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Research Article

DETERMINATION OF MEXILETINE IN PHARMACEUTICAL PREPARATIONS BY ZERO-, FIRST- AND SECOND-ORDER DERIVATIVE SPECTROPHOTOMETRIC METHODS

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ABSTRACT

Mexiletine is an orally effective agent useful in the treatment of serious ventricular arrhythmias. This paper describes zero-, first- and second-order derivative spectrophotometric methods for determination of mexiletine in pharmaceutical preparations. The solutions of the standard and pharmaceutical samples were prepared in acetonitrile. Absorbances of mexiletine were measured at 262 nm for zero-order by measuring height of peak from zero, at 260, 268 and 274 nm for first and 272 and 277 for second-order derivative spectrophotometric method by measuring peak to peak height. The linearity ranges were found to be 20-140 µg mL^{-1} for the zero, first- and second-order derivative spectrophotometric methods. The developed methods in this study are accurate, sensitive, precise, and reproducible and can be directly and easily applied to the pharmaceutical preparation. Also, the results obtained from three derivative spectrophotometric methods were compared and no significant difference was found statistically.

Keywords: Mexiletine, Zero-, First-, Second-order Derivative Spectrophotometric Method, Pharmaceutical Preparation.

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, antiarrhythic drugs therapy is preferred for patients with cardiac arrhythmia[1-3]. Mexiletine (Figure 1), 1-(2,6-dimethylphenoxy)-2-amino-propane, 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].

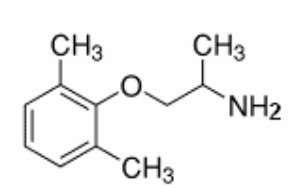

Figure 1: Chemical structure of mexiletine

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. To our knowledge, there is no derivative spectroscopic method for determination of mexiletine in pharmaceutical preparation in literature. Derivative spectrophotometry is an analytical technique of great utility for extracting both qualitative and quantitative information from spectra composed of unresolved bands, and for eliminating the effect of baseline shifts and baseline tilts. It consists of calculating and plotting one of the mathematical derivatives of a spectral curve[19]. In the last year, this technique has been rapidly gained its application in the analysis of pharmaceutical preparations.

We wanted to develop new spectrophotmetric methods for determination of mexiletine in pharmaceutical preparation without the necessity of sample pre-treatment. After developing zero-, first- and second-order derivative spectrophotometric methods were also carried out and all optimization parameters were also considered. Also, the developed methods were applied to commercial preparation as capsule.

MATERIALS AND METHODS

Chemicals

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. Mexitil capsule containing 200 mg mexiletine was obtained by pharmacy (Erzurum, Turkey).

Instrument

A Thermospectronic double-beam UV-Visible spectrophotometer (HEλIOSβ) with the local control software was used. Zero-, first- and second- derivative spectra of reference and sample solutions were recorded in 1 cm quartz cells at a scan speed of 600 nm min^{-1}, a scan range of 240-300 nm and fixed slit width of 2 nm.

Preparations of the standard and quality control solutions

The stock standard solution of mexiletine was prepared in acetonitrile to a concentration of 1000 μg 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 20, 40, 60, 80, 100, 120 and 140 μg mL^{-1} for mexiletine (n=6). For quality control samples containing concentration 30, 70, 110 μg mL^{-1} of mexiletine, the stock solution was diluted with acetonitrile.

Procedure for pharmaceutical preparations

The average capsule mass was calculated from the mass of capsules of Mexitil (200 mg mexiletine tablet, 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 100 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. Approximate dilutions were made at concentrations of 60 and 120 μg mL^{-1} with acetonitrile. Zero-, first- and second-order derivative spectra were recorded against acetonitrile.

Data analysis

All statistical calculations were performed with the Statistical Product and Service Solutions (SPSS) for Windows, version 10.0. Correlations were considered statistically significant if calculated P values were 0.05 or less.

RESULTS AND DISCUSSION

Method development

The derivative wavelength difference (Δλ) depends on the measuring wavelength range and n values (smoothing factor). Generally, the noise decreases by increasing Δλ. Optimal wavelength range should be chosen since the broad peaks become sharper, the ratio of signal/noise elevates and the sensitivity of the method increases by controlling the degree of low pass filtering or smoothing. Therefore, a series of *n* values (*n*=1-9) were tested in the first and second-order derivative spectra of mexiletine in acetonitrile. Optimum results were obtained in the measuring wavelength range 240-300 nm, *n*=5 (Δλ=17.5 nm) for first- and second- order derivative spectrophotometric methods.

Figure 2a presents the overlay of UV spectra of mexiletine in acetonitrile gives two characteristic maxima at 262 and 268 nm. These two shouldered peaks were separated by using derivative spectrophotometer. Figures 2b,c presents the overlay of first and second-order ultraviolet spectra of mexiletine standard samples in acetonitrile, respectively. As demonstrated in the Figure 2b, the spectra present characteristic a maxima and two minima. Maxima are represented at 260 nm and minima are shown at 268 and 274 nm. As demonstrated in the Figure 2c, the spectra present characteristic a maxima and a minimum are shown at 272 and 277 nm.

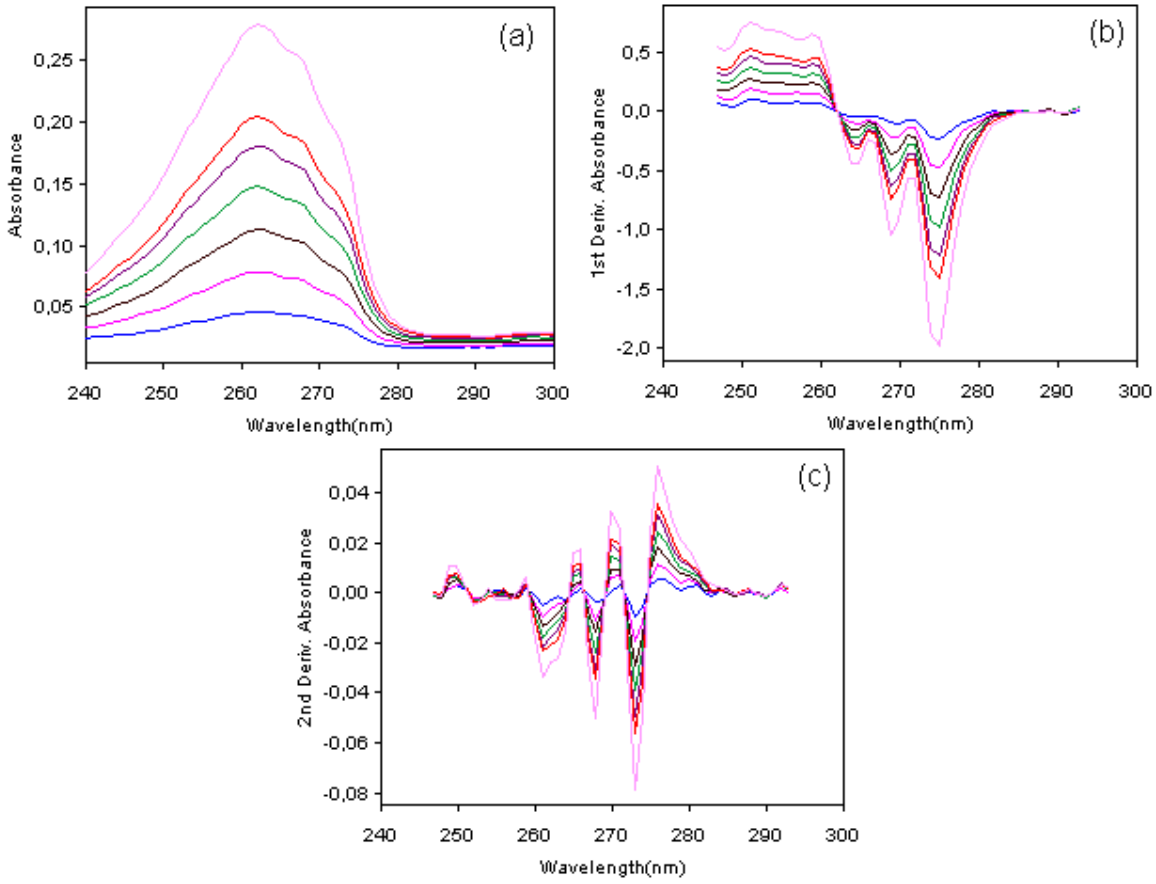

Figure 2: Spectrum of obtaining calibration graph point:
(a) Zero-order derivative spectrum of standard solution of mexiletine
(b) First-order derivative spectrum of standard solution of mexiletine
(c) Second-order derivative spectrum of standard solution of mexiletine

As no difference was observed between spectra of mexiletine standard and capsule solutions and in the maximum wavelengths of all spectra, it was suggested that the developed methods allowed complete elimination of the background absorption due to the capsule excipients at the chosen wavelengths both in zero-, first- and second-order derivative spectra of mexiletine (Figures 3a-c).

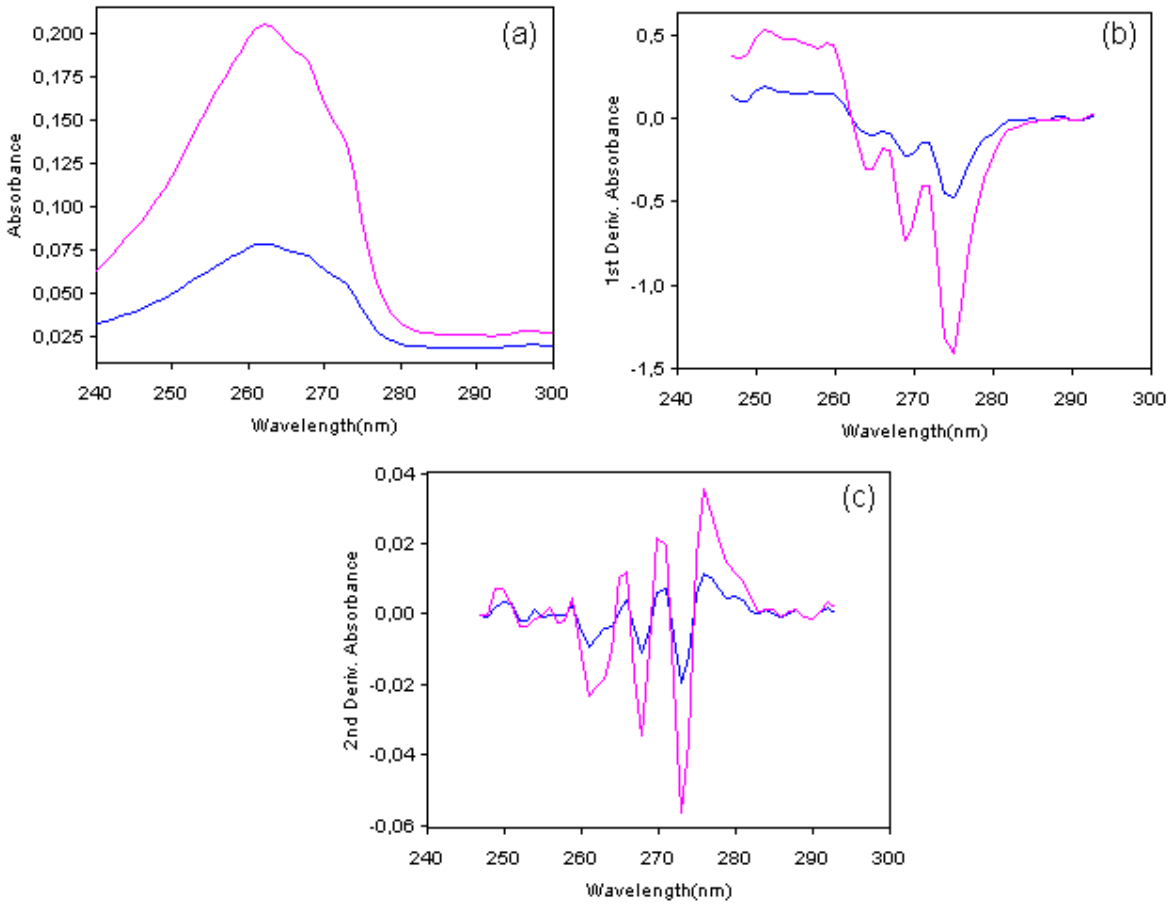

Figure 3: Spectrum of solutions of Mexitil capsule containing mexiletine (60 and 120 (µg mL^{-1}):

(a) Zero-order derivative spectrum
(b) First-order derivative spectrum
(c) Second-order derivative spectrum

Method validation

Linearity

For quantitative analysis of mexiletine, the calibration curves were plotted for each spectrophotometric method over the concentration ranges cited. The peak to zero method for calibration curve in the first- and second-order derivative spectrophotometric methods was

used. The linearity ranges of all spectrophotometric methods were found to be 20-140 µg mL^{-1}. The statistical parameters and regression equations which were calculated from the calibration curves along with the standard error of the slope and the intercept are given in Table 1.

Table 1: Results of regression analysis of mexiletine by the proposed methods

Methods	Range (µg mL^{-1})	LR[a]	Sa	Sb	R	LOD	LOQ
Zero-order Spectrophotometric Method	20-140	$A_{262\ nm}$=0.154x+0.084	0.027	0.004	0.9996	5.00	15.15
First-order Spectrophotometric Method	20-140	$1D_{260\ nm}$=0.442x+0.032	0.075	0.009	0.9988	6.00	18.18
	20-140	$1D_{268\ nm}$=0.268x-0.125	0.040	0.009	0.9974	6.00	18.18
	20-140	$1D_{274\ nm}$=1.034x-0.485	0.143	0.017	0.9997	5.00	15.15
Second-order Spectrophotometric Method	20-140	$2D_{272\ nm}$=0.016x-0.006	0.004	0.002	0.9964	6.00	18.18
	20-140	$2D_{277\ nm}$=0.031x-0.014	0.005	0.007	0.9939	5.00	15.15

λ: Wavelength, [a]Based on six 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, x: mexiletine concentration (µg mL^{-1}), LOD: Limit of detection, LOQ: Limit of quantitation, A: Absorbance, 1D: First-order absorbance, 2D: Second-order absorbance

Limits of detection (LOD) and quantitation (LOQ)

The LOD and LOQ of mexiletine by the proposed methods were determined using calibration standards. LOD and LOQ values were calculated as 3.3 σ/S and 10 σ/S, respectively, where S is the slope of the calibration curve and σ is the standard deviation of y-intercept of regression equation (n=6)[19] (Table 1).

Specificity

Comparison of the zero-, first- and second-order derivative spectrum of mexiletine in standard and drug formulation (Mexitil capsule) solutions show that the wavelength of maximum and minimum absorbance did not changed (Figures 3a-c). According to the results obtained, the zero-, first- and second-order derivative spectrophotometric methods are able to access mexiletine in presence of excipients and hence, methods can be considered specific.

Accuracy and precision

The precision of the analytic methods were determined by repeatability (within-day) and intermediate precision (between-day). Three different concentrations which were quality control samples (30, 70, 110 µg mL^{-1}) were analyzed six time in one day for within-day precision and once daily for three days for between-day precision. Repeatability was ≤2.99%, ≤2.31% and ≤3.77% (n=6) and intermediate precision was ≤3.09%, ≤3.22% and ≤4.29% (n=6) for zero-, first- and second-order derivative spectrophotometric methods, respectively (Table 2). Accuracy of zero-, first- and second order derivative spectrophotometric methods showed acceptable relative error values were ≤2.57%, ≤2.67% and ≤2.45% (n=6), respectively (Table 2).

Table 2: Precision and accuracy of mexiletine by the proposed methods

Method	λ (nm)	Added (µg mL^{-1})	Within-day			Between-day		
			Found±S.D (µg mL^{-1})	Accuracy	Precision R.S.D%[a]	Found±SD (µg mL^{-1})	Accuracy	Precision R.S.D%[a]
Zero-order Spectrophotometric Method	$A_{262\ nm}$	30	29.6±0.57	-1.33	1.93	30.3±0.68	1.00	2.24
		70	71.3±2.13	1.86	2.99	71.8±2.22	2.57	3.09
		110	108.8±1.71	-1.10	1.57	108.3±2.26	-1.55	2.09
First-order Spectrophotometric Method	$1D_{260\ nm}$	30	30.5±0.56	1.67	1.84	30.4±0.65	1.33	2.14
		70	70.7±1.51	1.00	2.14	69.7±2.07	-0.43	2.97
		110	109.5±2.14	-0.45	1.95	111.6±2.32	1.45	2.08
	$1D_{268\ nm}$	30	30.6±0.38	2.00	1.24	30.8±0.71	2.67	2.31
		70	70.1±1.62	0.14	2.31	71.2±2.29	1.71	3.22
		110	110.9±0.55	0.82	0.49	112.1±2.45	1.91	2.19
	$1D_{274\ nm}$	30	30.6±0.39	2.0	1.27	30.5± 0.72	1.67	2.36
		70	70.8±0.32	1.14	0.92	71.4±1.94	2.00	2.72
		110	110.7±1.02	0.64	0.97	111.6±1.43	1.45	1.28
Second-order Spectrophotometric Method	$2D_{272\ nm}$	30	30.8±0.08	2.67	0.26	30.7±0.97	2.33	3.16
		70	71.1±1.34	1.57	1.88	71.2±2.08	1.71	2.92
		110	112.6±4.25	2.36	3.77	112.7±4.84	2.45	4.29
	$2D_{277\ nm}$	30	30.5±0.44	1.67	1.44	30.6±0.86	2.00	2.81
		70	71.6±1.62	2.29	2.26	71.2±2.47	1.71	3.47
		110	112.1±2.25	1.91	2.01	112.1±3.42	1.91	3.05

S.D : Standard deviation of six replicate determinations, R.S.D: Relative standard derivation, [a]Average of six replicate determinations, Accuracy: (%relative error) (found-added)/addedx100

Recovery

To determine the accuracy of the zero-, first- and second-order derivative spectrophotometric methods and to study the interference of formulation additives, the recovery was checked as three different concentration levels (20, 60, 100 µg mL^{-1}) and analytical recovery experiments were performed by adding known amount of pure drugs to pre-analyzed samples of commercial dosage form (Mexitil). The percent analytical recovery values were calculated by comparing concentration obtained from the spiked samples with actual added concentrations. The recoveries of zero-, first- and second-order derivative spectrophotometric methods were between 101.5%-102.4%, 98.6%-101.0% and 97.0%-101.0 (Table 3).

Table 3: Recovery values of mexiletine in pharmaceutical preparation.

Commercial preparation			Mexitil capsule (20 µg mL^{-1})		
Method	λ (nm)	Added (µg mL^{-1})	Found±S.D (µg mL^{-1})	Recovery (%)	R.S.D [a] (%)
Zero-order Spectrophotometric Method	$A_{262\ nm}$	20	20.3±0.41	101.5	2.02
		60	61.2±1.56	102.0	2.55
		100	102.4±1.94	102.4	1.89
First-order Spectrophotometric Method	$1D_{260\ nm}$	20	19.8±0.34	99.0	1.72
		60	59.9±1.47	99.8	2.45
		100	98.6±2.14	98.6	2.17
	$1D_{268\ nm}$	20	19.8±0.39	99.0	1.97
		60	59.9±1.66	99.8	2.77
		100	98.7±2.22	98.7	2.25
	$1D_{274\ nm}$	20	20.2±0.69	101.0	3.42
		60	59.7±1.24	99.5	2.08
		100	100.6±2.72	100.6	2.70
Second-order Spectrophotometric Method	$2D_{272\ nm}$	20	19.4±0.64	97.0	3.29
		60	59.6±1.85	99.3	3.10
		100	99.7±3.89	99.7	3.90
	$2D_{277\ nm}$	20	20.2±0.88	101.0	4.36
		60	60.3±2.29	100.5	3.79
		100	98.9±3.47	98.9	3.51

S.D :Standard deviation of six replicate determinations, R.S.D: Relative standard derivation,
[a]Average of six replicate determinations

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 (4 °C) and frozen (-20 °C) temperature for 24 h and 72h. Stability measurements were carried out with zero-, first- and second-order derivative spectrophotometric methods. 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 -20 °C for at least 72h (Table 4).

Table 4: Stability of mexiletine in solution.

Stability (%)		Room temperature stability (Recovery % ± S.D)		Refrigeratory stability, +4°C (Recovery % ± S.D)		Frozen stability, - 20°C (Recovery % ± S.D)	
λ (nm)	Added ($\mu g\ mL^{-1}$)	24 h	72 h	24 h	72 h	24 h	72 h
A_{262} nm	30	99.3±0.09	98.5±0.21	101.2±0.64	98.4±0.68	99.6±1.54	98.0±4.50
	80	103.7±0.07	97.3±0.01	100.4±0.16	101.6±1.59	102.1±0.09	103.0±1.22
	140	101.2±2.88	101.2±0.06	102.4±0.41	99.7±2.54	101.2±2.57	101.1±1.99
$^1D_{274}$ nm	30	102.5±0.07	98.5±0.45	98.7±0.17	101.3±1.87	102.1±2.14	93.8±0.14
	80	102.3±1.97	101.2±0.08	101.5±0.07	99.4±0.724	101.4±2.51	102.0±0.14
	140	98.5±0.15	107.8±0.08	98.8±0.74	99.1±1.23	101.5±0.09	99.4±0.08
$^2D_{272}$ nm	30	102.1±0.04	100.5±0.12	101.2±0.09	103.8±1.30	99.9±1.78	102.8±0.20
	80	98.7±3.21	100.1±1.02	98.7±0.26	103.7±0.09	100.6±1.32	102.9±0.06
	140	100.8±2.03	98.2±0.65	99.4±0.57	98.3±1.02	101.8±4.71	102.1±1.84

S.D :Standard deviation of six replicate determinations

Comparison of three spectrophotometric methods

Zero-, first- and second-order derivative spectrophotometric methods were applied for determination of the commercial capsule (Table 5). The results show the high reliability and reproducibility of three methods. The best results obtained at 262 nm, 274 nm and 272 nm for zero-, first- and second-order derivative spectrophotometric methods were statistically compared using the F-test. At 95 % confidence level, the calculated *F*-values do not exceed the theoretical values (Table 6). Therefore, there is no significant difference between zero-,

first- and second-order derivative spectrophometric methods. This is suggested that the three methods are equally applicable.

Table 5: Determination of mexiletine in pharmaceutical preparation

Commercial preparation	Method	λ (nm)	n	Found ± S.D (mg)	Recovery (%)	R.S.D[a] (%)	Confidence interval
Mexitil capsule 200 mg/capsule	Zero-order Spectrophotometric Method	$A_{262\ nm}$	12	201.2±4.11	100.6	2.04	199.2-202.1
	First-order Spectrophotometric Method	$1D_{260\ nm}$	12	198.4±5.09	99.2	2.56	198.9-199.6
		$1D_{268\ nm}$	12	202.3±6.09	101.2	3.01	197.7-203.5
		$1D_{274\ nm}$	12	201.7±3.94	100.8	1.95	199.7-202.3
	Second-order Spectrophotometric Method	$2D_{272\ nm}$	12	202.4±4.37	101.2	2.16	197.9-203.4
		$2D_{277\ nm}$	12	201.6±4.92	100.8	2.44	198.8-202.6

S.D: Standard deviation of six replicate determinations, R.S.D: Relative standard derivation, [a]Average of six replicate determinations

Table 6: Statistical comparison (F-test) of the results obtained by proposed methods

Commercial preparation	Statistical Values	Zero-order derivative Spectrophotometric Method	First-order derivative Spectrophotometric Method	Second-order derivative Spectrophotometric Method	F Values
Mexitil capsule 200mg/capsule	n	12	12	12	F_c=1.32 Ft=3.29
	X	201.2	201.7	202.4	
	S.D	4.11	3.94	4.37	
	Std.Error	16.7	12.3	17.6	
	CI	(199.2-202.1)	(199.7-202.3)	(197.9-203.4)	

n: Number of determination. X: mean, S.D: Standard deviation, CI: Confidence interval, Fc: Calculated F values, Ft: Tabulated F values, H_o: Hypothesis: no statitically significant difference exists between three methods
$F_t > F_c$: H_o hypothesis in accepted (α=0.05)

The proposed methods are very effective for the assay of mexiletine in capsules. The validity of the proposed methods 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.

CONCLUSION

In conclusion, zero-, first- and second-order derivative spectrophometric methods were developed for determination of mexiletine in capsule dosage form. Mexiletine can be directly determined in capsules in presence of excipients without sample pre-treatment procedures by using spectrophotometric methods. The apparatus and reagents used seem to be accessible even for the simple laboratories. Also, no significant difference was found between the proposed spectrophotometric methods (F_c= 1.32> F_t=3.29). Therefore, developed methods can be recommended for routine and quality control analysis of mexiletine.

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