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Gas chromatographic–mass spectrometric determination of serum mexiletine concentration after derivatization with perfluorooctanoyl chloride, a new derivative

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Abstract

Mexiletine is an antiarrhythmic agent used in the treatment of ventricular arrhythmia. The drug has a narrow therapeutic window which necessitates monitoring its serum concentrations. We describe a gas chromatographic–mass spectrometric analysis of mexiletine using selected ion monitoring. Mexiletine was extracted from alkaline serum with dichloromethane and then derivatized with perfluorooctanoyl chloride. The derivatization reaction was completed in 20 min at 80°C. We used N-propylamphetamine as the internal standard. The ions monitored were m/z 122, 454 and 575 for the derivatized mexiletine and m/z 91, 118, 440 and 452 for the derivatized internal standard. The within-run precision at a serum mexiletine concentration of 1 mg/l was 1.9% (mean=0.98, S.D.=0.019 mg/l, n=7) and the between-run precision was 2.5% (mean=0.99, S.D.=0.025 mg/l, n=7). The assay was linear for serum mexiletine concentrations of 0.2 to 4 mg/l. The detection limit was 0.1 mg/l. The average recoveries of mexiletine and the internal standard were 80% and 84%, respectively at a mexiletine concentration of 1 mg/l. There was no carry over problem in our assay. We observed a good correlation between mexiletine concentrations measured by a reference laboratory (GC) and by our new GC–MS assay.

Keywords: Mexiletine; Perfluorooctanoyl chloride

1. Introduction

Mexiletine, 1-(2,6-dimethylphenoxy)-2-aminopropane is an antiarrhythmic agent used in the treatment of ventricular arrhythmia [1]. It is available in the form of hydrochloride salt, a single dose ranges from 50–400 mg and a daily dose up to 1500 mg can be prescribed. The bioavailability of mexiletine is 80–90% by the oral route. Peak plasma concentration occurs 1–4 h after ingestion [2]. The therapeutic range is 0.7–2.0 mg/l in plasma and the elimination half life is 9–12 h. The major urinary metabolites are *p*-hydroxymexiletine and hydroxymethylmexiletine, both of which undergo further metabolism to alcohols [3]. The monitoring of serum mexiletine concentration is essential because mexiletine concentrations over 2 mg/l are associated

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with a high incidence of side effect including hypotension, bradycardia, dizziness, blurred vision, tremor and confusion [4,5]. Four deaths have been reported in adults who acutely ingested 4–8 g of mexiletine [6,7].

Serum mexiletine concentration can be determined by high-performance liquid chromatography (HPLC) [8,9]. However, occasionally HPLC method lacks specificity in certain patients. In these patients compounds are extracted from the plasma samples that nearly coelute with mexiletine, and thereby distort interpretation of chromatogram and accurate quantitation [10]. Gas chromatographic procedures have utilized flame ionization, nitrogen–phosphorus and electron-capture detection [11–15]. Gas chromatography–mass spectrometry (GC–MS) using selected ion monitoring is a sensitive and highly specific method for analysis of mexiletine after extraction from serum and derivatization with pentafluoropropionic anhydride [10]. Kempton et al. [16] described a GC–MS method for mexiletine without derivatization. Rohrig and Harty [17] also described analysis of mexiletine in postmortem blood without derivatization. However, the molecular mass of mexiletine is only 179 and in the electron ionization (EI) mass spectrum of mexiletine the base peak was observed at m/z 44 with another strong peak at m/z 58. A relatively weak molecular ion was observed at m/z 179.

Because mexiletine was involved in several fatal overdoses, and serum mexiletine concentrations are also determined in forensic toxicology laboratories [16,17], a specific GC–MS assay for mexiletine would be useful. For medical–legal investigation, unambiguous structural identification of the compound is essential. GC–MS is considered as the gold standard for unambiguous identification of abused drugs in forensic toxicology laboratories. Therefore, a specific reference method using GC–MS for identification and quantitation of mexiletine in serum should complement existing HPLC and GC methods for routine analysis of mexiletine. In this report we describe a novel derivatization of mexiletine using perfluorooctanoyl chloride. The molecular mass of the perfluorooctanoyl derivative of mexiletine was 575. The derivative is less volatile than the parent compound and the assay is free from interferences from more volatile components in serum. In addition, unambiguous identification of mexiletine as the perfluorooctanoyl derivative can be easily achieved by observing characteristic ions in the higher molecular mass range. The base peak was observed at m/z 454.

2. Materials and methods

Mexiletine was obtained from Boehringer while the internal standard N-propylamphetamine was obtained from Altech Applied Science (College Park, PA, USA). The derivatizing agent perfluorooctanoyl chloride was obtained from PCR Chemicals (Gainesville, FL, USA). HPLC grade dichloromethane, the extraction solvent, sodium tetraborate decahydrate were obtained from Aldrich (Milwaukee, WI, USA). A stock solution of mexiletine (1 mg/ml) was prepared in dichloromethane and another stock solution of N-propylamphetamine, the internal standard (0.1 mg/ml) was prepared in methanol.

To extract mexiletine from serum (supplemented with various concentrations of mexiletine or patient's serum), we supplemented 1 ml of serum with 10 μl of the internal standard solution. Then 1 ml of borate buffer (pH 9.8) was added. The borate buffer was prepared by dissolving 20 g of sodium tetraborate decahydrate in 1 liter of deionized water. Mexiletine, along with the internal standard were extracted with 10 ml of dichloromethane. The sample was mixed in a rotating mixer for 15 min. After centrifuging for 5 min at 1500 *g*, the upper aqueous layer was discarded and the lower organic layer was transferred to a conical test tube and the organic phase was evaporated under nitrogen almost to dryness. Then 50 μl of the derivatizing agent (perfluorooctanoyl chloride) was added to the remaining organic phase. The reaction mixture was incubated at 80°C for 20 min. Then the organic phase was almost evaporated to dryness. The residue was reconstituted with 50 μl of ethyl acetate and the organic phase was further concentrated to approximately to half of the volume. We injected 2–3 μl of the reconstituted organic phase onto the GC–MS system.

The GC–EI-MS analysis was carried out using a Model 5890 gas chromatograph, with an Ultra-1 capillary column (25 m×0.2 mm) coupled with a 5970 series mass selective detector (Hewlett-Pac-

kard, Palo Alto, CA, USA). The Ultra-1 column was crosslinked with methyl silicone gum with a film thickness of 0.33 μm. The GC–chemical ionization (CI)-MS analysis was carried out using a Model 5890 series II gas chromatograph coupled to a 5972 series mass-selective detector (Hewlett-Packard). We used methane as the ionizing gas for CI mass spectrometric analysis.

The initial oven temperature for GC–MS analysis was 175°C. After maintaining that temperature for 5 min, the oven temperature was increased at a rate of 20°C/min to a final oven temperature of 300°C, which was maintained for an additional 2 min. The total run time was 13.25 min. Split less injection with an injector port temperature of 250°C was used. The carrier gas was helium with a column flow-rate of 0.29 ml/min and a linear velocity of 21 cm/s. In order to obtain full scan EI mass spectra a scan range of m/z 40–600 was used. However, for obtaining full scan CI mass spectra a scan range of m/z 50–600 was used in order to avoid a strong m/z 42 peak which could originate from ionized methane gas. The source pressure during CI was $1.9 \cdot 10^{-4}$ Torr (1 Torr=133.322 Pa). Because most clinical laboratories are equipped with an EI mass spectrometer only, further analysis of linearity, detection limit, precision and comparison of patient's specimens containing mexiletine were done using EI-MS. Selected ion monitoring with ions monitored at m/z 91, 118, 122, 440, 454, 482 and 575 were used. The ions m/z 122, 454 and 575 were originated from the derivatized mexiletine and the ions at m/z 91, 118, 440 and 482 from the derivatized internal standard.

3. Results and discussion

3.1. Chromatographic properties of derivatized mexiletine and the internal standard

Since the precedent had been set by other investigators, dichloromethane was also used for extraction of mexiletine in this study [8]. We observed baseline separation between perfluorooctanoyl derivative of mexiletine (retention time: 8.6 min) and the internal standard, N-propylamphetamine (retention time: 8.1 min). We also observed excellent peak shapes for both compounds and using selected ion monitoring we observed clean chromatograms. A typical ion chromatogram of a patient receiving mexiletine was given in Fig. 1. The reaction condition for derivatization showed quantitative yields for both mexiletine and the internal standard, because we did not observe any peak in the total ion chromatogram (using scan mode) for unreacted mexiletine or the internal standard.

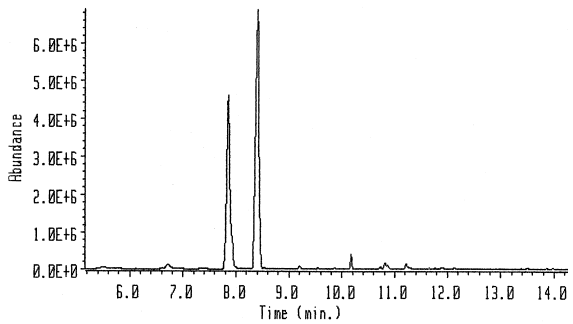

Fig. 1. Total ion chromatogram analysis of a patient sample containing 1.4 mg/l of mexiletine. The first peak was the derivatized internal standard (retention time 8.1 min) and the second peak was the derivatized mexiletine (retention time 8.6 min).

3.2. Mass spectral characteristics of derivatized mexiletine and the internal standard

Mexiletine is a small molecule with a molecular mass of 179. In this derivatizing technique the molecular mass of the perfluorooctanoyl derivative of mexiletine was 575. In the EI mass spectrum of the perfluorooctanoyl derivative of mexiletine we observed a weak molecular ion at m/z 575 (relative abundance: 1.5%). The base peak was observed at m/z 454 due to the loss of phenoxy moiety from the derivatized molecule. Another strong peak was observed at m/z 122 (relative abundance: 46.6%) due to the phenoxy group. The observation of two strong peaks at m/z 454 and 122 certainly aid in unambiguous identification of mexiletine as the perfluorooctanoyl derivative. In contrast, underivatized mexiletine showed strong peaks at m/z 44 and 58 only. The proposed fragmentation pattern using EI was given in Fig. 2 while the mass spectrum was given in Fig. 3. The molecular ion in the EI mass spectrum was relatively weak. In order to further confirm the

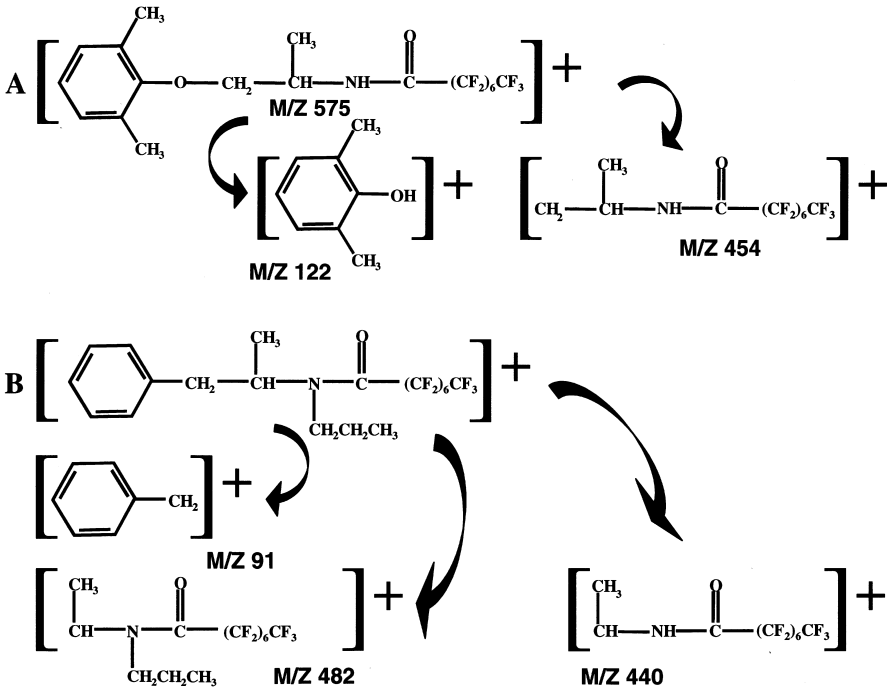

Fig. 2. Proposed fragmentation pattern (using electron ionization) of the perfluorooctanoyl derivative of (A) mexiletine, (B) N-propylamphetamine.

structure of the perfluorooctanoyl derivative of mexiletine, we studied the CI mass spectrum. We observed a strong protonated molecular ion at m/z 576 (relative abundance: 13.2%), thus aiding in characterization of the compound. Another strong peak was also observed at m/z 454 (relative abundance: 36.3%). The base peak was observed at m/z 123 (Fig. 4).

We did not observe a molecular ion in the EI mass spectrum of perfluorooctanoyl derivative of N-propylamphetamine, the internal standard. However, the base peak was observed at m/z 482 and another strong characteristic peak was observed at m/z 440 (relative abundance: 75.6%). As expected, we also observed two other characteristic peaks at m/z 118 (relative abundance: 20.4%) and m/z 91 (relative abundance: 27.8%). The proposed fragmentation pattern using EI is given in Fig. 2 and the mass spectrum of the derivatized internal standard is given in Fig. 5. Because no molecular ion was observed, we studied the CI mass spectrum of perfluorooctanoyl derivative of N-propylamphetamine. A protonated molecular ion at m/z 574 (relative abundance: 2.3%) was observed, thus aiding in unambiguous characterization of the compound. The base peak was observed at m/z 119 (Fig. 6).

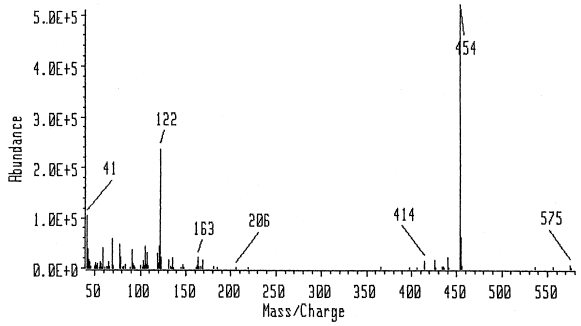

Fig. 3. Electron ionization full scan mass spectrum of perfluorooctanoyl derivative of mexiletine.

3.3. Precision, linearity and detection limit

The within- and between-run precisions of the mexiletine assay were determined by using a serum standard containing 1 mg/l of mexiletine. The

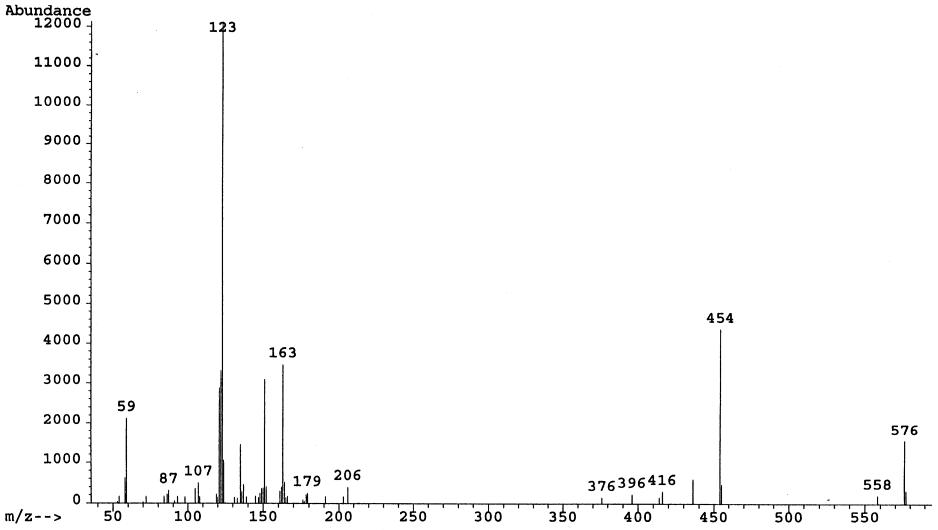

Fig. 4. Chemical ionization full scan mass spectrum of perfluorooctanoyl derivative of mexiletine.

within-run precision was 1.9% (mean=0.98, S.D.= 0.019 mg/l, $n=7$). The corresponding between-run precision was 2.5% (mean=0.99, S.D.=0.025 mg/l, $n=7$). The assay was linear for serum mexiletine concentration of 0.2 to 4 mg/l. Using the x-axis as the target mexiletine concentration and the y-axis as the observed mexiletine concentration, we observed the following regression equation:

$$y = 0.96x + 0.08 \quad (r = 0.99)$$

The detection limit of the assay was a serum mexiletine concentration of 0.1 mg/l.

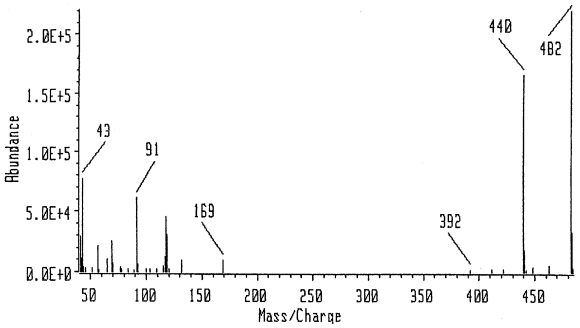

Fig. 5. Electron ionization full scan mass spectrum of perfluorooctanoyl derivative of N-propylamphetamine, the internal standard.

3.4. Carry over

In order to investigate carry over problem in our GC–MS assay, we injected 2 μl of ethyl acetate after analyzing serum standard containing 4 mg/l of mexiletine. We observed a flat baseline in the total ion chromatogram and observed no peaks. Therefore, we concluded that our GC–MS assay was free from carry over problem.

3.5. Recovery

The average recovery of mexiletine was 80% at a serum mexiletine concentration of 1 mg/l. The average recovery of the internal standard also at a concentration of 1 mg/l was 84.0%.

3.6. Comparison of the methods

In order to validate our method we compared mexiletine concentrations in six patients receiving mexiletine obtained by a reference laboratory (Associated Regional University Pathologists, Salt Lake City, UT, USA) and our GC–MS assay (Table 1). The reference laboratory uses GC with nitrogen–phosphorus detection for analysis of mexiletine. The

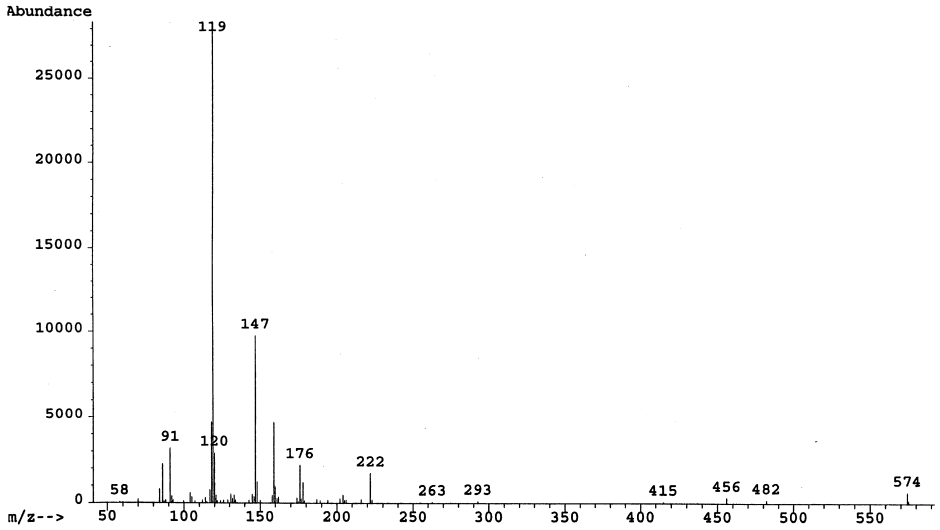

Fig. 6. Chemical ionization full scan mass spectrum of perfluorooctanoyl derivative of N-propylamphetamine, the internal standard.

Table 1
Mexiletine concentrations obtained in sera of six patients by a reference laboratory and GC–MS assay

Patient ID	Mexiletine concentration (mg/l)	
	Reference laboratory (GC)	GC–MS
1	0.6	0.5
2	0.9	0.9
3	1.0	1.2
4	1.1	1.1
5	0.5	0.6
6	1.5	1.4

protocol also uses N-propylamphetamine as the internal standard. Mexiletine along with the internal standard were extracted from alkaline serum (borate buffer was used) using ethyl acetate. The organic extract was directly injected into the GC system without derivatization.

Using the *x*-axis as the mexiletine concentration obtained by the reference laboratory and *y*-axis as the concentration obtained by our GC–MS assay, we observed the following regression equation:

$$y = 0.92x + 0.09 \quad (r = 0.95)$$

Therefore, we conclude that our GC–MS assay for mexiletine compared well with the GC method used in the reference laboratory.

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