ELSEVIER

Journal of Chromatography B, 708 (1998) 195–200

JOURNAL OF CHROMATOGRAPHY B

Determination of free concentration of sameridine in blood plasma by ultrafiltration and coupled-column liquid chromatography

Eivor Eklund, Carina Norsten-Höög, Torbjörn Arvidsson*

Concept Division, Bioanalytical Chemistry, Astra Pain Control AB, S-151 85 Södertälje, Sweden

Received 2 September 1997; received in revised form 9 December 1997; accepted 16 December 1997

Abstract

Sameridine is a new candidate drug with both local anaesthetic and analgesic properties. The free concentration of sameridine in blood plasma was determined by coupled-column liquid chromatography. Following adjustment of the pH and the temperature of the plasma samples, the free fraction was prepared by ultrafiltration. The coupled-column liquid chromatographic system consisted of a reversed-phase column, a cation-exchange extraction column and a cation-exchange analytical column. Sameridine was detected by UV determination at 205 nm and the system showed high selectivity. The limit of quantification was 1 n*M* and the within-day precision was 4.6% (R.S.D., $n=10$). © 1998 Elsevier Science B.V.

Keywords: Sameridine

1. Introduction

Sameridine hydrochloride (*N*-ethyl-1-hexyl-*N*-methyl-4-phenyl-4-piperidine carboxamide hydrochloride), c.f. Fig. 1, is a new class of compound with both local anaesthetic and analgesic properties [1]. The clinical use of sameridine is for intrathecal administration in order to provide anaesthesia for surgical purposes and prolonged postoperative analgesia [2,3].

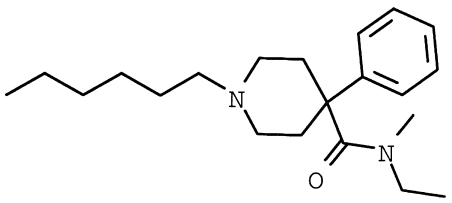

Fig. 1. Chemical structure of sameridine.

During the development of the new agent, it was of interest to determine the concentration of sameridine in biological samples in both animal and human studies. The total concentration of sameridine was determined by capillary gas chromatography with nitrogen-sensitive detection after liquid–liquid extraction [4]. Molecular imprints of sameridine were also tested as a sample preparation technique [5]. In human studies, it was also of interest to determine the free concentration of sameridine. When correlating pharmacological and toxicological effects with drug concentration, the free concentration is often a better estimate compared to the total concentration of the drug, and this is of special interest for local anaesthetic drugs [6–8]. This paper

*Corresponding author.

0378-4347/98/$19.00
PII S0378-4347(97)00671-3

will present a method for the determination of the free concentration of sameridine in human plasma using ultrafiltration and coupled-column liquid chromatography. The local anaesthetic agents ropivacaine and bupivacaine were determined using similar methodology [9]. The choice of conditions and material of the filter during ultrafiltration, as well as optimisation of the liquid chromatographic system, will be discussed and data from the validation of the method will be presented.

2. Experimental

2.1. Chemicals

Sameridine hydrochloride monohydrate was obtained from Astra Production Chemicals, Södertälje, Sweden (sameridine base, M_r 330.5). CO_2 (50% in O_2) came from AGA GAS (Stockholm, Sweden). Acetonitrile of LiChrosolv grade was obtained from Merck (Darmstadt, Germany). All the other chemicals were of analytical grade, obtained from the usual commercial sources.

2.2. Equipment

The pH was measured using a XEROLYTE microelectrode (Mettler-Toledo, Greifensee, Switzerland) and temperature-controlled centrifugation was performed using a Hettich Rotanta/TRC (Hettich, Tuttlingen, Germany) with a 35° fixed-angle rotor. For collecting blood plasma, heparinised Venoject tubes (Terumo, Leuven, Belgium) were used. The plasma was stored in Nunc Cryotubes (Intermed, Roskilde, Denmark). The ultrafiltrate was prepared using an ultrafiltration device with a centrifugal filter for sample preparation, Ultrafree-CL (low binding cellulose 30 000 NMWL) (Millipore, Bedford, MA, USA).

The liquid chromatographic system consisted of two Shimadzu LC-9A pumps (Shimadzu, Kyoto, Japan) connected to a CMA/200 autoinjector (CMA/Micro-dialysis, Stockholm, Sweden) with two six-port switching valves equipped with a 500-μl injection loop and an extraction column, Spherisorb 5SCX (10×3.2 mm I.D.) (Hichrom, Theale, PA, USA), respectively.

The columns were a Kromasil C_{18} 5 μm (100×4.6 mm I.D.; Hichrom) and a Spherisorb 5SCX 5 μm (100×2.1 mm I.D.; Hichrom). The column oven was a BAS LC-22C (Bioanalytical Systems, West Lafayette, IN, USA).

The detectors were a Spectra 100 UV–Vis (Spectra Physics, San José, CA, USA) for the assay and a LDC Spectro Monitor III (Riviera Beach, FL, USA) to check the switching time in the coupled-column system. PE Nelson Access* Chrom (Perkin-Elmer Nelson Systems, Cupertino, CA, USA) was used for the integration and calculation of the results.

2.3. Procedures

Phosphate buffer, pH 3 (ionic strength=0.02), was prepared by diluting 3.1 ml of 1 *M* phosphoric acid and 20 ml of 1 *M* sodium dihydrogen phosphate to 1000 ml with deionised water. Ammonium phosphate buffer, pH 2.6 (ionic strength=0.3), was prepared by diluting 149 ml of 2 *M* ammonium hydroxide and 348 ml of 1 *M* phosphoric acid to 1000 ml with deionised water. The eluents were prepared by mixing specified volumes of acetonitrile and buffer.

Phosphate buffer, pH 7.4 (ionic strength=0.2), was prepared by diluting 18 ml of 1 *M* sodium dihydrogen phosphate and 121 ml of 0.5 *M* disodium hydrogen phosphate to 1000 ml with deionised water.

Two standard stock solutions (0.01 and 0.005 mg/ml) of sameridine were prepared in 0.01 *M* phosphoric acid and stored in a refrigerator. To obtain a suitable concentration and a pH of 7.4, the external standards and control standards were prepared by dilution of the stock solutions of sameridine with 0.01 *M* phosphoric acid and the last dilution step (1:10, v/v) with phosphate buffer, pH 7.4 (ionic strength=0.2).

Plasma control samples (20–1000 n*M*, total concentration) were prepared by adding sameridine to pooled drug-free plasma, and these were stored frozen at −20°C.

2.4. Handling of plasma samples

Human blood was collected in heparinised Venoject tubes and centrifuged within 1 h. The plasma

was transferred to polypropylene tubes (Nunc) and frozen within 30 min. The plasma samples were kept frozen (−20°C) until analysis.

2.5. Analytical method

The blood plasma sample was brought to 37°C and the pH was adjusted to pH 7.4 by adding CO_2 (gas). A 2-ml volume of the sample was transferred to an ultrafiltration device, Ultrafree-CL, and centrifuged at 37°C and 7000 rpm (approx. 6800 *g*) for 15 min to give about 400 μl of the ultrafiltrate.

The ultrafiltrate (400 μl) was injected, without further pre-treatment, into a coupled-column LC system (Fig. 2). A heart-cut (approx. 1 ml) of the zone where the analyte was eluted (t_R approx. 5 min) from the first analytical column (C1, Kromasil C_{18}) was transferred to and enriched on the cation-exchange extraction column (C2, Spherisorb 5SCX) using an eluent of low ionic strength. In the next step, an eluent of high ionic strength was introduced and the analyte was eluted from the extraction column, in back-flush mode, into the second analytical column (C3, Spherisorb 5SCX) for final separation. The eluent for the first column (C1) consisted of acetonitrile–phosphate buffer, pH 3 (ionic strength=0.02; 35:65, v/v), while the eluent for the cation-exchange columns (C2 and C3) consisted of acetonitrile–ammonium phosphate buffer, pH 2.6 (ionic strength=0.3; 33:67, v/v). The flow-rate was 1.0 ml/min for column C1 and 0.4 ml/min for column C3, and the temperature was 27°C for both columns. The eluate was detected at 205 nm.

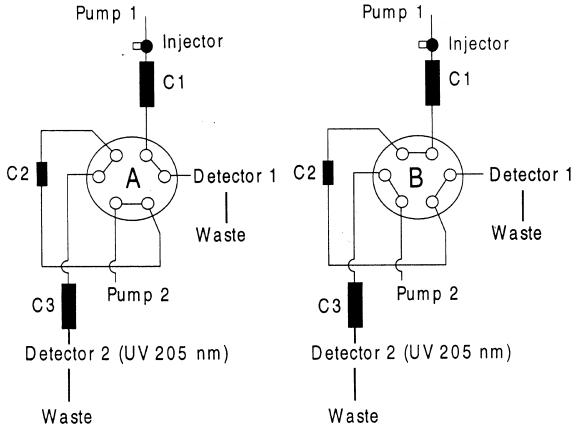

Fig. 2. Coupled-column liquid chromatographic system. Event 1: valve in position A for injection and rough separation on the first column (C1). Event 2: valve to position B for loading of the analyte onto the cation-exchange extraction column (C2). Event 3: valve back to position A for back-flush elution and final separation on column (C3). Injector, six-port valve with a 500-μl loop. Eluent 1, acetonitrile–phosphate buffer, pH 3 (ionic strength= 0.02; 35:65, v/v). Flow-rate, 1.0 ml/min. C1, Kromasil C_{18}. Detector 1, detector [for testing the retention (C1)]. C2, cation-exchange extraction column (Spherisorb 5SCX). Eluent 2, acetonitrile–ammonium phosphate buffer, pH 2.6 (ionic strength= 0.3; 33:67, v/v). Flow-rate, 0.4 ml/min. C3, Spherisorb 5SCX. Detector 2, UV detection at 205 nm.

2.6. Quantification

The standards were prepared within the concentration range 1–300 n*M* at seven concentration levels. The standard curve was not linear over the entire concentration range and quantification was obtained by linear regression in two concentration ranges, 1–20 n*M* and 10–300 n*M*. The peak heights were measured and the concentrations of sameridine in the ultrafiltrate were calculated by comparison with external standards. If quantification in the entire range is needed, a quadratic quantification model with a weighting of $1/x$ can be used.

On each occasion of analysis, ultrafiltrated aqueous control standards were determined to check the adsorption to the ultrafiltration device. Plasma control samples, with a free concentration of about 5–200 n*M* (n=5), were determined to measure within-day and between-day precision.

2.7. Stability

The stability of the free concentration of sameridine in plasma samples was tested at room temperature and at −20°C, for five days and for a period of two years, respectively. The plasma samples were spiked with 1000 n*M* of sameridine, i.e., giving a free concentration of about 200 n*M*.

3. Results and discussion

The free concentrations of drugs in blood plasma are usually determined under physiological conditions (pH 7.4, 37°C), in order to have reliable

correlations to pharmacological effects. Sameridine (Fig. 1) is a hydrophobic amine with a pK_a of 9.2. In aqueous solution at pH 7.4, sameridine shows a strong tendency to adsorb to various surfaces, e.g. glass, hence, to minimise errors in the handling of standards, the stock solution was prepared in buffer of low pH, and all dilutions, except for the final one, were also made with buffers of low pH. In the clinic, sameridine is administered intrathecally and the maximum total concentration (C_{max}) obtained will be about 100–200 n*M*; however, after i.v administration, the C_{max} will be 1000–2000 n*M*. In humans (healthy volunteers), under physiological conditions (pH 7.4, 37°C), the protein binding of sameridine is about 70–85%. Accordingly, in pharmacokinetic and pharmacodynamic evaluations, it is of interest to monitor free concentrations within the range 1–300 n*M*.

3.1. Drug–protein binding and ultrafiltration

Drug–protein binding is generally characterised by a reversible equilibrium and is affected by physicochemical parameters, such as protein concentration, temperature, pH and adsorption [7], which must be considered when setting up conditions for preparation of the free fraction.

Ultrafiltration is a simple technique for preparing the free fraction, due to the availability of commercial devices and, therefore, it is often the method of choice. When developing ultrafiltration conditions for sameridine, it is important to control both the pH and the temperature because the free fraction of sameridine varies, e.g. when the pH was raised from 7.0 to 7.8, the free fraction decreased from 23 to 14% and when the temperature was raised from 32 to 40°C, the free fraction decreased slightly from 20 to 18%. In the analytical procedure, the temperature and the pH were therefore fixed at 37°C and pH 7.4, respectively. The ultrafiltrated volume must also be considered since the amount of protein in the upper phase will continuously increase during ultrafiltration. The ultrafiltrated volume was therefore kept below 25% of the total plasma volume used, in order to avoid systematic errors. Sameridine, which is a strongly hydrophobic amine, shows a high tendency, at pH 7.4, to adsorb to various surfaces, e.g. the ultrafiltration membrane. The choice of membrane is critical, e.g. sameridine is completely adsorbed to polysulfone membranes (Millipore Ultrafree-Cl, polysulfon, 30 000 NMWL), whereas for regenerated cellulose (Millipore Ultrafree-Cl, low binding cellulose, 30 000 NMWL) or the Amicon, MPS-1, YMT 30 membrane, the adsorption is less than 15%. The Ultrafree-Cl device was chosen because it is disposable; however, the Amicon device may be used if the sample volume used is less than 1 ml. The adsorption will depend on the volume filtered (Fig. 3) and, at a given volume, adsorption will be saturated and recovery will be constant. Filtration of buffered samples of sameridine (pH 7.4) shows that the ultrafiltrated volume must be greater than 250 μl to obtain constant recovery. A similar profile to that shown in Fig. 3 was observed for plasma samples, therefore, the plasma sample volume must be at least 1 ml if the ultrafiltrate should be less than 25% of the total volume. If the total adsorption is higher than 10–15%, it is also advisable to filter the aqueous standards for quantitative determination and to use a total plasma volume of 2 ml, to minimise systematic errors.

3.2. Coupled-column liquid chromatography

Following intrathecal administration of sameridine, the free concentration levels in plasma are low and a limit of quantification (LOQ) of 1 n*M* is needed for pharmacokinetic evaluation. Liquid chromatography with UV detection at a low wave-

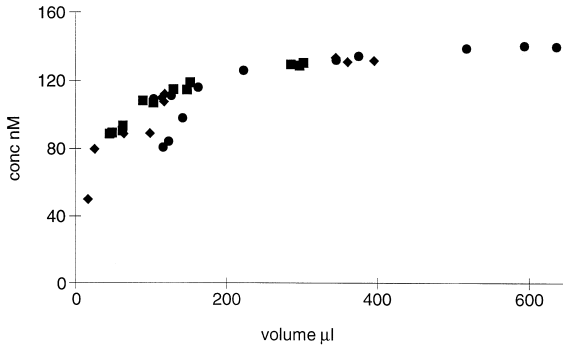

Fig. 3. Recovery of sameridine from different volumes of ultrafiltrate. Different sample volumes of sameridine (159 n*M* in phosphate buffer, pH 7.4; ionic strength=0.2) were ultrafiltrated. Sample volume: ♦, 0.5 ml; ■, 1.0 ml and ●, 2.0 ml.

length (205 nm) was chosen and a coupled-column system was developed for the purpose (Fig. 2). The methodology was based on the coupling of reversed-phase chromatography and ion-exchange chromatography, a technique similar to that described in our method for the determination of free concentrations of ropivacaine and bupivacaine [9]. Since it was necessary to determine ten times lower concentrations of sameridine compared to ropivacaine, the chromatographic conditions were modified. In order to obtain higher sensitivity, a larger injection volume (400 μl), an extraction column for enrichment and an analytical column with a smaller I.D. (2.1 mm) were used. A large volume of the ultrafiltrate was injected onto the first analytical column and a heart-cut of the analyte (1 ml) had to be transferred to the second analytical column. This was done via a small cation-exchange extraction column. The low ionic strength of the eluent from the first analytical column made it possible to enrich the analyte on the cation-exchange column, since retention of sameridine was very high ($k'>50$) at the low ionic strength used. The analyte was then finally resolved on the second analytical column after back-flush elution from the extraction column, using an eluent of high ionic strength. To avoid errors in the transfer of the analyte to the cation-exchange extraction column and to ensure the performance of the analytical system, the retention of sameridine on the first column was always checked before each batch of analysis. The switching time of the six-port valve coupled to the extraction column was set from the peak volume data.

3.3. Method validation

The selectivity of the method was established by comparison of the chromatograms obtained from drug-free human plasma and from human plasma after administration of sameridine. The chromatograms from drug-free plasma showed no peak that was liable to interfere with sameridine (Fig. 4).

The standard curves were linear, with a coefficient of determination (r^2) of >0.991 in the concentration ranges 1–20 and 10–300 n*M*, respectively, if an unweighted linear regression model was used. In order to cover the entire concentration range, 1–300 n*M*, a quadratic model with a weighting of $1/x$ may be used, giving an r^2 of >0.999.

The within-day and between-day precisions are given as the relative standard deviation (R.S.D.), determined by analysing human plasma control samples on each occasion of analysis, with a free concentration in the same range as that of the unknown samples. The within-day and between-day precisions were in the ranges of 2–8 and 4–9%, respectively (see Table 1).

The LOQ for the determination of sameridine in ultrafiltrate of human plasma samples was set at 1 n*M* (R.S.D.=4.6%, $n=10$).

The free concentration of sameridine in blood plasma was found to be stable during storage at room

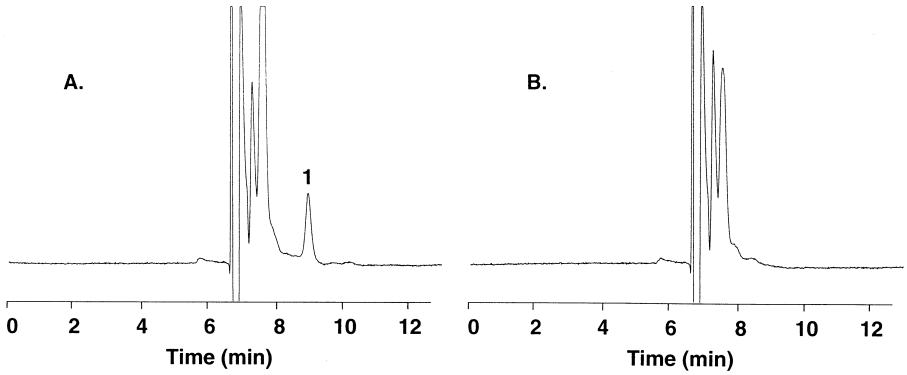

Fig. 4. Chromatograms of human plasma samples after intrathecal administration. Experimental conditions are according to the analytical method. (A) 11.2 n*M* sameridine, (1). (B) drug-free sample.

Table 1
Within-day and between-day precision of plasma control samples

Occasions of analysis	Added concentration (n*M*)								
	1064			47.3			21.0		
	Found concentration (n*M*)	R.S.D. (%)	*n*	Found concentration (n*M*)	R.S.D. (%)	*n*	Found concentration n*M*	R.S.D. (%)	*n*
1	212.0	3.7	5	9.85	4.6	5	5.19	6.2	5
2	205.5	3.6	5	10.07	1.8	5	5.34	8.3	5
3	218.4	3.0	5	10.29	2.8	5	4.62	5.4	5
4	211.2	2.4	5	9.98	5.5	4			
5	210.1	1.8	5	10.59	3.9	6			
6	220.4	2.9	5	10.41	2.3	5			
7	209.5	3.7	5						
8	212.7	4.3	5						
Σ		3.6	40		4.2	30		9.0	15

For each analysis, five human plasma control samples, in the range of the unknown samples, were analysed.

temperature and at −20°C, for at least five days and two years, respectively.

4. Conclusion

The free concentration of sameridine in blood plasma was determined by methodology involving ultrafiltration and coupled-column liquid chromatography. The method has been used for hundreds of samples and has shown high selectivity, sensitivity and precision. The chromatographic system also shows a high degree of ruggedness and stability and is suitable for routine analysis.

References

[1] A.-L. Ask, R. Sandberg, in preparation.
[2] L. Westman, P. Westerling, A. Valentin, A. Ekblom, Abstract at the World Congress of Anaesthesiologists, Sydney, Australia, 1996, D 474.
[3] T. Muldoon, M. Personne, K. Axelsson, U. Feuk, A. Ekblom, D. Connolly, K. Milligan, Abstract at the European Society of Anaesthesiology, Lausanne, Switzerland, 1997, A 265.
[4] P. Neidenström, C. Norsten-Höög, T. Arvidsson, in preparation.
[5] L.I. Andersson, A. Paprica, T. Arvidsson, Chromatographia 46 (1997) 57.
[6] G.T. Tucker, Acta Anaesthesiol. Belg. 39 (1988) 147.
[7] G.T. Tucker, Reg. Anesth. 17(3 suppl.) (1992) 1.
[8] G.T. Tucker, Reg. Anesth. 19 (1994) 155.
[9] T. Arvidsson, E. Eklund, J. Chromatogr. B 668 (1995) 91.