ELSEVIER

Journal of Chromatography B, 662 (1994) 118–122

JOURNAL OF CHROMATOGRAPHY B: BIOMEDICAL APPLICATIONS

Short communication

Determination of plasma mexiletine levels with gas chromatography–mass spectrometry and selected-ion monitoring

M. Beth Minnigh[a], John D. Alvin[b], Michael A. Zemaitis[b,*]

[a]*Pharmakon R and D Laboratories, 3400 Forbes Ave., Pittsburgh, PA 15213, USA*
[b]*Department of Pharmacology, University of Pittsburgh, School of Dental Medicine, 633-1 Salk Hall, Pittsburgh, PA 15261, USA*

First received 13 June 1994; revised manuscript received 29 August 1994

Abstract

Mexiletine, 1-(2,6-dimethylphenoxy)-2-aminopropane (Mexitil), is an orally effective agent useful in the treatment of serious ventricular arrhythmias. This paper describes a gas chromatographic–mass spectrophotometric assay with selected-ion monitoring for the measurement of plasma or serum mexiletine levels. The drug and internal standard (*p*-chlorophenylalanine methyl ester) were extracted from plasma into ethyl acetate–hexane–methanol (60:40:1, v/v). After separation and evaporation of the organic phase, the drug and internal standard were derivatized to their pentafluoropropyl derivatives prior to analysis. The reproducibility of the daily standard curve yielded mean inter- and intra-day coefficients of variability from 0.7 to 11.0%. The coefficients of variability for control plasma samples (0.5 and 1.0 μg/ml) ranged from 2.6 to 5.0% and the accuracy of the assay was 106 ± 6 and 100 ± 5% for the low and high level controls respectively. The limit of quantitation for the assay was 0.1 μg/ml. No interfering peaks were detected in any patient samples. This method can be used as a primary analytical method to measure mexiletine plasma levels or can serve as a convenient back-up method to HPLC procedures when contaminating peaks coelute with mexiletine.

1. Introduction

Mexiletine, 1-(2,6-dimethylphenoxy)-2-aminopropane (Mexitil), is an orally effective agent useful in the treatment of serious ventricular arrhythmias. The therapeutic steady-state concentration for the drug is in the range 0.5–2.0 μg/ml [1,2]. Levels below 0.5 μg/ml indicate suboptimal dosing or patient noncompliance, while levels above 2.0 μg/ml may herald the onset of adverse side effects. During the past 20 years, multiple methods for the determination of mexiletine in plasma have been published. Several gas chromatographic methods have been published with drug analysis by flame ionization, electron capture or nitrogen-sensitive detection [3–9]. Moreover, several high-performance liquid chromatographic (HPLC) methods have been developed, primarily to simplify technology and extraction so that mexiletine plasma monitoring is a procedure within the instrumental capabilities of most clinical laboratories [10–15].

* Corresponding author.

0378-4347/94/$07.00
SSDI 0378-4347(94)00395-5

Although we routinely measure mexiletine levels with HPLC methodology, it has become apparent that, on occasion, HPLC methodology lacks specificity in certain patients. In these patients, compounds are extracted from the plasma sample that nearly co-elute with mexiletine, and thereby distort interpretation of the chromatogram and accurate quantitation. Although tedious mobile-phase changes can allow accurate quantitation, we have found that evaporation and derivatization of the final solution injected on the HPLC system and reinjection onto a GC–MS operated in the selected-ion monitoring (SIM) mode provided a sensitive and highly specific method to analyze mexiletine. The following report outlines the GC–MS procedure for mexiletine analysis and analyzes the accuracy and reliability of the procedure. A typical chromatogram from a patient on chronic mexiletine therapy is also presented.

2. Experimental

2.1. Chemicals and reagents

Mexiletine was provided by Boehringer Ingelheim Pharmaceuticals (Ridgefield, CT, USA) and *p*-chlorophenylalanine methyl ester (used as the internal standard) was purchased from Aldrich (Milwaukee, WI, USA). Pentafluoropropionic anhydride was purchased from Pierce (Rockford, IL, USA). All reagents and solvents used were of analytical grade and were purchased from Fisher Scientific (Pittsburgh, PA, USA).

2.2. Stock solutions

Stock solutions of mexiletine and *p*-chlorophenylalanine methyl ester (PCPAMe) of 1 mg/ml were prepared in methanol. These solutions were stored at −30°C and aliquots were diluted as needed to prepare plasma standards and for use as an internal standard spiking solution.

2.3. Standard solutions and samples

The methanol stock solution of mexiletine was diluted to 0.1 mg/ml (spiking solution A) or 0.4 mg/ml (spiking solution B) with methanol. To 10 ml of blank plasma (anticoagulated with CPDA-1 [citrate–phosphate–dextrose–adenine]) was added either 10, 20 or 40 μl of spiking solution A or 20, 40 or 80 μl of spiking solution B. The resulting standard plasma samples contained mexiletine concentrations of 0.1, 0.2, 0.4, 0.8, 1.6, and 3.2 μg/ml respectively. The stock solution of PCPAMe was diluted 1:50 to a final concentration of 20 μg/ml for addition to plasma as an internal standard. During one study, duplicate samples were prepared in plasma and serum to determine the applicability of the method to a different matrix.

Quality control samples at two concentrations (0.5 and 1.0 μg/ml) were prepared by addition of 10 or 20 μl of mexiletine stock solution to 20 ml of blank plasma. These solutions were divided into 0.5-ml aliquots and were stored at −30°C until use.

2.4. Instrumentation and conditions

The instrument used in these studies was a Hewlett-Packard 5890 gas chromatograph connected to a Hewlett-Packard 5971a mass-selective detector. All injections were done in the splitless mode with a dwell time of 0.7 min before the purge valve was turned on. The mass-selective detector was autotuned daily and the assays were run at 400 V above the autotune voltage in the selected-ion monitoring mode (SIM). The mass ions specific for the pentafluoropropyl derivatives of mexiletine (*m/z* 122, 204 and 325) and PCPAMe (*m/z* 125,196 and 359) were determined in the full-scan mode by injection of authentic derivatized standards. Subsequently these ions were entered into the SIM program for sample analysis.

Separation of the pentafluoropropyl derivatives of mexiletine and PCPAMe was accomplished with a Supelco SPB-5 capillary column (30 m × 0.25 mm I.D.; 0.25 μm film thickness).

Gas chromatograph conditions were: injection port temperature, 240°C; mass spectrometer interface temperature, 280°C; the GC oven temperature was programmed from 100°C (0.5 min hold) to 280°C (2.0 min hold) at a rate of 25°C/min. The carrier gas was helium; head pressure was 70 kPa, purge flow-rate was 30 ml/min. Under these conditions, the two compounds were clearly separated by approximately 0.4 min with absolute retention times in the range of 6–7 min depending on the actual column length at the time of assay (varies due to periodic column "chipping").

2.5. Sample preparation

To 0.5 ml of plasma was added 100 μl of NaOH (1.5 mol/l) and 25 μl of PCPAMe internal standard (20 μg/ml) in a 15-ml screw-top glass tube. The samples were mixed and shaken for 10 min with extraction solvent (ethyl acetate–hexane–methanol, 60:40:1, v/v). The sample was then centrifuged at 2000 *g* for 10 min at room temperature and the organic layer was transferred to a 5-ml conical screw-top glass tube. The solvent was then evaporated under a gentle stream of nitrogen at room temperature. A 50-μl volume of pentafluoropropionic anhydride (PFPA) was added and the tube was capped tightly and heated at 80°C for 15 min. The tube was allowed to cool to room temperature and the PFPA was evaporated under a gentle stream of nitrogen. The sample was reconstituted in 50 μl of methanol and a 1-μl aliquot was injected onto the GC system.

2.6. Calibration curves

Blank plasma was spiked with appropriate amounts of mexiletine to produce final concentrations of 0.1, 0.2, 0.4, 0.8, 1.6, and 3.2 μg/ml. These calibration standards along with unspiked plasma were then subjected to the procedure described above. For statistical analysis and validation of the procedure, the calibration standards were prepared in triplicate (from separately prepared stock solutions) and run each day for three days along with quality control samples. During analysis of patient samples, the standard curve was run only once per day.

2.7. Calculations

Calibration curves were constructed by plotting the abundance ratio of [*m/z* 204 (mexiletine-PFP)]/[*m/z* 196 (PCPAMe-PFP)] as a function of plasma mexiletine concentration. The slope and intercepts of the resulting line were calculated by least squares regression analysis, and these values were used to calculate the concentration of quality control samples (0.5, 1.0 μg/ml) and patient samples.

3. Results and discussion

The total-ion chromatograph (full-scan mode) for authentic mexiletine-PFP and PCPAMe-PFP is shown in Fig. 1. The corresponding mass spectra of each compound, their chemical structures and fragmentation patterns are shown in Fig. 2a,b. As can be seen from the chromatogram, each compound is well separated from the other with retention times of 6.3 and 6.7 min. These times depend on the exact column length; for the full-scan study, the column was practically new and was 30 m long. Each compound

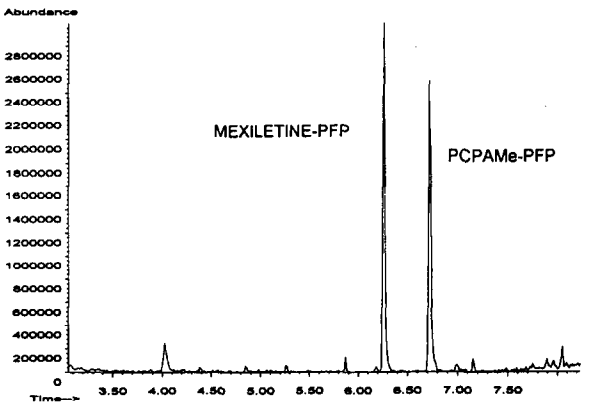

Fig. 1. The total-ion chromatogram (full scan) of authentic samples of mexiletine-PFP and *p*-chlorophenylalanine methyl ester-PFP (PCPAMe-PFP).

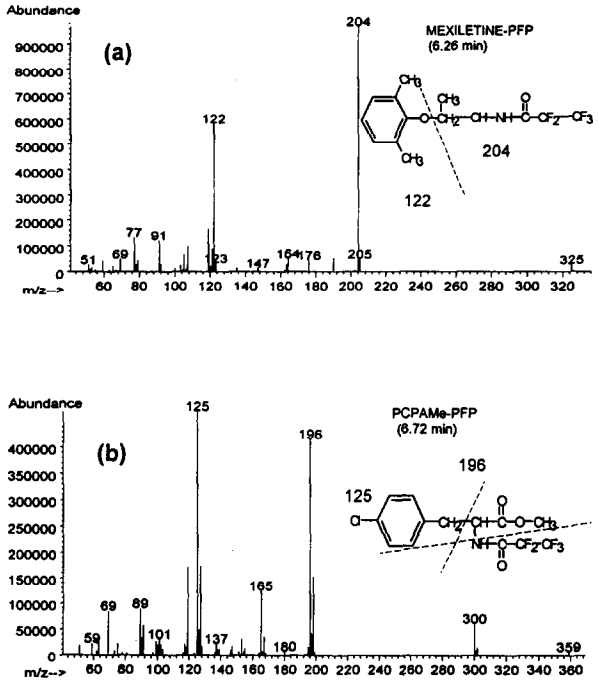

Fig. 2. Molecular structures and EI mass spectra of the pentafluoropropyl derivatives of (a) mexiletine and the internal standard (b) *p*-chlorophenylalanine methyl ester (PFPAMe).

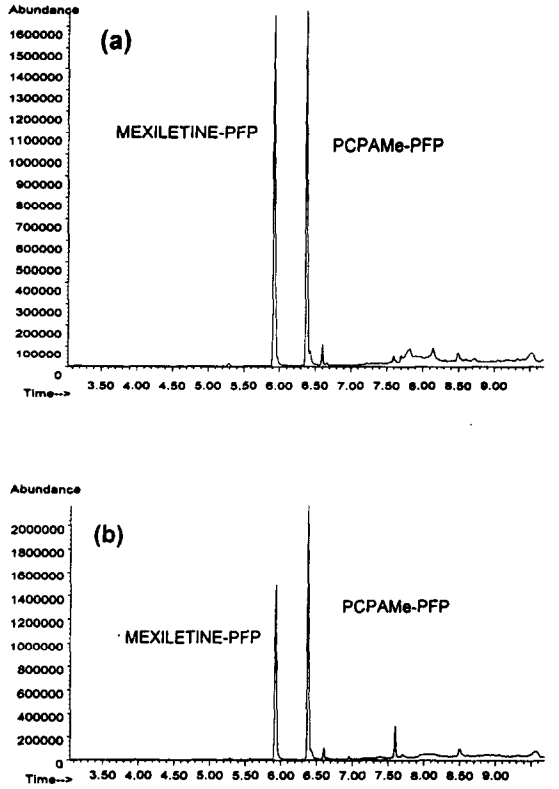

Fig. 3. The total-ion chromatogram (SIM mode) of mexiletine-PFP and *p*-chlorophenylalanine methyl ester-PFP (PCPAMe-PFP) extracted from human plasma: (a) seeded blank plasma, (b) from a patient taking mexiletine.

fragments into two major ions along with a small amount of parent ion. Mexiletine has prominent fragments at *m/z* 122 and 204, while PCPAMe-PFP has prominent fragments at *m/z* 125 and 196. For subsequent quantitative studies, the ratio of 204/196 was used to establish a standard curve and to calculate mexiletine concentrations in patient samples and quality control samples.

Typical total-ion chromatograms from mexiletine seeded plasma (1.0 μg/ml) and from plasma of a patient taking mexiletine (calculated concentration of 0.8 μg/ml) are shown in Fig. 3a,b. These chromatograms were generated with the mass spectrometer in the SIM mode as previously described. As is apparent from these tracings, this procedure is quite selective; no significant interference peaks were noted. This is true for virtually all patient samples analyzed by this procedure.

Extraction recovery was calculated by comparing the mexiletine/internal standard area ratio obtained from a pure mixture of 0.5 μg of mexiletine and 1.0 μg of internal standard with the ratio obtained upon extraction of 0.5 μg of mexiletine from plasma and its addition to 1.0 μg of internal standard. The procedure was reversed to analyze extraction of the internal standard. Recovery was calculated to be 93% for mexiletine and 82% for *p*-chlorophenylalanine methyl ester.

The validity of the assay procedure was established through experiments designed to study the accuracy and precision of the method. The six-point calibration curve was linear with a typical slope of 3.1 and *x*-intercepts of approximately zero ($r > 0.996$). The mean ($n = 3$) intra- and inter-day coefficients of variation for the calibration plasma samples of 0.1, 0.2, 0.4, 0.8, 1.6 and 3.2 μg/ml were: 11.0/10.3, 10.9/10.4, 9.7/2.7, 3.1/1.4 and 2.7/0.7%, respectively. The

accuracy of the assay was determined by calculating mexiletine concentrations in seeded plasma controls of 0.5 μg/ml and 1.0 μg/ml ($n = 9$). For the low control, the accuracy was 106 ± 6% (mean ± S.D.) and for the high control it was 100 ± 5% (mean ± S.D.). On the basis of the signal-to-noise ratio of the GC–MS and the lowest standard run, the limit of quantitation for the assay, as described, is 0.1 μg/ml. The limit of detection is well below 0.1 μg/ml; for single dose kinetic studies the limit of quantitation could be lowered by increasing the plasma volume extracted to 1 ml, decreasing the reconstitution volume, or increasing the injection volume.

The above method provides an accurate reproducible method to analyze the antiarrythmic agent mexiletine. Comparison of plasma and serum samples run together showed no difference in extraction or analytical results. It is useful as a simple primary method if sufficient mass spectrometer time is available in the laboratory. In addition, it can serve as a valuable back-up procedure for more commonly used HPLC procedures when interfering peaks are present. The remaining solution after injection onto the HPLC can be evaporated, derivatized, and injected in the GC–MS for verification and quantitation of mexiletine.

References

[1] L.F. Prescot, J.A. Clements and A. Pottage, *Postgrad. Med. J.*, 53 (1977) 50.
[2] N.P.S. Campbell, J.G. Kelly, A.A.J. Adgey and R.G. Shanks, *Br. J. Clin. Pharmacol.*, 6 (1978) 103.
[3] S. Willox and B.N. Singh, *J. Chromatogr.*, 128 (1976) 196.
[4] A. Frydman, J.-P. Lafarge, F. Vial, R. Rulliere and J.-M. Alexandre, *J. Chromatogr.*, 145 (1978) 401.
[5] D.W. Holt, R.J. Flanagan, A.M. Hayler and M. Loizou, *J. Chromatogr.*, 169 (1979) 295.
[6] K.J. Smith and P.J. Meffin, *J. Chromatogr.*, 181 (1980) 469.
[7] O. Groch-Belanger, *J. Chromatogr.*, 309 (1984) 165.
[8] D.F. LeGatt, R.J. Audette, O. Youzwishen and T. Stewart, *J. Pharm. Biomed. Anal.*, 9 (1991) 1147.
[9] S.G. Ji, Q.H., X.L. Li and P. Li. *Biomed. Chromatogr.*, 7 (1993) 196.
[10] R.K. Bhamra, R.J. Flanagan and D.W. Holt, *J. Chromatogr.*, 307 (1984) 439.
[11] O. Groch-Belanger, J. Turgeon and M. Gilbert, *J. Chromatogr.*, 337 (1985) 172.
[12] R. Kelly, D. Christmore, R. Smith, L. Doshier and S.L. Jacobs, *Ther. Drug Monit.*, 3 (1981) 279.
[13] B.K. Kramer, K.M. Ress, F. Mayer, V. Kuhlkamp, H.M. Liebich, T. Risler and L. Seipel, *J. Chromatogr.*, 493 (1989) 414.
[14] M.C. Contreras de Condado, A. Quintana de Gainzarain, I. Mendoza Mujica and J.A. Condado Rodriguez, *J. Chromatogr.*, 530 (1990) 164.
[15] E. vasBinder and T. Annesley, *Biomed. Chromatog.*, 5 (1991) 19.