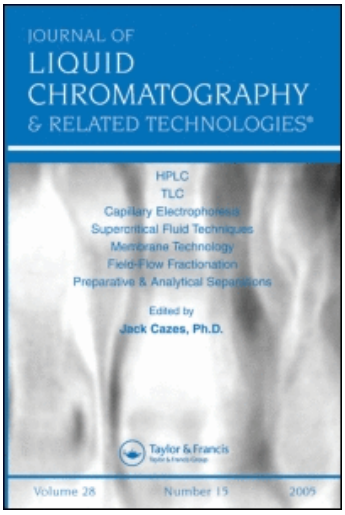

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A Modified Reverse-Phase HPLC Method for the Analysis of Mexiletine Hydrochloride

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ABSTRACT

Mexiletine hydrochloride is an orally active class I antiarrythmic agent. The purpose of this work is to develop a sensitive, selective, and stability indicating reverse phase HPLC assay for the quality control analysis and stability testing of mexiletine hydrochloride dosage forms. The method was developed to quantify the drug for various formulations. Analysis was carried out on a 4 mm × 150 mm C-18 microsorb column, using a 50 : 50 methanol : 0.053 M sodium acetate buffer. The pH of the mobile phase was adjusted to 4.8. The flow rate was set at 1 mL/min and the analysis was carried out at 254 nm. The internal standard used in the analysis was thiamine hydrochloride. The drug eluted at about 8 min, while the internal standard eluted at about 3 min. The stability of the assay was studied by subjecting the drug to extreme pH conditions. The limit of quantification (LOQ) and limit of detection (LOD) were found to be

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0.06 μg and 0.02 μg, respectively. The reproducibility of the method was tested by carrying out multiple injections on the same day, as well as, on five consecutive days. The coefficient of variation for the intra-day and inter-day studies was found to be 0.81% and 1.47%, respectively.

Key Words: Mexiletine HCL; HPLC assay; Stability indicating.

INTRODUCTION

Mexiletine hydrochloride is a class IB orally active antiarrthymic agent. Chemically it is 1-methyl-2-(2,6-xylyloxy)-ethylamine hydrochloride[1]. Mexiletine hydrochloride has been shown to be effective in the suppression of induced ventricular arrhythmias, including those induced by glycoside toxicity and coronary artery ligation. It is available as 150 mg, 200 mg, and 250 mg capsules. It is not available in a liquid dosage form. The USP/NF suggests a reverse phase HPLC assay method for the analysis of mexiletine hydrochloride using a 60 : 40 mixture of methanol : sodium acetate buffer[2]. This method, however, provides too low of a retention time for mexiletine hydrochloride, namely, 1 min. Due to the short retention time, there is a possibility of the solvent peaks overlapping with the drug peak[3]. Hence, an attempt was made to achieve a longer and reasonable retention time for the drug[4–6]. The main purpose for this study is to develop a stability indicating reverse phase HPLC assay method for mexiletine hydrochloride, which could be used to analyze the extemporaneously formulated dosage form.

EXPERIMENTAL

Chemicals and Reagents

Mexiletine hydrochloride, (1-[2,6-xylyloxy]-2-aminopropane), Lot No. 66H0668, Sigma-Aldrich Chemical, St. Louis, MO. Thiamine hydrochloride, Lot No. 3410, Nutritional Biochemicals Corporation, Cleveland, OH. Sodium acetate, Certified A.C.S, Fused Anhydrous, Lot No. 007024, Fisher Scientific, Fairlawn, NJ. Hydrochloric acid, 38% (w/v), A.C.S, Chempure™ Brand, Lot No. M152KPHA, Curtin Matheson Scientific, Houston, TX. Acetic acid, Glacial, Lot No. K08815, J. T. Baker, Phillipsburg, NJ. Methanol, HPLC grade, (U.V. cutoff 205 nm), Lot No. 011868, Fischer Scientific, Fairlawn, NJ.

Instrumentation

The HPLC system consisted of: two 501 Waters HPLC pumps with a 20 μL loop injector; Waters 486 tunable UV detector; and a Waters 712 WISP autosampler. Data was acquired and processed using the Millenium® (Version 2.1) software obtained from Waters. The separation was carried out using a Microsorb C-18 column, 4 × 150 mm with a particle size of 100A.

High Performance Liquid Chromatographic Parameters

The wavelength for detection was set to 254 nm. The mobile phase was a 50 : 50 mixture of sodium acetate buffer (0.053 M) : methanol mixture. The flow rate of the mobile phase was set to 1 mL/min. The mobile phase was degassed using inert helium gas, and filtered through a 45 μm FP Vericel® Membrane Filter, HPLC certified, Lot No. 2092010 supplied by Gelman Sciences, Ann Arbor, MI, with the help of a Millipore Filter Holder, part #4, obtained from Millipore Filter Corporation, Bedford, MA. The sample was filtered through a 0.45 μm nylon syringe filter, Lot No. 0001962045. In each run, 20 μL of the sample was injected.

Calibration Curve for the High Performance Liquid Chromatography Assay Method

The stock solution was prepared by dissolving 1.502 g of mexiletine hydrochloride in 100 mL of water, giving a 15.02 mg/mL solution. The stock solution was subsequently diluted to obtain concentrations of 1.502 mg/mL, 3.004 mg/mL, 4.506 mg/mL, 6.008 mg/mL, 7.510 mg/mL, 9.012 mg/mL, and 10.514 mg/mL of mexiletine hydrochloride, respectively. The internal standard solution was prepared by dissolving 1.132 g of thiamine hydrochloride in 1000 mL of the mobile phase. A 1 mL sample of each dilution was spiked with 100 μL of the internal standard solution and injected. Each dilution was injected three times. The mean peak area for the three runs was calculated. The ratio $(\text{area-under-the-curve})_{\text{drug}}/(\text{area-under-the-curve})_{\text{internal standard}}$ was computed for each dilution. The ratio of the peak areas was used as a marker to assure the same instrument performance on a daily basis. The peak areas were plotted against their respective concentrations for the five dilutions and a calibration curve was obtained. A linear regression analysis was carried out on the calibration curve. The R^2 and the equation for the line were calculated, using Beer's Law.

In order to test the inter-day variation for the method, five replicate injections of the same dilution were performed over a period of five days.

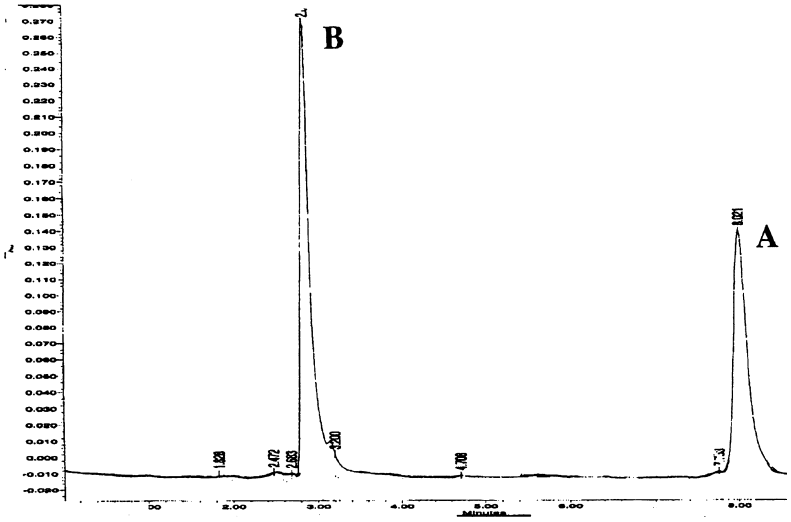

Figure 1. Chromatogram showing peaks for mexiletine hydrochloride (Peak A) retention time of 8.201 and the internal standard thiamine hydrochloride (Peak B) with a retention time of 2.951.

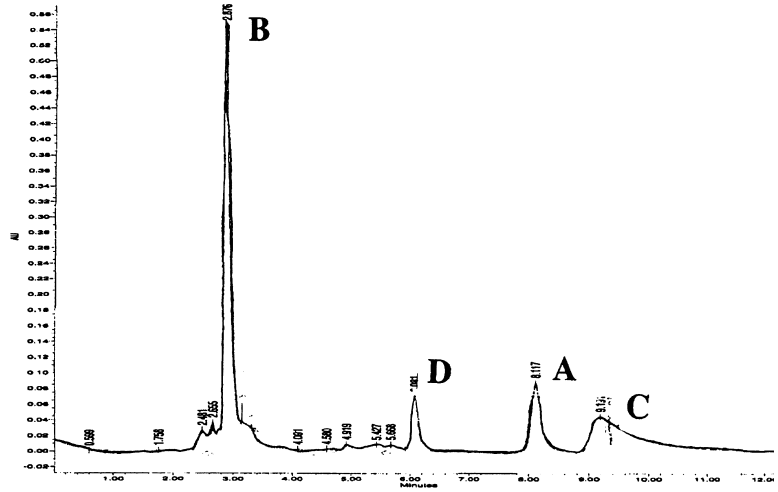

Figure 2. Chromatogram showing peaks for mexiletine hydrochloride (Peak A) retention time of 8.117, its degradation products obtained on treatment with HCl (Peak C and D) retention times of 9.138 and 6.081, respectively, and the internal standard thiamine hydrochloride (Peak B) with a retention time of 2.876.

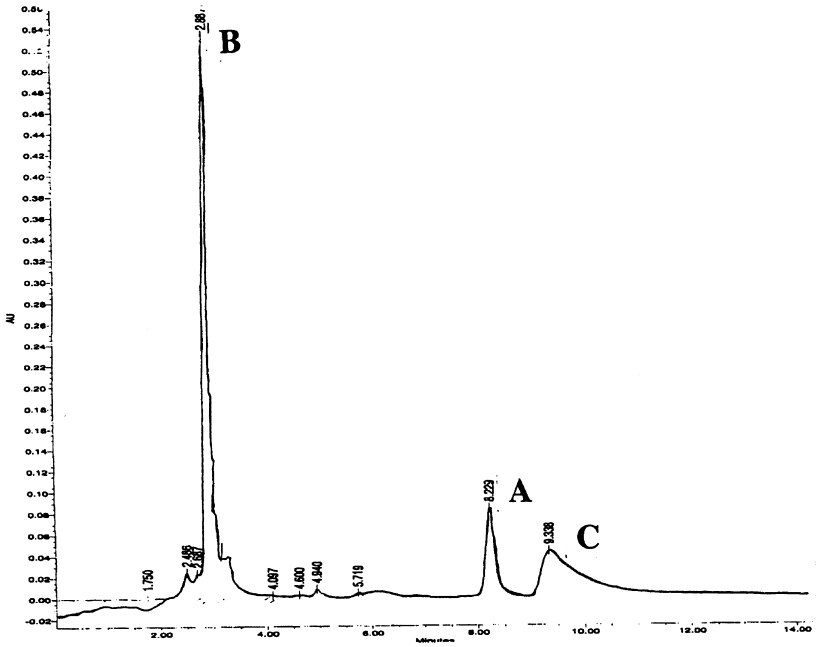

Figure 3. Chromatogram showing peaks for mexiletine hydrochloride (Peak A) retention time of 8.229, its degradation product obtained on treatment with NaOH (Peak C) retention time of 9.338, and the internal standard thiamine hydrochloride (Peak B) with a retention time of 2.887.

The peak area obtained for each run was divided by the area obtained for the internal standard and the ratio $(\text{area-under-the-curve})_{\text{drug}}/(\text{area-under-the-curve})_{\text{internal standard}}$. The relative standard deviation between the peak areas was determined.

According to the USP, the lowest concentration for which the percentage relative standard deviation (RSD) of multiple injections is <5.0% is the limit

Table 1. Data showing intra-day variation for peak areas of mexiletine hydrochloride.

Peak areas	Standard deviation between peak areas ($n = 3$)	Coefficient of variation
4,199,808 4,187,338 4,135,719	33978.97	0.81%

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Table 2. Mexiletine hydrochloride concentrations vs. peak areas to establish the Beer's curve.

Concentration of mexiletine hydrochloride (μg/mL)	Peak area 1	Peak area 2	Peak area 3	Mean peak area of the drug	Mean peak area of internal standard	Ratio of peak areas
30.04	1,406,478	1,356,195	1,304,461	1355711.3	1,949,041	0.696
60.08	2,992,930	2,977,859	2,889,762	2,953,517	2,018,047	1.464
90.12	4,199,808	4,187,338	4,135,719	4174288.3	1,926,586	2.167
120.16	5,764,438	5,629,977	5,762,388	5718934.3	1,934,801	2.956
150.2	7,253,080	7,076,575	6,898,002	6215148.3	1,957,849	3.614
180.24	8,519,886	8,269,108	8,580,807	8456600.3	2,146,801	3.939
210.28	10,067,059	9,980,420	9,981,341	10009606.6	1,986,164	5.040

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of quantification (LOQ). The limit of detection (LOD) value, by convention is taken to be $0.3 \times \text{LOQ}$[5]. The LOD and LOQ were accordingly calculated.

Stability Indicating Nature of the Assay

The stock solution of mexiletine hydrochloride was subjected to degradation under acidic and basic conditions. A 15 mL sample of the mexiletine hydrochloride stock solution was treated with 0.87 M HCL solution, prepared by adding 8.3 mL of 38% (w/v) hydrochloric acid and the volume was made up to 100 mL with reverse osmosis water. The solution was heated for 30 min, cooled, and neutralized with 100 mL of 1 M NaOH solution obtained by dissolving 4 g of sodium hydroxide in 100 mL of reverse osmosis water. The solution was injected into the column. Simultaneously, a 15 mL sample of the mexiletine hydrochloride stock solution was treated with 1 M NaOH solution, prepared by dissolving 4 g of sodium hydroxide in 100 mL of RO water. The solution was heated for 30 min, cooled, and neutralized with 0.87 M HCl. It was filtered and then injected into the column. The acid and base treated solutions were spiked with the internal standard and reinjected into the column.

RESULTS AND DISCUSSION

Stability Indicating Reverse-Phase High Performance Liquid Chromatography Assay Method

A typical chromatogram for the sample containing pure mexiletine hydrochloride and thiamine hydrochloride (internal standard) is provided in Fig. 1.

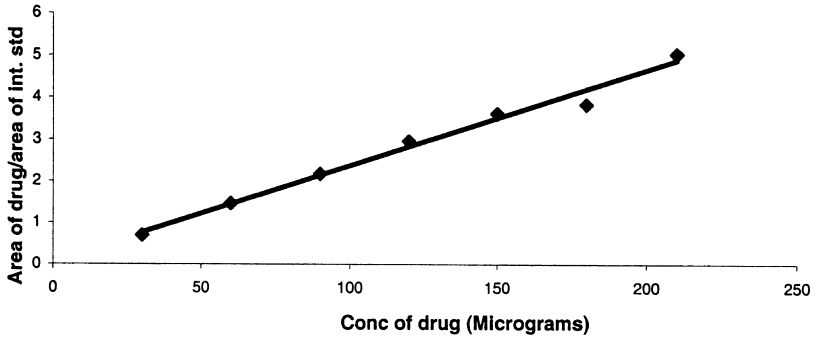

Figure 4. Calibration plot establishing linear relationship between the concentration of the drug and peak area, with a slope = 0.0229; intercept = 0.0775 and $R^2 = 0.9861$.

Table 3. Inter-day variation for peak areas of mexiletine hydrochloride.

Concentration of mexiletine hydrochloride (μg/mL)	Day 1	Day 2	Day 3	Day 4	Day 5	Standard deviation between peak areas	Coefficient of variation
90.12	4,199,808	4,240,749	4,317,169	4,167,277	4,083,116	61753.85	1.47%
	4,187,338	4,218,319	4,227,578	4,194,108	4,162,834		
	4,135,719	4,270,256	4,221,587	4,193,457	4,128,388		
	4,235,761	4,178,241	4,225,896	4,190,178	4,053,582		
	4,167,989	4,200,564	4,235,687	4,224,477	4,057,292		

The retention times for mexiletine hydrochloride (Peak A) and thiamine hydrochloride (Peak B) were about 8 min and about 3 min, respectively. The peaks are well separated and resolved. Under these conditions there is no overlap with the drug or the internal standard peak. An experiment to show the stability indicating nature of the assay was also performed. This was done in order to determine if the degradation products under these conditions overlap with the drug or the internal standard peak. On treatment of the drug with an acid (HCl), a degradation peak was seen at 6.081 min (Fig. 2), which is well resolved from the drug and internal standard peaks. Alkaline degradation of the drug resulted in an additional peak at 9.338 min (Fig. 3), which is again well resolved from the drug and internal standard peaks.

Intra-Day and Inter-Day Studies

Three replicate injections for each dilution were carried out on the same day, in order to test the intra-day variance and linearity of the assay. The coefficient of variation for the peak areas was determined to be 0.81% (Table 1). The calibration plot showed a good linear relationship between the peak areas and the concentration of the drug, with a R^2-value of 0.9861 (Table 2 and Fig. 4). The coefficient of variation between the peak areas for the inter-day studies was determined to be 1.47% (Table 3). The LOD and LOQ for this assay were calculated to be 0.0002 μg and 0.06 μg, respectively (Table 3).

CONCLUSIONS

A modified stability indicating method for mexiletine hydrochloride was developed, which gave a longer retention time of 8 min for the drug as compared to an extremely short retention time of 1 min as reported in the USP. The method was found to be linear, reproducible, and stability indicating.

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