ELSEVIER

Journal of Chromatography B, 660 (1994) 191–195

JOURNAL OF CHROMATOGRAPHY B: BIOMEDICAL APPLICATIONS

Short communication

High-performance liquid chromatographic assay for sematilide in plasma using solid-phase extraction microcolumn technology

Sylvie Laganière*, Lynne Goernert, Greg Beatch

Biopharmaceutics and Pharmacodynamics Division, Bureau of Drug Research, Drugs Directorate, Health Protection Branch, Department of Health, 2-West, Sir Frederick Banting Building, Tunney's Pasture, Ottawa, Ont. KIA-OL2, Canada

First received 8 April 1994; revised manuscript received 24 May 1994

Abstract

A simple and sensitive high-performance liquid chromatographic assay for quantification of sematilide in rabbit plasma was developed. After extraction of samples via solid-phase extraction on C_8 microcolumns, baseline resolution was achieved on a reversed-phase 5 μm Inertsil ODS-2 column using isocratic conditions with mobile phase consisting of water–glacial acetic acid–acetonitrile–methanol–triethylamine (93.5:4.0:1.5:0.5:0.5) and UV detection at 254 nm. The assay did not require evaporation or reconstitution steps. The injection interval was 8 minutes. The inter-day coefficient of variation for replicate analysis of spiked samples was less than 7.6% and the accuracy was more than 97% over the standard curve range (0.128 to 3.191 μM) using 0.5 ml of plasma. The assay has been successfully applied to pharmacokinetic studies in rabbits.

1. Introduction

Sematilide hydrochloride (SEM) is being developed as a class III antiarrhythmic agent [1]. A sensitive analytical assay was necessary to determine the full drug concentration–time profile of sematilide in rabbits. There is only one published method for sematilide in human plasma using a liquid–liquid extraction procedure followed by chromatographic resolution by HPLC with UV detection [2]. Another author reported using the same extraction procedure with electrochemical detection [3], but validation data was not provided. We have developed a method for the quantification of sematilide in rabbit plasma using solid-phase extraction microcolumns, eliminating evaporation and reconstitution steps which are time consuming, followed by reversed-phase HPLC, and provided validation data for a biological assay application.

2. Experimental

2.1. Materials

Sematilide hydrochloride, N-[2-(diethylamino) ethyl]-4-[(methylsulfonyl)amino]benzamide hydrochloride (Fig. 1), was provided by Berlex Laboratories (Lachine, Canada). The internal standard (I.S.) N-acetylprocainamide (NAPA) was purchased from Aldrich Chemicals (Milwaukee, WI, USA). The two standards were certified by the Health Protection Branch labora-

* Corresponding author.

SSDI 0378-4347(94)00261-3

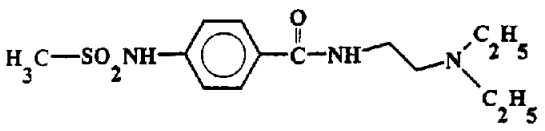

Fig. 1. Structure of sematilide.

tory using MS, IR, and NMR. Organic solvents (acetonitrile, methanol) were of high-purity grade (Baker, John's Scientific, Toronto, Canada). Triethylamine, sodium acetate (Fisher Scientific, Nepean, Canada), trifluoroacetic acid (Mallinckrodt, Montreal, Canada), glacial acetic acid, hydrochloric acid (Baker), sodium hydroxide (BDH Chemicals, Toronto, Canada) and boric acid (Baker) were reagent grade. The New Zealand white male rabbit plasma was obtained from Lampire Biologicals (Pipersville, PA, USA).

2.2. Preparation of standards

Sematilide (0.207 m*M*) stock solution and the I.S. solution (0.541 m*M*) were prepared in 0.1% glacial acetic acid (pH 3.5). Six working standard solutions (range from 1.276 to 31.91 μM) were prepared by diluting the stock solution with the same solvent. Working standard solutions were stable up to 3 months at +4°C. Standard curve samples were prepared by spiking 0.5 ml of blank rabbit plasma with 50 μl of the appropriate working standard solution of sematilide and 50 μl of the I.S. standard solution to achieve final concentrations ranging from 0.128 to 3.191 μM.

2.3. Calibration

On each analytical day, a set ($n = 6$) of standard curve samples was analyzed in duplicate along with each batch of samples. Calibration plots were constructed by log–log linear least-squares regression of the peak area ratio of sematilide to the I.S. versus sematilide plasma concentrations. The intra-day ($n = 6$) and inter-day ($n = 12$) precision and accuracy were evaluated at the low, medium and high drug concentrations, namely at 0.128 or 0.160, 0.798, and 3.191 μM. Inter-day assay variation was obtained over a one month period. Stability of in vitro samples was assessed in duplicate under several storage and processing conditions, and over a 6-month period.

2.4. Solid-phase extraction procedure

A 0.5 ml aliquot of rabbit plasma was placed in 13 × 100 mm glass tubes (Maple Leaf, John's Scientific, Toronto, Canada). A 50-μl aliquot of I.S. solution (75 ng) was added to all samples except the blank which received 50 μl of 0.1% acetic acid. A 50-μl aliquot of sematilide standard solution was added to standard curve and quality control samples. Boric acid buffer solution (0.5 ml, 0.4 *M*, pH 8.5) was added and the tubes were vortexed.

Disposable solid-phase extraction (SPE) columns (SPEC C_8, 15 mg, from Toxi-Lab, Irvine, CA, USA) were selected for the extraction procedure. Prior to application, the prefilters were removed and rinsed with boric acid buffer solution. The columns were mounted on a Lida vacuum manifold apparatus (Wennick Scientific, Ottawa, Canada). Each column was solvated with 1 ml of methanol followed by 1 ml of boric acid buffer. Upon reinstalling the pre-filters onto the columns, the plasma mixture was added to the column and aspirated through by slight vacuum (1.693 kPa). The column was washed with 0.5 ml of buffer using gentle vacuum. The prefilter was discarded and the column washed with 300 μl of 5% trifluoroacetic acid applying initially a slight vacuum and then a stronger vacuum (3.386 kPa) for 20 s to remove all traces of the wash solution. Analytes were eluted with 3 × 100 μl of a solution of 10% acetonitrile in 4% acetic acid using gentle vacuum. Eluates were collected in auto-sampler vials, (1.5 ml, 12 × 32 mm, Varian, Toronto, Canada), vortexed and transferred into a 200-μl flat-bottom glass inserts suitable for the autosampler vial and 50 μl was injected on the HPLC column.

2.5. Instrumentation and chromatographic conditions

The chromatographic system consisted of an SP8800 HPLC pump from Spectra Physics (Toronto, Canada), an SP8780 autosampler fitted with a 50-μl Rheodyne sample loop, an SP 4270 integrator and a Spectroflow 773 UV–VIS detector set at 254 nm (1 AUFS, 1 s). Chromatographic separation was achieved with a 5 μm Inertsil ODS-2 (150 × 4.6 mm I.D., CSC, Montreal, Canada) maintained at 35°C with a Waters (Mississauga, Canada) column heater. A precolumn μBondapack C_{18} insert from Waters was used. The mobile phase consisted of water–glacial acetic acid–acetonitrile–methanol–triethylamine (93.5:4.0:1.5:0.5:0.5, v/v/v/v/v) at a flow-rate of 2 ml/min. Data was collected on a 386SX33 microcomputer equipped with a SP ChromNet Data controller.

3. Results and discussion

3.1. Selection of the SPE columns

Disposable solid-phase extraction columns were selected due to their design having an extraction disk instead of a bedding, which allows for a bed mass of 1.5 to 30 mg compared to 100 to 1000 mg in conventional columns. It is owing to this small bed mass that the columns can be solvated, washed and finally eluted with small volumes, allowing for combined eluates to be directly injected onto the LC column. The advantages of this feature are the elimination of the evaporation and reconstitution steps usually required for concentrating the eluate, use of minimal volume of solvents, and reduction in time required to extract the samples.

3.2. Extraction recovery

The extraction recovery was determined by comparing peak areas from plasma extracts (n = 6) with those from directly injected solutions (n = 3) of the analyte made in the same eluent solvents. The percentage recovery was 71.7 + 6.4% and 71.5 + 2.8% for sematilide at 0.160 μM and 3.191 μM, respectively, and 77.6 ± 2.2% for the I.S. at 0.541 μM.

3.3. Assay validation

Fig. 2 shows typical chromatograms of a plasma sample spiked with 1.595 μM of sematilide, and of a rabbit's plasma sample containing 1.340 μM of sematilide. Retention times of 5.9 and 7.2 min correspond to sematilide and the I.S., respectively. Chromatograms from extracted blank rabbit plasma were free of interfering peaks.

Calibration curves for sematilide, using log–log linear least-squares regression analysis, were linear over the concentration range of 0.128–3.191 μM (n = 6), with a standard error of fit for individual deviations about the line ($S_{y.x}$) of 0.06 (indicating a 6% variation of peak area ratio responses about the regression line), a mean r^2 of 0.9971, a mean slope of 0.9983 (S.E. 0.0073)

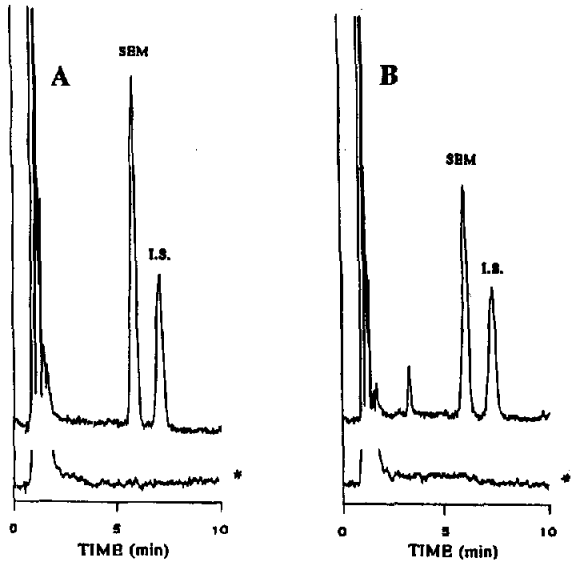

Fig. 2. Chromatograms of rabbit plasma extracts. (A, upper trace) Plasma spiked with 1.595 μM of sematilide (SEM), (B, upper trace) rabbit plasma sample containing 1.34 μM of SEM after intravenous administration, and blank plasma samples (A and B, lower trace). The amount of internal standard (I.S.) added to the sample was 75 ng. Experimental conditions as stated in the text.

Table 1
Intra-day assay precision and accuracy for sematilide in plasma ($n = 6$)

Actual concentration (μM)	Precision		Accuracy, mean deviation from nominal (%)
	Mean calculated concentration (μM)	R.S.D. (%)	
3.191	3.169	2.6	−0.7
1.595	1.575	4.5	−1.3
0.798	0.735	2.4	−7.9
0.128	0.126	3.8	−1.6

and a mean y-intercept of −5.99 (S.E. 0.039) over a one-month period.

The mean intra-day accuracy, defined as mean percent deviation from the nominal value, and the mean C.V. for precision are listed in Table 1. The inter-day accuracy and mean C.V. for precision obtained over a one-month period are presented in Table 2. The limit of quantitation determined from the standard curves was 0.144 μM (C.V. 8.98%), based on the 95% upper prediction limit of the predicted response Y (Y_i) at the lowest standard curve concentration (X_i) [4].

3.4. Stability

The stability of sematilide in plasma was determined with samples spiked at 0.160, 0.798 and 3.19 μM. Stability of the analyte was tested (1) for long term storage of plasma samples at −80°C over a 6-month period, (2) for unprocessed plasma samples at ambient temperature (20°C) for 24 h, (3) for run-time stability of processed samples left at ambient temperature (20°C) for 24 h before injection on the HPLC and (4) for 2 freeze–thaw cycles of spiked plasma samples. The stability was assessed by a comparison with duplicates of plasma samples freshly made with the analytes. Data are presented in Table 3.

No significant degradation of the analytes was observed for spiked rabbit plasma samples stored at −80°C over a 6-month period. In addition to the long-term stability study, the analytes were also stable under all the conditions listed above.

3.5. Biological application

The method has been applied successfully to the analysis of sematilide in rabbit plasma, and has allowed us to follow the pharmacokinetic disposition over several half-lives following an intravenous bolus of 3.0 mg/kg. Fig. 3 represents

Table 2
Inter-day assay precision and accuracy for sematilide in plasma ($n = 12$)

Actual concentration (μM)	Precision		Accuracy, mean deviation from nominal (%)
	Mean calculated concentration (μM)	R.S.D. (%)	
3.191	3.277	4.2	+2.7
0.798	0.779	6.0	−2.3
0.160	0.160	7.6	+0.3

Measurements were obtained over one month.

Table 3
Stability studies

Actual concentration (μM)	Accuracy (mean deviation from nominal) (%)($n = 2$)				
	Fresh reference sample day 0	Long term storage [a] (−80°C) 6 months	Plasma samples unprocessed [a] (20°C, 24 h)	Run-time samples processed [a] (20°C, 24 h)	Freeze–thaw [a] (2 cycles)
3.191	+1.8	−4.9	+4.1	+1.0	+1.9
0.798	+1.3	+6.7	−0.2	−3.9	−0.6
0.160	+9.1	+0.6	+1.2	−10.4	+9.2

[a] Comparison was made with fresh standards processed on each analytical day.

a typical concentration–time profile of sematilide in rabbits.

4. Conclusions

The procedure utilizes recent advancements made in the size of the bed mass of solid-phase extraction columns which allow small volumes of solvents to be used. These columns allow for injection of the eluent directly into the HPLC system, therefore eliminating the need for evaporation and reconstitution steps.

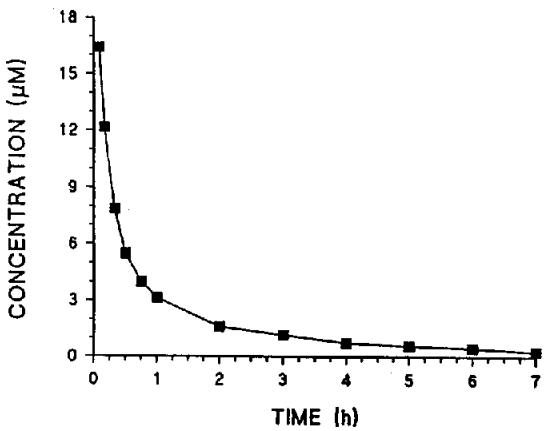

Fig. 3. Concentration versus time profile of sematilide in rabbit plasma following an IV administration of 3 mg/kg.

This analytical assay has been validated for rabbit plasma. The low limit of quantitation of 0.144 μM for sematilide (C.V. 8.98%, 0.5 ml of plasma) has allowed us to apply the method to pharmacokinetic studies. This method is simple, reproducible, sensitive and requires minimal solvent quantities and time.

Acknowledgements

We would like to thank Dr G.A. Neville and his colleagues, Bureau of Drug Research, Drugs Directorate, HPB Canada, for his Good Laboratory Practice certification of the standards. We would also like to thank G. Teoli for her help in the preparation of this manuscript.

References

[1] T.M. Argentieri, *Cardiovascular Drug Rev.*, 10 (2) (1992) 182–198.
[2] S. Dancik, T. Koziol, E. Nisperos and E. Woolf, *J. Pharm. Sci.*, 80 (1991) 157–159.
[3] P.H. Hinderling, C. Dilea, T. Koziol and G. Millington, *Drug Metab. Dispos.*, 21 (1993) 662–669.
[4] G.T. Wernimont, in W. Spendley (Editor), *Use of statistics to develop and evaluate analytical methods*, Association of Official Analytical Chemists, VA, USA, 1985.