SPECTROPHOTOMETRIC METHODS FOR DETERMINATION OF ETHAMSYLATE IN THE PRESENCE OF ITS DEGRADATION PRODUCT HYDROQUINONE

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Ethamsylate was determined in the presence of its known degradation product, hydroquinone, as an impurity using spectrophotometric methods. Four spectrophotometric methods; second derivative, derivative ratio, ratio difference, and dual wavelength, were evaluated. The accuracy, precision, and linear range were determined for each method, and the methods were validated as per ICH guidelines. The specificity of each method was assessed by analyzing synthetic mixtures containing different proportions of the degradation product and drug. The developed methods were applied to the determination of ethamsylate in bulk powder and two dosage forms.

Keywords: *ethamsylate, hydroquinone, degradation, spectrophotometry.*

СПЕКТРОФОТОМЕТРИЧЕСКИЕ МЕТОДЫ ОПРЕДЕЛЕНИЯ ЭТАМСИЛАТА В ПРИСУТСТВИИ ПРОДУКТА ЕГО ДЕГРАДАЦИИ ГИДРОХИНОНА

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Для определения этамсилата в присутствии гидрохинона – известного продукта его разложения, рассматриваемого в качестве примеси, – использованы четыре спектрофотометрических метода, основанные на второй производной, отношении производных, разности отношений и паре длин волн. Методы проверены в соответствии с рекомендациями Международного совета по гармонизации технических требований к фармацевтическим препаратам для человека, определены точность, дисперсия и линейный диапазон для каждого метода. Специфичность каждого метода оценивалась путем анализа синтетических смесей, содержащих различные пропорции продукта деградации и препарата. Разработанные методы применены для определения этамсилата в сыпучих порошках и двух лекарственных формах.

Ключевые слова: *этамсилат, гидрохинон, деградация, спектрофотометрия.*

Introduction. Ethamsylate (ETM) or diethylammonium 2,5-dihydroxybenzene sulfonate is the most widely prescribed drug for prophylaxis and control of hemorrhages from small blood vessels [1]. It is a hemostatic drug that appears to maintain the stability of capillary walls and correct abnormal platelet adhesion [1]. A literature review revealed that many analytical methods are available for ETM assays. These methods

include spectrophotometry [2, 3], spectrofluorimetry [4], high-performance thin-layer chromatography [5, 6], electrochemical methods [7, 8], chemiluminescence [9, 10], and capillary electrophoresis [11, 12].

Hydroquinone (HQ, benzene-1,4-diol) is a degradation product of and major impurity in ETM [13, 14]. HQ is possibly carcinogenic and suspected to cause genetic defects. It is very toxic to aquatic life and may cause allergic skin reactions and serious eye damage [15]. Some methods have been applied as stability-indicating assays for ETM; however, they are not sufficiently sensitive to detect trace amounts of HQ present as an impurity in ETM [14]. To the best of our knowledge, no spectrophotometric methods have been reported for the simultaneous determination of ETM and HQ.

Therefore, our aim was to develop a highly sensitive stability indicating assay for the detection of trace amounts of HQ as an impurity in ETM.

Experimental. Spectrophotometric measurements were carried out on a dual beam UV-Vis spectrophotometer (UV-1650 PC; Shimadzu, Kyoto, Japan). The bundled software (UV PC personal spectroscopy software version 3.7, Shimadzu) was used to process absorption and derivative spectra. Scans were carried out in the range from 200 nm to 400 nm at 0.1 nm intervals using 1.00 cm quartz cells.

We obtained standards of ETM (purity 99.0–101.0%) from Memphis Chemical Co. (Cairo, Egypt) and HQ (minimum purity of 98 %) from Sisco Research Laboratories Pvt. Ltd (Mumbai, India). We also purchased ETM in pharmaceutical dosage forms as Dicynone® ampoules (batch number GLE2557; Minapharm, Cairo, Egypt) containing 250 mg of ETM per 2 mL and Dicynone 250 tablets (batch number CLE2878, Minapharm, Cairo, Egypt) containing 250 mg of ETM per tablet. Deionized water was used in all experiments.

Stock standard solutions (1 mg/mL) of ETM and HQ were prepared in deionized water. Working standard solutions (100 μg/mL) of ETM and HQ were prepared by diluting the stock solutions with deionized water. Solutions containing ETM and HQ in different ratios (containing 10–80 % of HQ) were prepared by transferring aliquots of the working solutions into a set of 10-mL volumetric flasks. Each flask was then filled to the mark with deionized water.

For construction of the calibration curves aliquots of the ETM working standard solution were accurately measured and transferred into a set of 10-mL volumetric flasks. Each flask was filled to the mark with deionized water to obtain solutions with ETM concentrations between 10 and 100 μg/mL.

Aliquots of the HQ working standard solution were accurately measured and transferred into a set of 10-mL volumetric flasks. Each flask was filled to the mark with deionized water to obtain HQ concentrations between 10 and 60 μg/mL.

The second derivative (D2) absorption spectra of ETM (10–100 μg/mL) were recorded with $\Delta\lambda = 8$ nm and a scaling factor of 100. The peak amplitudes of the D2 spectra were measured at 322.5 nm

In the derivative ratio (DR) method, the zero-order absorption spectra of ETM (10–100 μg/mL) were measured and recorded. These spectra were then divided by the absorption spectrum of 60 μg/mL HQ to obtain the first derivatives. Then, a calibration curve was constructed using the amplitude at 317.0 nm and the concentrations of ETM, and the regression equation was computed.

Ratio difference (RD) spectra were obtained by dividing the spectra of ETM (10–100 μg/mL) by the spectrum of 60 μg/mL HQ and used for the determination of ETM. A calibration curve was constructed using the difference in amplitude at 311.9 and 327.0 nm and the concentrations of ETM, and the regression equation was computed.

For the dual wavelength (DW) method, the zero-order spectra of ETM (10–100 μg/mL) were measured. Absorbance values for each spectrum were measured at 300.4 and 274.2 nm. Then a calibration curve was constructed using the difference in absorbance and the concentrations of ETM, and the regression equation was computed.

Dicynone ampoules containing 250 mg of ETM were diluted in a 50-mL volumetric flask with deionized water and then diluted again in 10-mL volumetric flask to obtain final concentrations of 50 and 75 μg/mL. Five tablets of Dicynone (250 mg) were accurately weighed and ground to a fine powder. An accurately weighed portion of the powdered tablets equivalent to 250 mg of ETM was transferred into a 100-mL beaker, sonicated in 20 mL of deionized water for 30 min, and filtered into a 50-mL volumetric flask. The residue was washed three times with 10 mL of deionized water and the solutions were made up to the mark with deionized water. Aliquots of this solution were transferred to 10-mL volumetric flasks and diluted with deionized water to prepare tablet solutions containing 50 and 75 μg/mL ETM.

The calibration curve construction steps were repeated in acidic (pH 5.65 adjusted with 0.1 M HCl) and alkaline (pH 6.65 adjusted with 0.1 M NaOH) aqueous solutions.

Results and discussion. The present methods could determine ETM at concentrations up to 100 μg/mL in solutions that were composed of up to 80% HQ. The zero-order absorption spectra of ETM and HQ showed severe overlapping, which would prevent the use of spectrophotometry directly for drug analysis without a preliminary separation step (Fig. 1a).

The D2 spectra of ETM and HQ were recorded with Δλ = 8 nm and a scaling factor of 100. The D2 spectra of HQ (10–60 μg/mL) showed no absorption at 322.5 nm (Fig. 1b). Hence, we choose this point to estimate the ETM concentration. A calibration curve was constructed for the relationship between the peak amplitude of D2 at 322.5 nm and the concentration of ETM. The regression equation was D2 = 0.0077*C* + + 0.0277 (*r* = 0.999), where D2 is the peak amplitude and *C* is the ETM concentration in μg/mL.

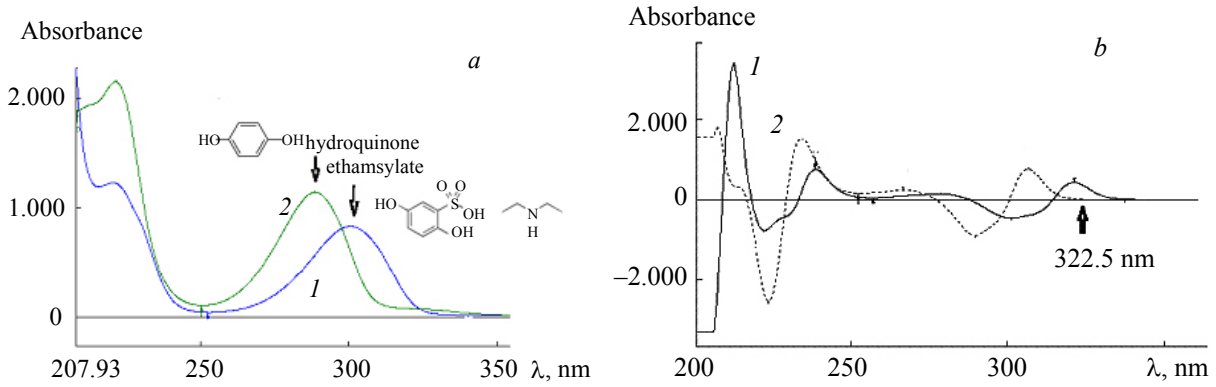

Fig. 1. Zero-order spectra (a) and second derivative spectra (b) of 50 μg/mL ETM (*1*) and 50 μg/mL HQ (*2*).

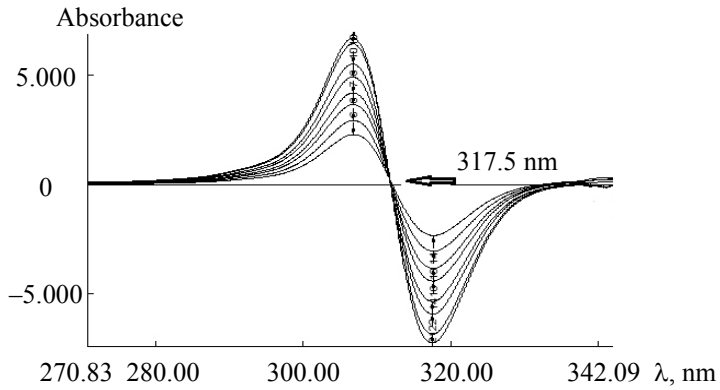

Fig. 2. Derivative ratio method, first-order of ratio spectra of ETM (10–100 μg/mL) divided by the spectrum of HQ (60 μg/mL).

In DR method, the absorption spectra of ETM were divided by the absorption spectrum of 60 μg/mL HQ and the first derivative of ratio spectra were calculated (Fig. 2). To optimize the DR method, the influences of different variables were studied. These variables were the divisor concentration, Δλ, and scaling factor. Careful selection of the divisor and the working wavelengths were of great importance [16]. Three different concentrations of the HQ solution (10, 50, and 60 μg/mL) were evaluated as divisors. The least noise and best selectivity were obtained with the spectrum of 60 μg/mL HQ as the divisor. A calibration curve was constructed representing the relationship between the peak amplitude of DR at 317.6 nm and the ETM concentration. The regression equation was DR = 0.0708*C* + 0.214 (*r* = 0.999), where DR is the peak amplitude and *C* is the ETM concentration in μg/mL.

For RD method we evaluated the differences in amplitudes (ΔP) in the ratio spectra at λ_1 = 311.9 nm and λ_2 = 327.0 nm using the spectrum of 60 μg/mL HQ as the divisor. The ratio spectrum of HQ will be a parallel line to the *x*-axis, and hence the difference in amplitude at any two wavelengths will be zero [17, 18]. A calibration curve was constructed representing the relationship between ΔP and the corresponding concentrations (Fig. 3). The regression equation was RD = 0.0727*C* + 0.1329 (*r* = 0.999), where RD is the peak amplitude and *C* is the ETM concentration in μg/mL.

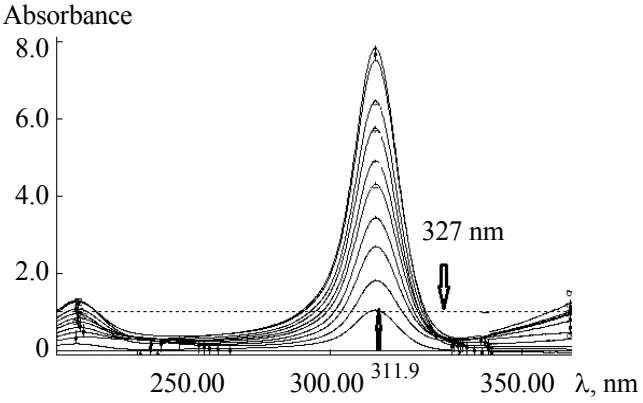

Fig. 3. Ratio difference method, zero-order spectra of ETM (10–100 μg/mL) divided by the spectrum of HQ (60 μg/mL, solid line), and the spectrum of HQ (60 μg/mL) divided by itself (dashed line).

We evaluated the differences in amplitudes (ΔP) in the ratio spectra at λ1 (311.9 nm) and λ2 (327.0 nm) using the spectrum of 60 μg/mL HQ as the divisor. The ratio spectrum of HQ will be a parallel line to the *x*-axis, and hence the difference in amplitude at any two wavelengths will be zero [17, 18]. A calibration curve was constructed representing the relationship between ΔP and the corresponding concentrations. The regression equation was RD = 0.0727*C* + 0.1329 (*r* = 0.999), where RD is the peak amplitude and *C* is the ETM concentration in μg/mL.

For DW method HQ had equal absorbance values at 300.4 and 274.2 nm for all the studied concentrations (10–60 μg/mL). The absorbance values were measured at both wavelengths for each spectrum to obtain absorbance differences (ΔP) in the zero-order spectra of ETM (10–100 μg/mL). A calibration curve was constructed representing the relationship between ΔP and the corresponding ETM concentration. The regression equation was DW = 0.0121*C* + 0.034 (*r* = 0.999), where DW is the peak amplitude and *C* is the ETM concentration in μg/mL.

The spectra of ETM and HQ did not change under either acidic or alkaline conditions.

Method validation. Validation of the proposed methods was performed according to International Conference on Harmonization guidelines [19]. The linearity of each method was evaluated by analyzing different concentrations of ETM (10–100 μg/mL). The analyses were performed according to the described experimental conditions. The parameters of the linear equations and their relative standard errors are summarized in Table 1. The accuracy was checked by blind analysis of pure samples of ETM by the proposed methods, and satisfactory results were obtained. The mean recoveries are provided in Table 1. Three different concentrations of ETM (20, 30, and 40 μg/mL) were analyzed three times per day using the proposed methods. The relative standard deviations were calculated (Table 1), and showed low deviations and high repeatability. The procedures were repeated on three different days for ETM at three concentrations (20, 30, and 40 μg/mL). The relative standard deviations were calculated (Table 1) and showed high intermediate precision.

Three concentrations of ETM (20, 30, and 40 μg/mL) were determined by measuring the peak amplitudes at the specified wavelengths ±0.2 nm for each proposed method. Lower relative standard deviation (RSD%) values were obtained for the DR, RD, and DW methods, and higher values were obtained for the D2 method because the measurements were not taken at peak maxima or minima (Table 1). The specificity of each method was evaluated by analysis of different mixtures prepared in the laboratory that contained different proportions (10%–80%) of HQ. The recovery of ETM (%) and RSD% were acceptable (Table 2).

TABLE 1. Assay Parameters and Validation Data for Determination of ETM by the Proposed Spectrophotometric Methods According to ICH Guidelines

Parameter	D2	DR	RD	DW
Range, μg/mL	10–100	10–100 [1]	10–100	10–100
Slope	0.0077	0.0719	0.0722	0.0120
Intercept	0.0259	0.1862	0.1531	0.0349
Correlation coefficient (*r*)	0.9990	0.9990	0.9990	0.9990
Accuracy Recovery (%)	100.0400	99.9900	99.8600	100.1000
Robustness (RSD%)	1.8780	1.3480	1.7980	1.7490
Repeatability (RSD%)	1.2340	1.4560	0.4350	0.7120
Intermediate precision (RSD%)	1.5950	1.2300	0.7680	0.7230

TABLE 2. Determination of ETM in the Laboratory-Prepared Mixtures by the Proposed Spectrophotometric Methods

Proportion of HQ (%)	ETM recovery (%)			
	D2	DR	RD	DW
10	98.600	99.300	99.100	99.300
20	98.900	99.000	100.200	98.900
30	100.460	98.900	100.500	99.400
40	99.000	99.300	100.000	99.500
50	99.430	99.300	101.600	100.100
60	99.330	100.000	100.800	100.100
70	99.330	100.700	101.600	99.100
80	101.000	102.000	101.000	98.600
Mean	99.380	100.025	100.600	99.375
SD	0.916	1.113	0.843	0.531
RSD%	0.922	1.112	0.837	0.534

TABLE 3. Recovery of ETM from Tablet (250 mg) and Ampoules (250 mg) Using the Proposed Spectrophotometric Methods

Method	D2	DR	RD	DW
Tablet				
Recovery%	98.500	99.000	101.200	100.900
RSD%	1.342	1.071	1.093	0.672
Ampoule				
Recovery, %	98.680	100.700	101.300	101.800
RSD, %	1.182	1.098	0.823	0.781

TABLE 4. Statistical Comparison of the Results Obtained by the Proposed Spectrophotometric Methods and a Reported Method for the Analysis of ETM

Method	D2	DR	RD	DW	Reported method[**]
Mean recovery, %	100.040	99.990	99.860	100.103	98.720
SD	0.762	0.698	0.575	0.482	0.776
Variance	0.580	0.487	0.330	0.232	0.603
n	10	10	10	10	6
Student's *t*-test	2.070 (2.145)[*]	1.780 (2.145)[*]	2.059 (2.145)[*]	0.171 (2.145)[*]	
F-test	1.039 (2.145)[*]	1.238 (2.145)[*]	1.827 (2.145)[*]	2.059 (2.145)[*]	

[*] The values in parentheses are the corresponding tabulated values of *t* and *F* at $p = 0.05$.
[**] Reported method [14]: HPLC on a kromasil column (C_{18}; 25 cm × 4.6 mm, i.d., 5-μm) using a mobile phase of methanol and water (50:50, v/v) at 0.6 mL/min.

The proposed methods were successfully applied to determination of ETM in two pharmaceutical dosage forms (tablets and ampoules). The mean recoveries and RSD% are shown in Table 3. The results obtained by the proposed methods were statistically compared to those obtained by a reported HPLC method [14]. The calculated *t*-value and *F*-value were less than theoretical ones, and the proposed and reported methods were not significantly different for both the accuracy and precision (Table 4) [20].

Conclusion. The proposed methods can determine ETM at concentrations up to 100 μg/mL and in solutions composed of up to 80% HQ. All the methods are sensitive, precise, and accurate and could be easily applied in quality control laboratories as stability-indicating methods for the determination of ETM in the presence of HQ. The proposed methods could be applied for routine analysis of ETM in pure bulk powders and its dosage forms.

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