average linear velocity 32 cm/s, at 120°C). The gas flow-rates at the

detector for hydrogen, make-up and air were 3, 100 and 30 ml/min, respectively. The purge valve was closed for 1 min.

2.3.4. Quantification

A single-level calibration curve (40.0 or 3000 n*M*) extrapolated through the origin was obtained by plotting the concentrations of six plasma standards at one concentration level vs. peak-height ratios of the analyte and internal standard. The calibration curve was accepted if the relative standard deviation (RSD) of the six standard samples was <8%. The concentration of sameridine in each unknown sample was calculated by means of unweighted linear regression analysis. In addition, in each batch of analysis QC samples at three different concentration levels (low, medium and high) were determined in duplicate. The QC concentrations in the low-level range were 4.00, 20.0 and 60.0 n*M* and in the high-level range 50.0, 300 and 2000 n*M*. The QC samples were accepted if they were within ±20% of the theoretical value, although two out of six samples (not at the same concentration) could lie outside the criteria, according to Shah et al. [9]. In the automatic sampler injector the standards and QC samples were spread among the unknown samples.

2.4. Validation of the method

2.4.1. Determination of recovery

The recovery of the extraction procedure was determined by comparing slopes from calibration curves. In the first experiment the standard samples were processed according to the method, while in the second experiment drug-free plasma including internal standard was extracted and sameridine was added just before the evaporation step, in exactly the same amount as in the first experiment. The extraction recovery was determined from the ratio of the slopes of the calibration curves.

2.4.2. Linearity

The linearity of the method was tested in two different concentration ranges in blood plasma, 1.00–75.0 n*M* (low-level range) and 10.0–3000 n*M* (high-level range), by running multilevel calibration curves.

2.4.3. Determination of precision and accuracy

The within-day and between-day accuracy and precision of the method were determined by performing analyses of QC samples in plasma with known concentrations of sameridine at three concentration levels and on at least three occasions of analysis. The concentrations were chosen to cover the ranges of the standard curves, 1.00–75.0 n*M* and 10.0–3000 n*M*. The QC concentrations in the low-level range were 4.00, 20.0 and 60.0 n*M* and in the high-level range 50.0, 300, and 2000 n*M*.

The QC samples were accepted if they were within ±15% of the theoretical value, except at the LOQ, where it should not deviate by more than ±20%. The precision around the mean value given as the RSD should not exceed 15%, except for the LOQ, where it should not exceed 20%, according to Shah et al. [9].

2.4.4. Stability in whole blood and plasma

The stability of sameridine in whole blood was determined at room temperature and on ice for 30, 60, 90 and 120 min. Sameridine was added to a final concentration of 2.4 μ*M* before incubation under different conditions. Plasma samples were prepared and analyzed according to the method described.

The stability of sameridine in plasma was determined after the addition of known amounts of sameridine to plasma, at three concentration levels (40, 1000 and 4000 n*M*), in duplicate. The samples were stored at room temperature and at −20°C for different lengths of time.

3. Results and discussion

3.1. Method development

Sameridine (Fig. 1) is a strongly hydrophobic basic amine with a pK_a of 9.2. The approach used for sample preparation was simple liquid–liquid extraction and it was possible to use a single-step extraction for plasma samples with a base added to the water phase and *n*-heptane as the organic phase. The selection of *n*-heptane as the organic phase gave high recoveries and the least interferences. Extraction solvents with hydrogen binding properties, e.g., methylene chloride, were also tested and high re-

coveries were obtained, although the gas chromatograms were not as clean as when using pure heptane as extraction solvent. The extraction recovery of sameridine in plasma to heptane was 89%. For the extraction, a rotary mixer was preferred to a vortex-mixing device because the latter caused the formation of emulsion, resulting in a lower recovery. The internal standard was added to plasma before extraction to avoid the need for accurate measurement of volumes during extraction. The choice of LPB 143 as the internal standard was based on the similarity of the structure of LPB 143 and sameridine, allowing a comparable extraction ratio and chromatographic behaviour.

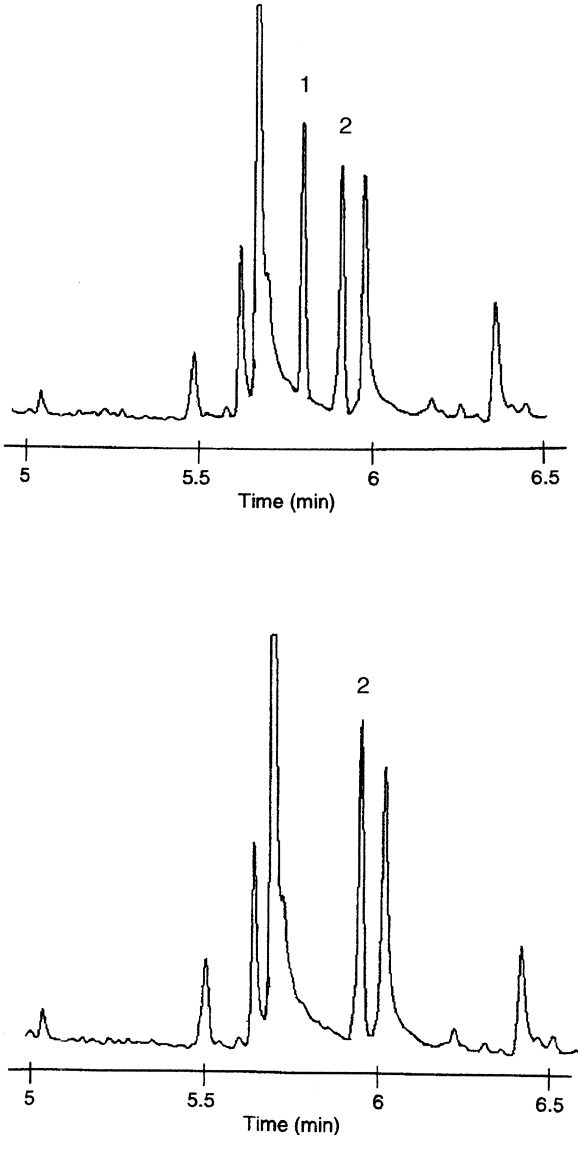

Fig. 2. Gas chromatograms of blood plasma samples. The experimental conditions were in accordance with the analytical method. Upper: human blood plasma sample taken after intrathecal administration in man, with a sameridine concentration of 28 n*M*. Lower: drug-free blood plasma sample with internal standard. Peaks: 1=sameridine, 2=LPB-143 (internal standard).

Due to the physicochemical characteristics of sameridine the nitrogen–phosphorus detector was chosen because of its good selectivity for and sensitivity to nitrogen-containing substances. Since a low LOQ was required, careful optimization of the chromatographic conditions was needed. Splitless injection of 1–3 μl was preferred on an Ultra-2 column (25 m×0.32 mm I.D., 0.17 μm) from Hewlett-Packard. However, most kinds of non-polar column materials can be used with this method. To get a short analysis time and narrow peaks, a thin film thickness, a fast and steeply rising temperature program and a constant flow of the carrier gas were used. The temperature program used started with an initial column temperature of 120°C and a rate of 40°C/min, to give a final temperature of 280°C.

Representative gas chromatograms from a human plasma sample after the administration of sameridine and from a drug-free human blood plasma sample are shown in Fig. 2.

3.2. Validation of the method

Sameridine is stable in whole blood for at least 120 min both at room temperature and on ice. However, after 60 min, haemolysis was observed in some cases. Accordingly the recommended conditions for handling blood samples during collection are at room temperature, followed by separation of plasma within 60 min of collection. In plasma sameridine has been determined to be stable during storage at room temperature for at least 7 days and at −20°C for at least 22 months.

The results for the between-day accuracy and precision of QC samples in plasma, in the low- and high-level ranges, are summarized in Table 1.

The LOQ for the method in the low-level range was set at 1 n*M* (100.3±7.6% RSD, $n=10$) and for

Table 1
Between-day precision and accuracy of sameridine QC samples at different concentration levels

Quantification range (n*M*)	Concentration (n*M*)	Accuracy (%)	RSD (%)	*n*
1.00–75.0[a]	4.00	98.6	5.6	15
	20.0	103.7	3.4	15
	60.0	103.5	3.8	15
10.0–3000[b]	50.0	94.8	9.8	14
	300	92.8	4.9	13[c]
	2000	96.9	4.8	14

[a] Five replicates per day, for 3 days.
[b] Two replicates per day, for 7 days.
[c] One QC sample rejected, not included in the calculations.

the high-level range at 10 n*M* (93.8±9.0% RSD, $n=10$).

Peak height ratios of sameridine and the internal standard versus sameridine concentrations were subjected to least-squares regression analysis. The multi-level calibration curves were found to be linear within the tested ranges of 1.00–75.0 n*M* and 10.0–3000 n*M*, respectively. The back-calculated concentrations were within ±20% of nominal values near the LOQ and within ±10% of nominal values at higher concentration levels.

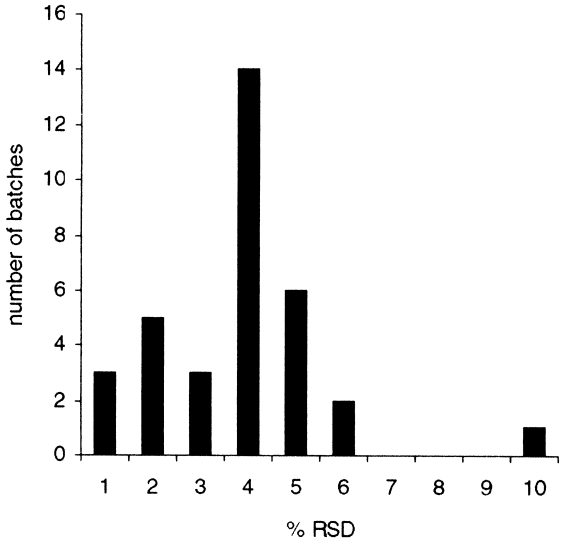

Fig. 3. Within-day precision (RSD), calculated from peak-height ratios ($n=6$) of standard and internal standard, during long-term use of the GC–NPD method.

3.3. Application of the method

The method presented has been applied to the determination of sameridine in blood plasma samples at different concentration levels. The method described in the present paper is for the analysis of human plasma samples. It is, however, possible to determine sameridine from other species, e.g., dog and rat, using the same method. Several thousand blood plasma samples have been assayed. Generally, the method is very robust and more than 95% of the batches of analysis performed were accepted directly, using the criteria proposed by Shah et al. [9], both in the low- and the high-concentration ranges. Results from 34 batches for analysis in the low-concentration range (Fig. 3) show that the within-day precision (RSD) was generally less than 5%. The within-day precision for the high-concentration range shows similar results.

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