

DETERMINATION OF MEXILETINE IN PHARMACEUTICAL PREPARATIONS BY GAS CHROMATOGRAPHY WITH FLAME IONIZATION DETECTION

Bilal YILMAZ^{a,*}, Vedat AKBA^b

^aDepartment of Analytical Chemistry, Faculty of Pharmacy, Ataturk University, 25240, Erzurum, Turkey

^bCriminal Police Laboratory, 25060, Erzurum, Turkey

*E-mail: bilalylmaz@yahoo.com

ABSTRACT

A simple and rapid method for determination of mexiletine in pharmaceutical preparations was developed and validated using gas chromatography with flame ionization detection (GC-FID). GC separation was performed in about 5 min using a HP-5 capillary column (30 m x 0.320 mm, 0.25 μ m). Nitrogen was used as carrier gas at a flow-rate of 2 mL min⁻¹. After injection of the sample at inlet temperature 250 °C, the temperature of GC oven was as follows: initial temperature was 150 °C, held for 1 min, increased to 180 °C at 40 °C min⁻¹ held for 1 min, and finally to 300 °C at 30 °C min⁻¹ with a final hold of 1.5 min. Calibration curve was linear between the concentration range 2.0-14 μ g mL⁻¹. The method was found to be specific, precise and accurate. The method was applied for the quality control of commercial mexiletine capsule form to quantify the drug and to check the formulation content uniformity.

Keywords: Mexiletine, GC-FID, Pharmaceutical preparation, Validation

INTRODUCTION

Arrhythmias, commonly observed as atrial fibrillation, atrial flutter, atrial tachycardia, ventricular tachycardia and premature beats, are the consequences of abnormal autorhythmicity or conduction disturbance of heart. Generally, antiarrhythmic drugs therapy is preferred for patients with cardiac arrhythmia [1-3].

Mexiletine (Figure 1), is an antiarrhythmic agent used in the treatment of ventricular arrhythmia [4]. 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 [5].

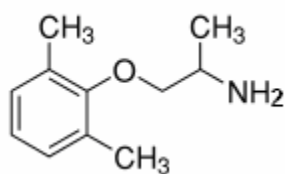


Figure 1. Chemical structure of mexiletine.

Several methods have been reported for determination of mexiletine including high-performance liquid chromatography (HPLC) [6-14], LC-MS-MS [15] and gas chromatography-mass spectrophotometry (GC-MS) [16-18] in plasma and other biological fluids. The USPXXIII [19] describes a reverse phase HPLC method for its assay while the BP 93 [20] describes a non aqueous titration method.

To our knowledge, there is no GC-FID method for determination of mexiletine in pharmaceutical preparations in literature. Therefore, we wanted to develop a new GC-FID method for determination of mexiletine in pharmaceutical preparations without the necessity of

sample pre-treatment. Also, the developed method was applied to commercial preparation as capsule.

MATERIALS AND METHODS

Chemicals and reagents

Mexiletine HCl (99.6% purity) was obtained by Eczacıbaşı Pharmaceutical Industry (Istanbul, Turkey). Acetonitrile (HPLC grade) was purchased from Fluka (Buchs, Switzerland), and other chemicals and solvents used were of analytical grade. Mexilit capsule containing 200 mg mexiletine was obtained by pharmacy (Erzurum, Turkey).

Instrumentation

The GC-FID method was performed an Agilent 6890 N Network GC equipped with a flame ionization detector, Agilent 7683 series autosampler, Agilent chemstation and HP-5 column with 0.25 μ m film thickness (30 m x 0.320 mm I.D.). Injection and detector temperature are 250 and 300 °C, respectively. The carries gas (N₂) flow-rate was kept constant during the run at 2 mL min⁻¹. Nitrogen (30 mL min⁻¹), hydrogen (35 mL min⁻¹) and synthetic air (350 mL min⁻¹) were used as auxiliary gases for the flame ionization detector. All gases were supplied by Havas (Ankara, Turkey).

Preparations of the standard and quality control solutions

The stock standard solution of mexiletine was prepared in acetonitrile to a concentration of 100 μ g mL⁻¹ and kept stored at -20 °C in dark glass flasks. Working standard solutions were prepared from the stock standard solutions. A calibration graph was constructed in the range of 2, 4, 6, 8, 10, 12 and 14 μ g mL⁻¹ for mexiletine (n=6). For quality control samples containing concentration 3, 5, 7 μ g mL⁻¹ of mexiletine, the stock solution was diluted with acetonitrile.

Procedure for pharmaceutical preparation

The average capsule mass was calculated from the mass of capsules of Mexitil (200 mg mexiletine capsule, which was composed of mexiletine and some excipients). They were then finely ground, homogenized and portion of the powder was weighed accurately, transferred into a 10 mL brown measuring flask and diluted to scale with acetonitrile. The mixture was sonicated for at least 10 min to aid dissolution and then filtered through a Whatman 42 paper. An appropriate volume of filtrate was diluted further with acetonitrile so that the concentration of mexiletine in the final solution was within the working range and then analyzed by GC-FID.

RESULTS AND DISCUSSION

Method development and optimization

Mexiletine is a polar molecule so a capillary column coated with 5% phenyl and 95% dimethylpolysiloxane was used separation. During method development, the injection port and detector temperatures were set to 250°C and 300°C, respectively. Different temperature programs were investigated to give an optimum temperature program as follows; initial temperature was 150°C, held for 1 min, increased to 180°C at 40°C min⁻¹ held for 1 min, and finally to 300°C at 30°C min⁻¹ with a final hold of 1.5 min. The injector volume was 2 µL in splitless mode.

The retention time of mexiletine was approximately 3.87 min with good peak shape. No further optimisation of the method was required. Additionally, preliminary precision and linearity studies performed during the development of the method showed that the 2 µL injection volume was reproducible and the peak response was significant at the analytical concentration chosen. Typical chromatograms obtained with standard mexiletine and capsules are presented in Figures 2 & 3.

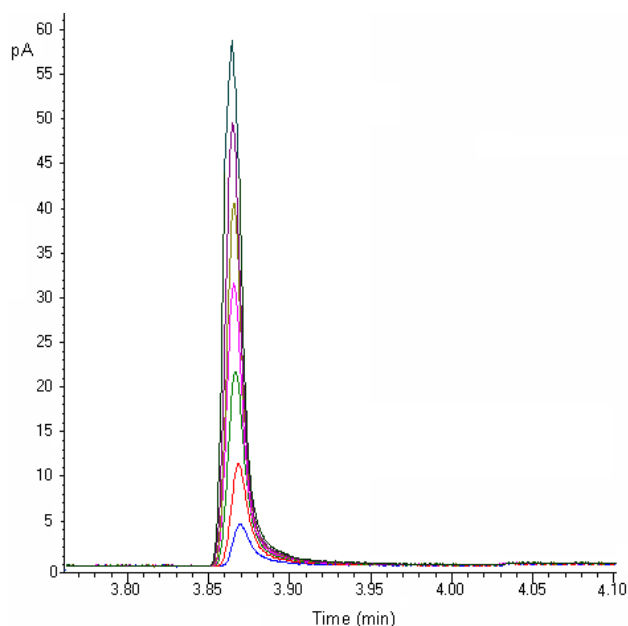


Figure 2. GC-FID chromatogram of mexiletine (2, 4, 6, 8, 10, 12 and 14 µg mL⁻¹).

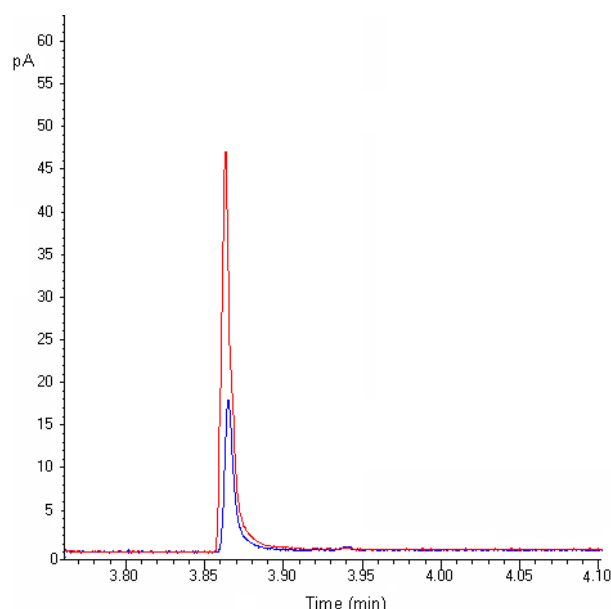


Figure 3. GC-FID chromatogram of Mexitil capsule containing mexiletine (5 and 12 µg mL⁻¹).

Method validation

Linearity

The linearity of peak area response versus concentration for mexiletine was studied over concentration range of 2-14 µg mL⁻¹. The calibration curve constructed was evaluated by its correlation coefficient. The correlation coefficients (*r*) of all the calibration curves were consistently greater than 0.999. Standard deviations of the slope and intercept for the calibration curves were 0.324 and 1.264, respectively (Table 1).

Precision and accuracy

The precision of GC-FID method was determined by repeatability (within-day) and intermediate precision (between-day). Three different concentrations which were quality control samples (3, 7, 11 µg mL⁻¹) were analyzed six time in one day for within-day precision and once daily for three days for between-day precision. The RSD values for within-day precision was ≤3.42% and for between-day precision was ≤3.29%. The bias values for within-day accuracy was ≤3.00% and for between-day accuracy was ≤2.45%. These data are summarised in Table 2.

Recovery

To determine the accuracy of the proposed method and to study the interference of formulation additives, the recovery was checked as three different concentration levels (2, 6, 10 µg mL⁻¹) and analytical recovery experiments were performed by adding known amount of pure drugs to pre-analyzed samples of commercial dosage forms. The percent analytical recovery values were calculated by comparing concentration obtained from the spiked samples with actual added concentrations. These values are also listed in Table 3.

Table 1. Linearity of of mexiletine by GC-FID method.

Methods	Range ($\mu\text{g mL}^{-1}$)	LR ^a	Sa	Sb	R	LOD	LOQ
GC-FID	2.0-14	$y=2.417x+5.842$	1.264	0.324	0.9996	0.30	1.50

^aBased on three calibration curves, LR:Linear regression, Sa: Standard deviation of intercept of regression line, Sb: Standard deviation of slope of regression line, R: Coefficient of correlation, y: peak-area, x: mexiletine concentration ($\mu\text{g mL}^{-1}$), LOD: Limit of detection, LOQ: Limit of quantitation

Table 2. Precision and accuracy of mexiletine by GC-FID method.

Method	Added ($\mu\text{g mL}^{-1}$)	Within-day			Between-day		
		Found \pm SD ($\mu\text{g mL}^{-1}$)	Accuracy	Precision RSD% ^a	Found \pm SD ($\mu\text{g mL}^{-1}$)	Accuracy	Precision RSD% ^a
GC-FID	3	2.91 \pm 0.052	-3.00	1.79	3.05 \pm 0.070	1.67	2.29
	7	7.08 \pm 0.242	1.14	3.42	7.11 \pm 0.234	1.57	3.29
	11	10.92 \pm 0.184	-0.73	1.68	10.73 \pm 0.246	-2.45	2.29

SD: Standard deviation of six replicate determinations, R.S.D: Relative standard derivation, ^aAverage of six replicate determinations, Accuracy: (%relative error) (found-added)/added \times 100

Table 3. Recovery values of mexiletine in pharmaceutical preparation.

Commercial preparation		Mexitil capsule ($2 \mu\text{g mL}^{-1}$)		
Method	Added ($\mu\text{g mL}^{-1}$)	Found \pm SD ($\mu\text{g mL}^{-1}$)	Recovery (%)	RSD ^a (%)
GC-FID	2	2.03 \pm 0.055	101.5	2.71
	6	6.07 \pm 0.184	101.2	3.03
	10	10.14 \pm 0.201	101.4	1.98

SD:Standard deviation of six replicate determinations, RSD: Relative standard derivation, ^aAverage of six replicate determinations

Table 4. Stability of mexiletine in solution.

Stability (%)	Room temperature stability (Recovery % \pm SD)		Refrigeratory stability, 4°C (Recovery % \pm SD)		Frozen stability, - 20°C (Recovery % \pm SD)	
	24 h	72 h	24 h	72 h	24 h	72 h
Added ($\mu\text{g mL}^{-1}$)						
3	99.3 \pm 0.584	99.2 \pm 0.058	98.4 \pm 0.746	101.3 \pm 1.426	98.6 \pm 0.176	101.1 \pm 1.626
8	101.4 \pm 0.152	102.1 \pm 0.522	101.2 \pm 0.043	99.4 \pm 0.064	101.8 \pm 4.430	98.2 \pm 0.462
14	98.2 \pm 0.042	98.5 \pm 0.530	97.9 \pm 0.075	102.8 \pm 0.246	103.8 \pm 0.680	98.1 \pm 0.671

Table 5. Determination of mexiletine in capsules containing mexiletine.

Commercial preparation	Method	% Recovery \pm SD	Confidence limits	P value	F-test
Mexitil capsule (200 mg)	Official method (BP 93) (Titration method)	100.09 \pm 0.760	200.19 \pm 0.690	0.342	$F_c=1.34$ $F_t=3.00$
	Official method (USPXXIII) (HPLC)	99.40 \pm 0.399	198.8 \pm 0.275		
	GC-FID	101.4 \pm 4.234	202.8 \pm 3.243		

Limit of detection and quantitation

The limit of detection (LOD) and limit of quantitation (LOQ) were determined by an empirical method that consisted of analyzing a series of standard solutions containing decreased amounts of mexiletine. LOD and LOQ were evaluated by serial dilutions of mexiletine stock solutions in order to obtain signal to noise ratios of

3:1 for LOD and 10:1 for LOQ. The LOD and LOQ values for analyte were found to be 0.30 and 1.50 $\mu\text{g mL}^{-1}$, respectively (Table 1).

Stability

To evaluate the stability of mexiletine, standard solutions were prepared separately at concentrations covering the

low, medium and higher ranges of calibration curve for different temperature and times. These solutions were stored at room temperature, refrigeratory (4⁰C) and frozen (-20⁰C) temperature for 24 h and 72h. Stability measurements were carried out with GC-FID method. The results were evaluated comparing these measurements with those of standards and expressed as percentage deviation and mexiletine was found as stable at room temperature, 4 and -20⁰C for at least 72h (Table 4).

Comparison of the methods

A survey of literature reveals that no GC-FID method for determination of mexiletine in pharmaceutical preparations. The present work describes the validation parameters stated by the CPMP/ICH guideline [21] to achieve GC-FID method for determination of mexiletine.

The proposed method is very effective for the assay of mexiletine in capsules. The validity of the proposed method was presented by recovery studies using the standard addition method. For this purpose, a known amount of reference drug was spiked to formulated capsules and the nominal value of drug was estimated by the proposed methods. Each level was repeated six times. The results were reproducible with low SD and RSD. No interference from the common excipients was observed.

The proposed method was applied to commercially available capsules. The results were compared with those obtained by USPXXIII [19] and BP 1993 [20] official methods. Statistical comparisons in terms of F-test for these methods were given in the Table 5. There are no significant differences between the proposed methods and the official methods with respect to the mean values and standard deviations at a 95% confidence level.

CONCLUSION

The present study describes a simple, rapid, sensitive, reliable, specific, accurate and precise GC-FID method for determination of mexiletine in pharmaceutical preparations. The method described in the present report has been effectively and efficiently used to analyze mexiletine pharmaceutical dosage form without any interference from the pharmaceutical excipients. Therefore, this validated GC-FID method can be used for the routine quality control analysis of the investigated drug in pharmaceutical preparations.

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