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Stability indicating methods for assay of mequitazine in presence of its degradate

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Abstract

Six procedures have been suggested for the determination of the antihistaminic agent, mequitazine, in the presence of its degradate. Mequitazine, having a phenothiazine group, undergoes peroxide oxidation and the corresponding sulphone is produced. Its identity was confirmed by IR and MS. The first procedure is based on determination of mequitazine by HPLC with UV detection at 256 nm. The mobile phase used is acetonitrile, ortho phosphoric acid (50:50) using caffeine as an internal standard. Linearity range is 1.00–9.00 μg/ml. The second determination is a densitometric procedure based on the determination of mequitazine in the presence of its degradate at 256 nm using the mobile phase, chloroform:methanol:ammonia (50:18:3). Linearity range is 1.25–7.50 μg per spot. The third procedure is spectrophotometric, where a mixture of mequitazine and its degradate are resolved by first derivative ratio spectra. Linear calibration graphs of first derivative values at wavelengths 210.2, 247 and 259.8 nm are obtained. On carrying out measurements at the three mentioned wavelengths, the linearity range is found to be 1.00–10.00 μg/ml. The fourth procedure is based on first derivative spectrophotometry, where D_1 measurements are carried out at 290 nm. Linearity range is 1.00–10.00 μg/ml. The fifth procedure is based on the reaction of mequitazine with 3-methyl-2-benzothiazolinone hydrazone (MBTH) in the presence of ferric chloride. A stable violet colored oxidative coupling product is formed, which is measured spectrophotometrically at 685 nm. The optimum experimental parameters for the reaction have been studied and assigned. Linearity range is 1.00–16.00 μg/ml. The sixth procedure is based on the reaction of mequitazine in the presence of its degradate with 2,6-dichloroquinone-4-chloroimide (Gibbs reagent) in aqueous methanolic medium. The reddish-brown colored condensation product is measured at 405 nm. The optimum experimental conditions for the reaction have been studied. Linearity range is 50.00–600.00 μg/ml. The validity of the described procedures was assessed by applying the standard addition technique. Statistical analysis of the results has been carried out revealing high accuracy and good precision. The suggested procedures could be used for the determination of mequitazine, both in pure and dosage forms, as well as in the presence of its degradate.

Keywords: Antihistaminic drugs; Mequitazine; HPLC; Densitometry; Derivative ratio; First derivative spectrophotometry; 3-Methyl-2-benzo-thiazolinone hydrazone (MBTH); 2,6-Dichloroquinone-4-chloroimide (Gibbs reagent)

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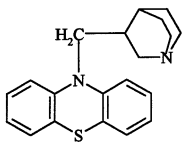
1. Introduction

Mequitazine, metaplexan; primalan; zesulan is a histamine H_1-receptor antagonist [1]. It is 10-(1-azabicyclo [2.2.2] oct-3-yl-methyl)-10-H phenothiazine. Mequitazine occurs as white crystals, m.p. 130–131 °C, soluble in alcohol and ether. It has the following structural formula [2].



Mequitazine
$C_{20}H_{22}N_2S$
Mol. Wt 322.47

Few procedures have been published about the determination of mequitazine including spectrophotometry [3,4], H^1NMR [5] and GLC [6].

This manuscript deals with the determination of mequitazine, the intact molecule, in the presence of its oxidative degradate, the corresponding sulphone. Degradation was enhanced by refluxing mequitazine with 30% hydrogen peroxide for 4 h and extraction with ether. During hydrolysis, the sulfur atom of the phenothiazine ring of mequitazine undergoes oxidation, leading to the formation of the corresponding sulphone.

Elucidation of the structure of the degradation product was carried out using mass spectroscopy and IR.

The main goal of the present work is to introduce stability indicating methods for the determination of mequitazine in the presence of its degradate. Six procedures have been introduced: HPLC, densitometry, derivative ratio spectrophotometry, first derivative spectrophotometry and colorimetrically using MBTH and Gibbs reagent. The suggested procedures are considered a new approach for routine analysis of mequitazine in its bulk form.

The procedures were applied for the determination of mequitazine in pure form, in dosage forms and in the presence of its degradate.

2. Experimental

2.1. Instruments

- μ Bondapak™ C_{18} 125° A 10 μm 4.6 × 250 mm HPLC cartridge column, SPD-10 A UV-Vis detector, LC-10 Ad liquid chromatograph Shimadzu.
- Shimadzu CS, 9000 flying spot densitometer.
- Precoated TLC plates silica G F_{254} (20 × 20 cm) from Merck.
- UV-1601 PC UV-Visible spectrophotometer Shimadzu.

2.2. Samples and pharmaceutical dosage forms

Mequitazine, pure sample (Rhone-Poulenc, France). Its purity was checked by determination of its m.p. (130–131 °C) [2] and analyzed to contain 99.90 ± 0.73% using A (1%, 1 cm) [7]. Primalan tablets: labeled to contain 5 mg of mequitazine per tablet; supplied and manufactured by Amriya for Pharmaceutical Industries, Alexandria, Egypt under license of Rhone-Poulenc; batch No. 206701. Primalan syrup: labeled to contain 1.25 mg of mequitazine per 2.5 ml syrup, supplied and manufactured by Amriya, batch No. 252301.

2.3. Standard drug solutions and reagents

All solvents used were HPLC and spectroscopic grade.

Mequitazine standard solution 1 mg/100 ml methanol (HPLC procedure) was prepared by dissolving 10 mg of the drug into 100 ml methanol and diluting ten times using the same solvent. Mequitazine standard solution 50 mg/100 ml methanol (densitometric procedure). Mequitazine standard solution 10 mg/100 ml ethanol (derivative ratio and first derivative spectrophotometry procedures). Mequitazine standard solution 5 mg/100 ml prepared by dissolving in the least quantity of ethanol and complete to volume with 0.1 M HCl (MBTH procedure). Mequitazine standard solution 100 mg/100 ml methanol (Gibbs reagent procedure). Caffeine

standard solution 10 mg/100 ml methanol. Acetonitrile:orthophosphoric acid mobile phase (50:50). Hydrogen peroxide (30%) v/v. (Prolabo). 0.1 M HCl E. Merck, Germany. Ferric chloride (1 g% in 0.1 M HCl) E. Merck. Ether from E. Merck. 3-Methyl-2-benzo-thiazolinone hydrazone (MBTH) (0.35 g% in 0.1 M HCl) E. Merck. 2,6-Dichloroquinone-4-chloroimide (Gibbs reagent) (0.4 g% in methanol) E. Merck.

2.4. Procedures

2.4.1. Method of preparation of the degradation product

Dissolve 1 g of mequitazine in 90 ml ethanol, add 10 ml 30% hydrogen peroxide and heat by refluxing for 4 h. Remove excess hydrogen peroxide by boiling. Extract the degradation product three times each with 10 ml ether. Evaporate under vacuum and dry in a desiccator. The degradate identity was checked and confirmed by carrying out mass spectroscopy and IR. Its m.p. was also determined.

2.4.2. Determination of mequitazine in the presence of its degradate using HPLC procedure

For construction of the calibration graph, take aliquot portions of mequitazine standard solution 1 mg/100 ml in methanol (1–9 ml) into a series of 10 ml measuring flasks. Add 1 ml of caffeine as an internal standard (10 mg% in methanol) and complete to volume with methanol. Inject 20 μl of the solution from each flask and record the chromatograms, maintaining the flow rate at 1.5 ml/min. Monitor the effluent at 256 nm using acetonitrile:orthophosphoric acid (50:50) as a mobile phase. Measure the peak area corresponding to the concentration of each and divide by the peak area of internal standard. Construct a calibration graph representing the relation between concentration and ratio of peak area. Concentration of unknown samples could be derived from the calibration graph or calculated from the following regression equation:

$$Y = 9.31 \times 10^{-2} X - 1.2 \times 10^{-3} \quad r = 1$$

where

$$Y = \frac{\text{Peak area of sample}}{\text{Peak area of internal standard}}$$

X is the concentration of mequitazine in μg/ml and r is the correlation coefficient.

Laboratory mixtures were prepared containing mequitazine and its degradate in the ratios of 10–80% of the degradate. They were treated similarly to determine mequitazine content.

2.4.2.1. Determination of mequitazine in primalan tablets using HPLC procedure. Accurately weigh 20 tablets and pulverize in a small mortar. Transfer a weighed quantity of powder equivalent to 10 mg mequitazine into a beaker and extract with 80 ml methanol using a magnetic stirrer. Filter into a 100-ml measuring flask and complete to volume with methanol. Measure 10 ml of this extract and dilute in a 100-ml measuring flask using methanol as a solvent. Determine mequitazine concentration by taking (1–9 ml) into 10 ml measuring flasks, add 1 ml caffeine as internal standard (10 mg% in methanol). Complete to volume with methanol and proceed as previously described in Section 2.4.2 starting from 'inject 20 μl of the solution…'.

2.4.2.2. Determination of mequitazine in primalan syrup using HPLC procedure. Into a 50 ml separating funnel, accurately measure 2 ml of primalan syrup and extract three times each with 10 ml of chloroform. Evaporate to dryness and dissolve in 100 ml methanol. Determine mequitazine concentration by taking (1–9 ml) into 10 ml measuring flasks, add 1 ml caffeine as internal standard (10 mg% in methanol). Complete to volume with methanol and proceed as previously described in Section 2.4.2 starting from 'inject 20 μl of the solution...'.

2.4.3. Determination of mequitazine in the presence of its degradate using densitometric procedure

For construction of calibration graph, apply 2.5, 5, 7.5, 10, 12.5 and 15 μl from stock solution 50 mg/100 ml of mequitazine in methanol on a TLC plate. Place in chromatographic tank previously saturated for 1 h with the developing mobile phase, chloroform:methanol:ammonia (50:18:3).

Develop the plate by ascending chromatography for a distance of 16 cm then remove and dry in air. Detect the spots under UV lamp and scan the plate at 256 nm. Construct the calibration curve, representing the relation between concentration and integrated peak area from which the concentration of unknown samples can be deduced or the following regression equation is used:

$$Y = 1.5682X + 6.273 \times 10^{-1} \quad r = 0.9996$$

where Y is the area under the peak, X is the concentration of mequitazine in μg per spot and r is the correlation coefficient.

Laboratory mixtures were prepared containing mequitazine and its degradate in the ratios of 10–80% of the degradate. They were treated similarly to determine mequitazine content.

2.4.3.1. Determination of mequitazine in primalan tablets using densitometric procedure. Accurately weigh 20 tablets and pulverize in a small mortar. Transfer a weighed quantity of powder equivalent to 5 mg and extract with 8 ml methanol, filter into a 10 ml measuring flask and complete to volume with methanol. Determine mequitazine concentration by applying 2.5–15 μl of this solution on a TLC plate and proceed as previously described in Section 2.4.3 starting from 'Place in chromatographic tank…'.

2.4.3.2. Determination of mequitazine in primalan syrup using densitometric procedure. Into a 50 ml separating funnel, accurately measure 10 ml of primalan syrup claimed to contain 5 mg of mequitazine and extract three times each with 10 ml of chloroform. Evaporate to dryness and dissolve in 10 ml methanol. Determine mequitazine concentration by applying 2.5–15 μl of this solution on a TLC plate and proceed as previously described in Section 2.4.3 starting from 'Place in chromatographic tank…'.

2.4.4. Spectrophotometric determination of mequitazine in the presence of its degradate using derivative ratio spectrophotometric procedure

For construction of calibration graph, take aliquot portions of mequitazine standard solution 10 mg/100 ml ethanol (1–10 ml) into a series of 100 ml measuring flasks. Dilute to volume with ethanol. Each absorption spectrum of mequitazine solution is divided by the spectrum of degraded mequitazine (10 μg/ml) as a divisor. The first derivative of ratio spectra was recorded; $\Delta\lambda$ 4 nm and scanning rate 1.5. The values of the derivatives were measured at 210.2, 247 and 259.8 nm. The concentration of mequitazine is proportional, respectively, to the amplitude of the signal at previously selected wavelengths. Construct a calibration graph representing the relation between concentration and peak amplitude from which the concentration of unknown samples can be calculated. The following regression equations can also be used:

At 210.2 nm

$$Y = -1.49 \times 10^{-2}X + 0.0 \quad r = 0.9999$$

At 247 nm

$$Y = 4.88 \times 10^{-2}X - 4.0 \times 10^{-4} \quad r = 0.9999$$

At 259.8 nm

$$Y = 6.28 \times 10^{-2}X - 5.0 \times 10^{-4} \quad r = 0.9999$$

where Y is the peak amplitude, X is the concentration of mequitazine in μg/ml and r is the correlation coefficient.

Laboratory mixtures were prepared containing mequitazine and its degradate in the ratios of 10–80% of the degradate. They were treated similarly to determine mequitazine content.

2.4.4.1. Spectrophotometric determination of mequitazine in primalan tablets using derivative ratio spectra procedure. Accurately weigh 20 tablets and pulverize in a small mortar. Transfer a weighed quantity of powder equivalent to 10 mg mequitazine into a small beaker and extract with 80 ml ethanol using a magnetic stirrer. Filter into a 100-ml measuring flask and complete to volume with ethanol. Determine mequitazine concentration by measuring different volumes of this solution (1–10 ml) into 100 ml measuring flasks. Complete to volume with ethanol and proceed as previously described in Section 2.4.4 starting from 'each absorption spectrum…'.

2.4.4.2. Determination of mequitazine in primalan syrup using derivative ratio spectra procedure. Into a 50 ml separating funnel, accurately transfer 20 ml of primalan syrup claimed to contain 10 mg of mequitazine. Extract three times each with 10 ml chloroform, evaporate to dryness and dissolve in 100 ml ethanol. Determine mequitazine concentration by measuring different volumes of this solution (1–10 ml) into 100 ml measuring flasks. Complete to volume with ethanol and proceed as previously described in Section 2.4.4 starting from 'each absorption spectrum…'.

2.4.5. Spectrophotometric determination of mequitazine in the presence of its degradate using first derivative procedure

Transfer accurately aliquot portions (1–10 ml) of mequitazine from its stock solution (10 mg/100 ml ethanol) into a series of 100 ml measuring flasks and complete to the mark with ethanol. Record the first derivative curves of each solution against ethanol as a blank. Measure D_1 at 290 nm and plot the calibration curve representing the relationship between the measured D_1 values and the corresponding concentration. The concentration of unknown samples can be deduced by using such calibration curve or by using the following regression equation:

$$D_1 = 4.4 \times 10^{-3} X - 1.0 \times 10^{-4} \quad r = 0.9991$$

where D_1 is first derivative value, X is the concentration in μg/ml and r is the correlation coefficient.

Laboratory mixtures were prepared containing mequitazine and its degradate in the ratios of 10–80% of the degradate. They were treated similarly to determine mequitazine content.

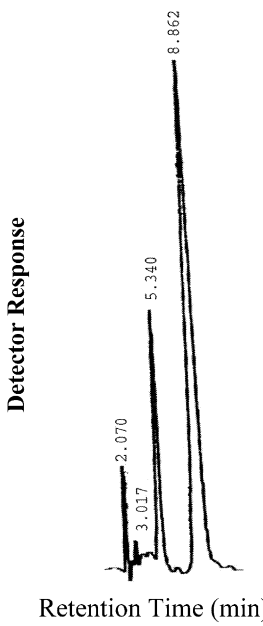


Fig. 1. HPLC spectra of mequitazine (at 5.340 min) in the presence of 60% degraded mequitazine (at 3.017 min) using caffeine as an internal standard (at 8.862 min).

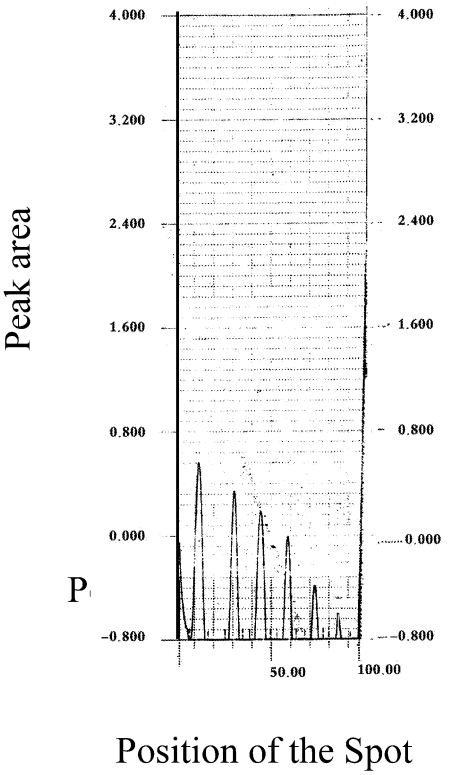


Fig. 2. Scanning profile of mequitazine (1.25–7.50 μg per spot) at 256 nm.

2.4.5.1. Spectrophotometric determination of mequitazine in primalan tablets using first derivative procedure. Accurately weigh 20 tablets and pulverize in a small mortar. Transfer a weighed

quantity of powder equivalent to 10 mg mequitazine into a small beaker and extract with 80 ml ethanol using a magnetic stirrer. Filter into a 100-ml measuring flask and complete to volume with ethanol. Determine mequitazine concentration by measuring different volumes of this solution (1–10 ml) into 100 ml measuring flasks. Complete to volume with ethanol and proceed as previously described in Section 2.4.5 starting from 'record the first derivative curves…'.

2.4.5.2. Spectrophotometric determination of mequitazine in primalan syrup using first derivative procedure. Into a 50 ml separating funnel, accurately transfer 20 ml of primalan syrup claimed to contain 10 mg of mequitazine and extract three times each with 10 ml chloroform. Evaporate to dryness and dissolve in 100 ml ethanol. Determine mequitazine concentration by measuring different volumes of this solution (1–10 ml) into 100 ml measuring flasks. Complete to volume with ethanol and proceed as previously described in Section 2.4.5 starting from 'record the first derivative curves…'.

2.4.6. Spectrophotometric determination of mequitazine in the presence of its degradate using MBTH

Transfer accurately aliquot portions (0.5–8 ml) of mequitazine from its standard solution (5 mg/100 ml) into a series of 25 ml measuring flasks, then add 2 ml of MBTH solution (0.35 g% w/v) and 5 ml ferric chloride solution (1 g%). Keep at room temperature for 30 min, then complete to volume with distilled water. Record the absorption spectrum of the solution against blank. Measure absorbance at 685 nm and construct a calibration graph. The concentration of unknown samples can be deduced by using the calibration curve or by using the following regression equation:

$$Y = 6.69 \times 10^{-2} X + 2.0 \times 10^{-4} \quad r = 1.$$

where Y is the absorbance at 685 nm and X is the concentration of mequitazine in μg/ml.

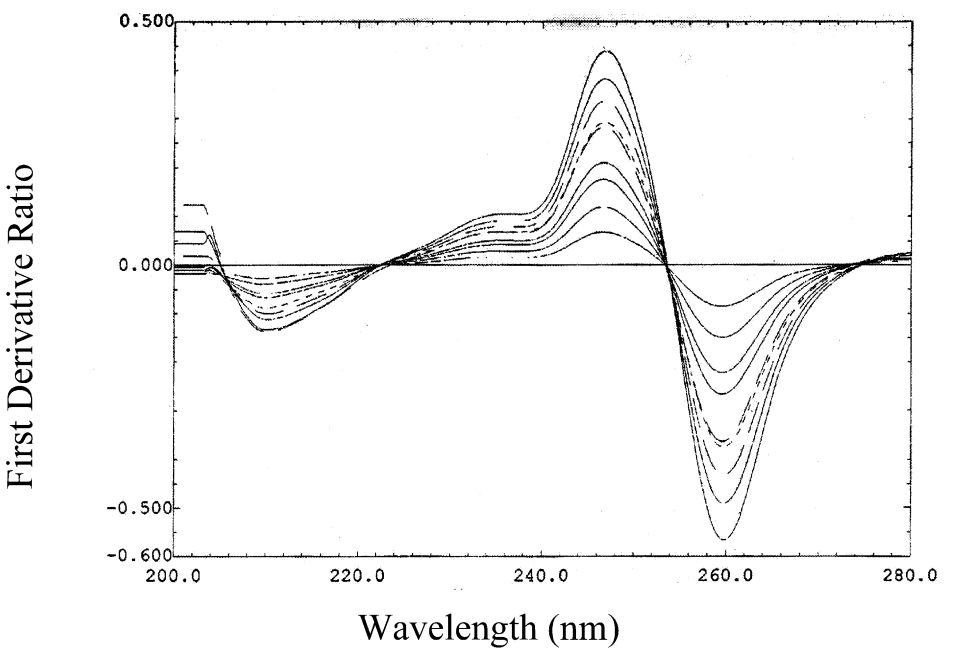


Fig. 3. Derivative ratio spectra of mequitazine in the presence of its degradate using 1–10 μg/ml mequitazine in ethanol and 10 μg/ml of its degradate.

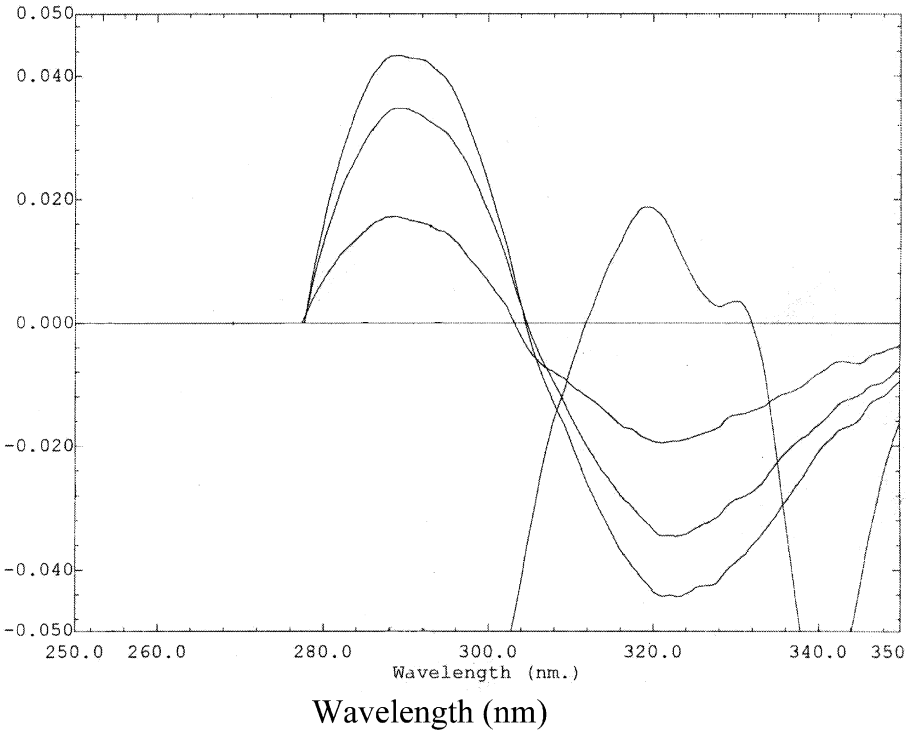


Fig. 4. First derivative spectra of mequitazine: 4, 8 and 10 μg/ml and its degradate (8 μg/ml) using ethanol as solvent.

Laboratory mixtures were prepared containing mequitazine and its degradate in the ratios of 10–80% of the degradate. They were treated similarly to determine mequitazine content.

2.4.6.1. Spectrophotometric determination of mequitazine in primalan tablets using MBTH. Accurately weigh 20 tablets and pulverize in a small mortar. Transfer a weighed quantity of powder equivalent to 5 mg mequitazine into a small beaker and extract with 5 ml ethanol and 75 ml 0.1 M HCl using a magnetic stirrer. Filter into a 100 ml measuring flask and complete to volume with 0.1 M HCl. Determine mequitazine concentration as previously described in Section 2.4.5 starting from 'Transfer accurately aliquot portions…'.

2.4.6.2. Spectrophotometric determination of mequitazine in primalan syrup using MBTH. Into a 50 ml separating funnel, accurately transfer 10 ml of primalan syrup claimed to contain 5 mg of mequitazine and extract three times each with 10 ml chloroform. Evaporate to dryness and dissolve in 5 ml ethanol, then complete to 100 ml using 0.1 M HCl. Determine mequitazine concentration as previously described in Section 2.4.6 starting from 'Transfer accurately aliquot portions…'.

2.4.7. Spectrophotometric determination of mequitazine in the presence of its degradate using Gibbs reagent

Transfer accurately aliquot portions (0.5–6 ml) of mequitazine standard solution (100 mg/100 ml methanol) into a series of 10 ml measuring flasks, then add 4 ml Gibbs reagent and complete to volume with methanol. Record the absorption spectrum of the solution against a blank after 90 min. Measure absorbance at 405

nm and construct a calibration graph. The concentration of unknown samples can be deduced by using the calibration curve or by using the following regression equation:

$$Y = 1.84 \times 10^{-3}X - 7.9 \times 10^{-3} \quad r = 0.9999$$

where Y is the absorbance at 405 nm and X is the concentration of mequitazine in μg/ml.

Laboratory mixtures were prepared containing mequitazine and its degradate in the ratios of 10–80% of the degradate. They were treated similarly to determine mequitazine content.

2.4.7.1. Spectrophotometric determination of mequitazine in primalan tablets using Gibbs reagent. Accurately weigh 25 tablets and pulverize in a small mortar. Transfer a weighed quantity of powder equivalent to 100 mg mequitazine, into a small beaker and extract with 80 ml methanol using a magnetic stirrer. Filer into a 100-ml measuring flask and complete to volume with methanol. Determine mequitazine concentration as previously described in Section 2.4.7 starting from 'Transfer accurately aliquot portions…'.

2.4.7.2. Spectrophotometric determination of mequitazine in primalan syrup using Gibbs reagent. Into a 500 ml separating funnel, accurately transfer 100 ml of primalan syrup claimed to contain 50 mg of mequitazine and extract three times each with 10 ml chloroform. Evaporate to dryness and dissolve in 50 ml methanol. Determine mequitazine concentration as previously described in Section 2.4.7 starting from 'Transfer accurately aliquot portions…'.

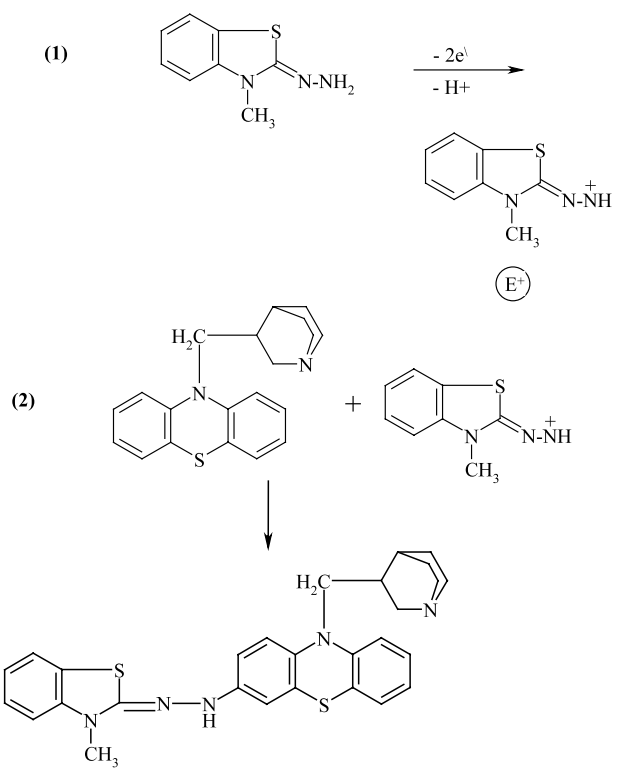


Fig. 5. Scheme for the suggested mechanism for the reaction of mequitazine with 3-methyl -2-benzothiazolinone hydrazone (MBTH).

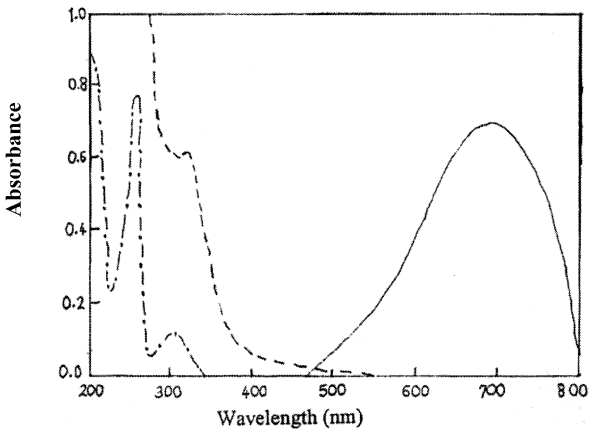


Fig. 6. Absorption spectra of MZ (-.-), MBTH (----) and their reaction product (—) (using 10 μg/ml of MZ) in 0.1 M hydrochloric acid under optimum conditions.

3. Results and discussion

3.1. HPLC method

Various solvent systems were tried to find out the best mobile phase for separation of mequitazine and its degradation product. Acetonitrile and orthophosphoric acid (50:50) gave better resolution, as shown in Fig. 1. Under the experimentally described conditions, the analyte peak was well defined, resolved and free from tailing. The retention time was ($t_r = 5.340$ min) at flow rate of 1.5 ml/min. The optimum wavelength for detection was 256 nm, at which good detector response was obtained for mequitazine. Linearity to concentration was obtained in the concentration range 1.00–9.00 μg/ml. The calibration curve could be represented by the regression equation and correlation coefficient, as mentioned in Section 2.4.2.

3.2. Densitometric method

This is concerned with the application of the densitometric technique for determination of mequitazine. The best separation of the studied drug from its degradate was obtained using chloroform:methanol:ammonia (50:18:3) as the developing mobile phase. The R_f value was found to be 0.61 for mequitazine and 0.40 for its degradate. Quantitatively, the chromatogram was scanned at 256 nm, as shown in Fig. 2. By applying this technique, a linear correlation was obtained between the concentrations 1.25–7.50 μg per spot of mequitazine and the spot area. The linear regres-

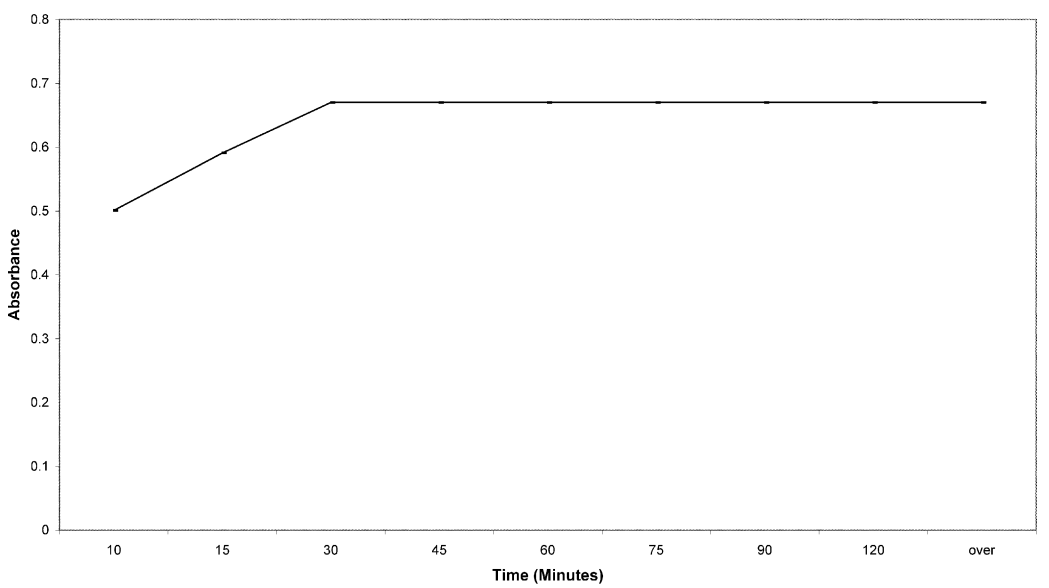


Fig. 7. Effect of time on stability of color produced by the reaction product of mequitazine (6 mg/ml) at 685 nm.

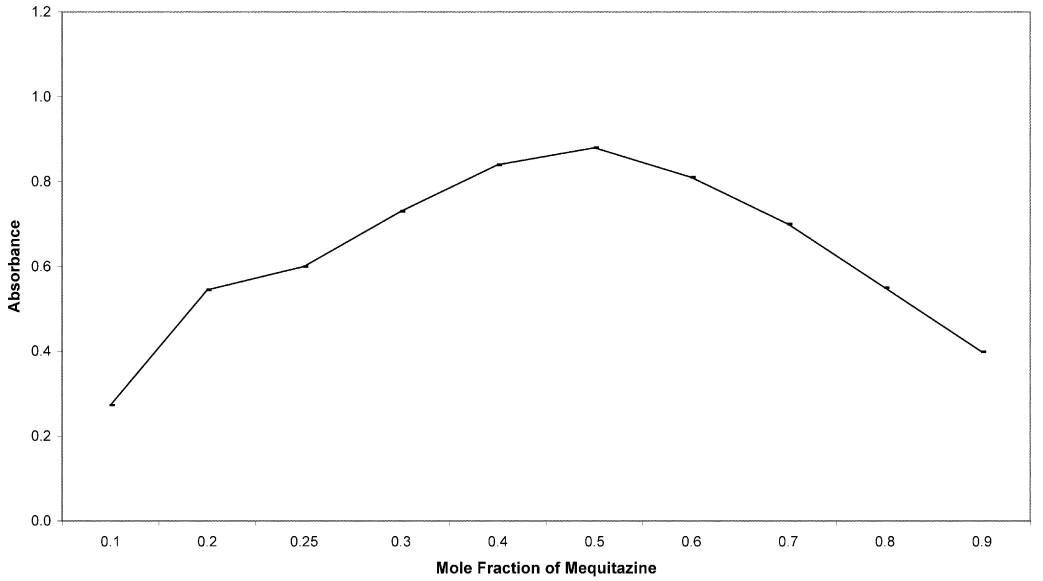


Fig. 8. Determination of the stoichiometry of the reaction of mequitazine with MBTH using 5×10^{-4} M solutions at 685 nm.

sion equation was calculated as mentioned in Section 2.4.3.

3.3. Derivative ratio spectrophotometric method

The present work is concerned with the application of a derivative ratio technique for determination of mequitazine in the presence of its degradation product. In practice, measurements were carried out at three wavelengths 210.2, 247 and 259.8 nm, as shown in Fig. 3. Linear correlations were obtained between the concentration 1.00–10.00 μg/ml of mequitazine and the peak amplitude using the three wavelengths. The corresponding linear regression equations were calculated as mentioned in Section 2.4.4.

3.4. First derivative spectrophotometric method

First derivative spectrophotometric method is used for the determination of mequitazine in the presence of its degradation product. The zero order absorption spectra of mequitazine and its degradation product in ethanol suffer an overlapping which interferes with the direct determination of pure mequitazine. The present work is devoted to the application of first derivative spectrophotometry to resolve such spectral overlapping for the determination of intact mequitazine in the presence of its degradation product at 290 nm, as shown in Fig. 4.

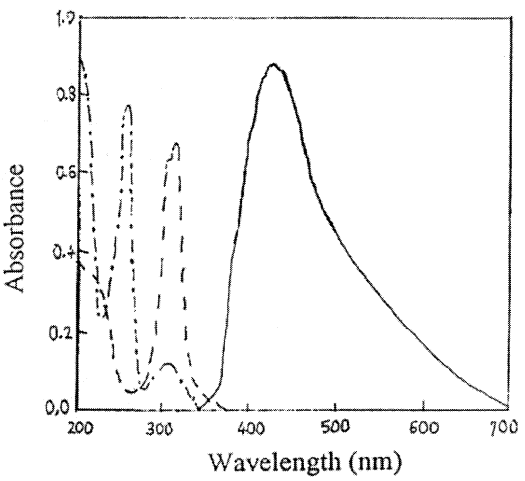


Fig. 9. Absorption spectra of Mz (-.-), Gibbs reagent (-----) and their reaction product (—) (using 500 μg/ml of Mz) in methanol under optimum conditions.

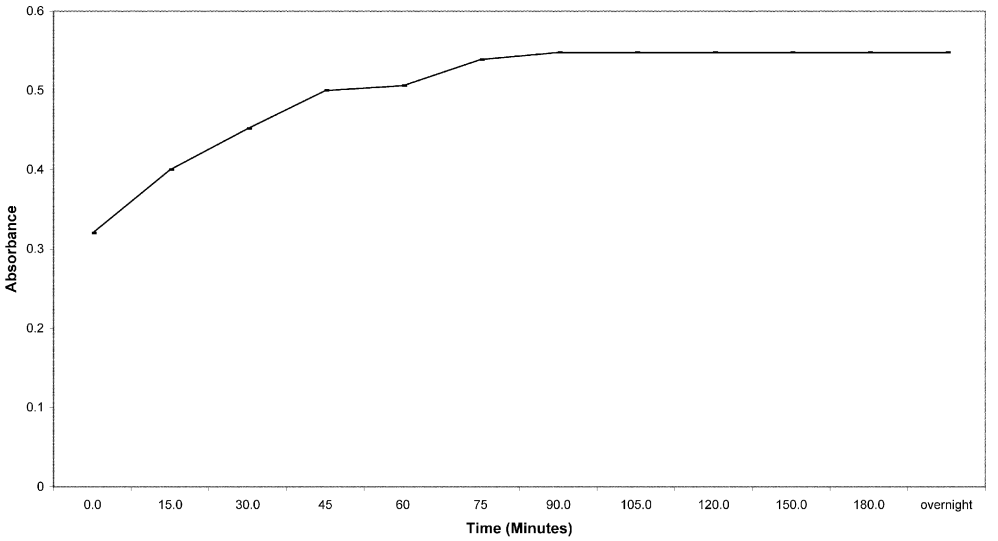


Fig. 10. Effect of time on the stability of color produced of reaction product of mequitazine (300 mg/ml) with Gibbs reagent (0.4 g%) at 405 nm.

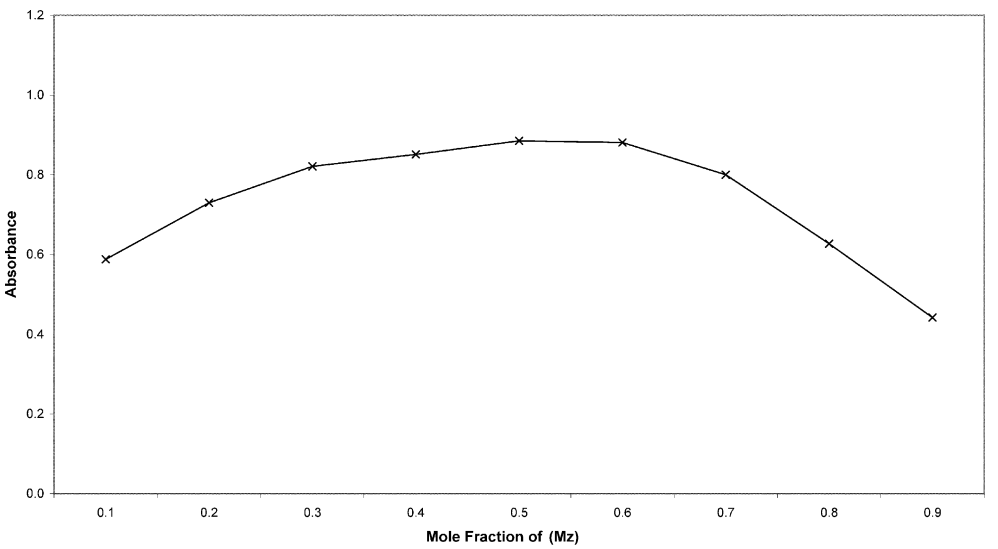


Fig. 11. Determination of the stoichiometry of the reaction of mequitazine with Gibbs reagent using 5×10^{-3} M solutions at 405 nm.

A linear correlation was obtained between the concentration of mequitazine and the D_1 values in the range of 1.00–10.00 μg/ml at 290 nm. The regression equation was calculated as mentioned in Section 2.4.5.

3.5. Spectrophotometric determination of mequitazine using MBTH

MBTH is used for the determination of mequitazine in the presence of its degradation

product. The reaction of MBTH with mequitazine in the presence of an oxidant proceeds via oxidative coupling as shown in Fig. 5. MBTH (1) loses two electrons and one proton on oxidation with oxidizing agent (ferric chloride), forming the electrophilic intermediate (2), which is the active coupling species [8]. The reagent would be expected to attack a carbon atom with maximum electron density, it attacks the position para to the nitrogen of the ring, to produce compound (3).

The reaction of mequitazine with MBTH produces a bluish-green color having maximum absorbance at 685 nm, as shown in Fig. 6.

Maximum color intensity was obtained after 30 min and it was stable for up to more than 24 h, as shown in Fig. 7.

Job's method [9] was applied to study the stoichiometry of the reaction, using 5×10^{-4} M solutions. Results revealed that mequitazine reacts with MBTH in 1:1 ratio, as shown in Fig. 8.

Applying the above experimentally approached optimum conditions, standard calibration graph was constructed. Beer–Lambert law was obeyed in the concentration ranges of 1.00–16.00 μg/ml. The regression equation was calculated as mentioned in Section 2.4.6.

3.6. Spectrophotometric determination of mequitazine using Gibbs reagent

Gibbs reagent is used for the determination of mequitazine in the presence of its degradation

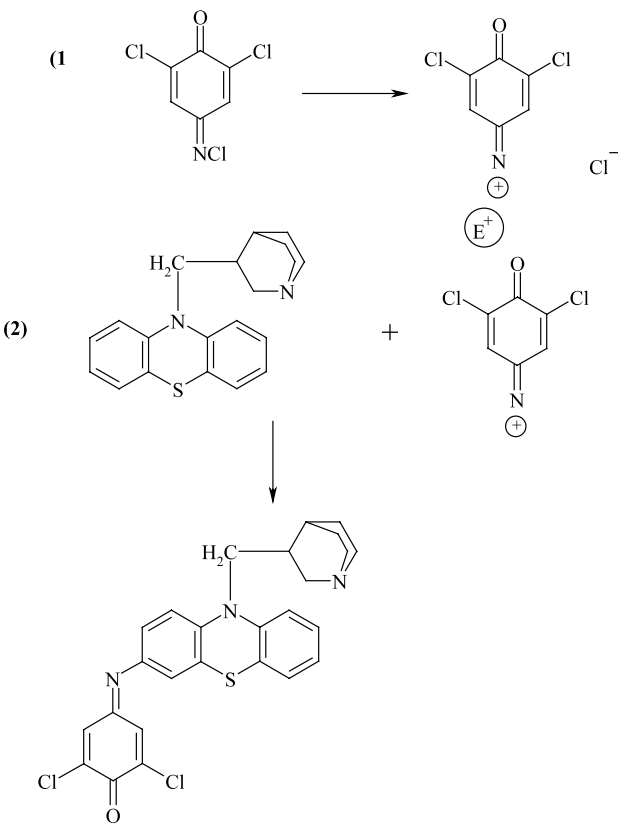


Fig. 12. Scheme for the suggested mechanism of the reaction between mequitazine and Gibbs reagent.

Table 1
Regression equations and S.E. of slopes and intercepts using proposed procedures

Procedure	Regression equation	S.E. of slope	S.E. of intercept
HPLC	$Y = 9.31 \times 10^{-2}X - 1.2 \times 10^{-3}$	294×10^{-4}	1.565×10^{-3}
Densitometry at 256 nm	$Y = 1.5682X + 6.273$	1.6306×10^{-2}	7.938×10^{-2}
Derivative ratio			
At 210.2 nm	$Y = -1.49 \times 10^{-2}X \pm 0.0$	5.2486×10^{-5}	3.256×10^{-4}
At 247 nm	$Y = 4.88 \times 10^{-2}X - 4.0 \times 10^{-4}$	1.22×10^{-4}	7.59×10^{-4}
At 259.8 nm	$Y = 6.28 \times 10^{-2}X - 5.0 \times 10^{-4}$	1.83×10^{-4}	1.133×10^{-3}
First derivative at 290 nm	$D_1 = 4.4 \times 10^{-3} - 1.0 \times 10^{-4}$	4.6156×10^{-5}	2.86×10^{-4}
MBTH at 685 nm	$Y = 6.69 \times 10^{-2}X + 2.0 \times 10^{-4}$	1.37×10^{-4}	1.276×10^{-3}
Gibbs reagent at 405 nm	$Y = 1.84 \times 10^{-3}X - 7.9 \times 10^{-3}$	6.2407×10^{-6}	2.253×10^{-3}

product. Gibbs reagent is more commonly called 2,6-dichloroquinone-4-chloroimide and less commonly, *N*,2,6-trichlor-*p*-quinoneimine. The use of Gibbs reagent as a delicate color reagent for phenols was first claimed by H.D. Gibbs [10].

It is apparent, however, that the reaction is a substitution reaction and is dependent upon the peculiar reactivity of the ortho and para positions of phenols in alkaline medium (pH = 9 using borate buffer) to electrophilic attack [11–14]. Such an activation is also present in aromatic amines [12]. Preliminary work has shown that aromatic amines primary, secondary and tertiary undergo reaction with 2,6-dichloroquinone-4-chloroimide and the reagent is directed to the ortho and para position of amino group [12]. Salicylamide also undergoes reaction with Gibbs reagent and yields an indophenol having maximum absorbance at 650 nm [15].

Ranitidine in aqueous-isopropanol medium reacts with Gibbs reagent at 90 °C and yields a purple color having maximum absorbance at 520 nm [16].

In the present work, mequitazine reacts with Gibbs reagent in aqueous methanolic medium at room temperature and yields a yellowish-brown colored product, having maximum absorbance at 405 nm, as shown in Fig. 9.

Maximum color intensity was obtained after 90 min and it was stable for up to more than 24 h, as shown in Fig. 10.

Job's method [9] was applied to study the stoichiometry of the reaction, using 10^{-3} M solutions. Results revealed that mequitazine reacts with Gibbs reagent in 1:1 ratio, as shown in Figs. 11 and 12.

The reaction of 2,6-dichloroquinone-4-chloroimide is affected by temperature, time of heating and the quantity of 2,6-dichloroquinone-4-chloroimide utilized. However, when the analytical solutions were heated at room temperature, 30, 40, 65 and or 100 °C for 10–120 min, the results showed that the reaction of mequitazine with Gibbs reagent needs no heat, but is stable after 90 min. It is sufficient to work at room temperature.

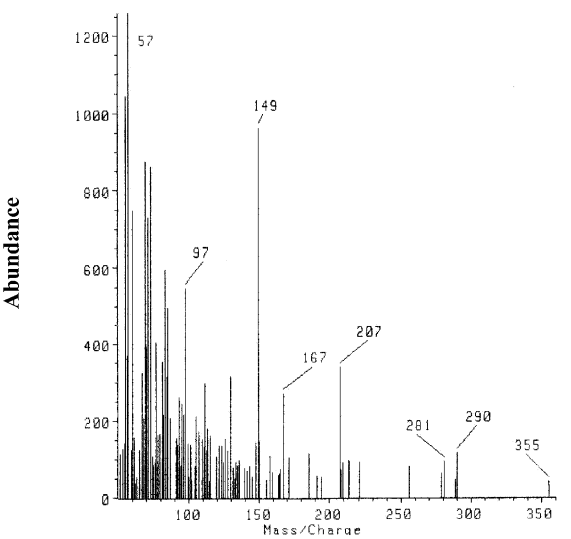


Fig. 13. Mass spectroscopy of the degradation product of mequitazine.

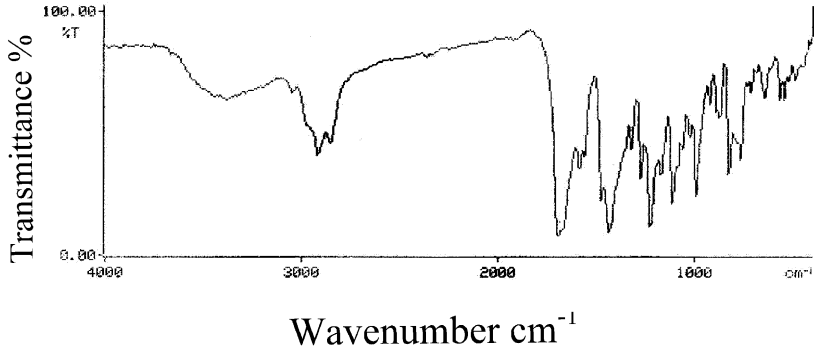


Fig. 14. IR spectroscopy of the degradation product of mequitazine.

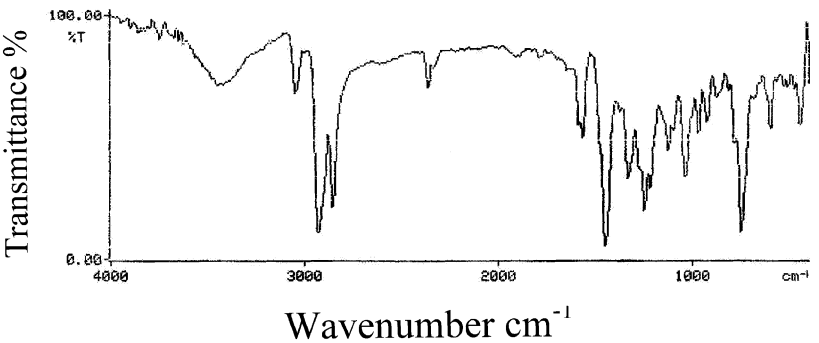


Fig. 15. IR spectroscopy of the pure mequitazine.

Table 2
Determination of mequitazine via the suggested procedures

Procedure	Sensitivity range (μg/ml)	L.O.D.* (μg/ml)	L.O.Q. (μg/ml)	Mean % recovery + S.D.
HPLC	1.00–9.00	0.122	1.00	100.15 ± 0.581
Densitometry at 256 nm	1.25–7.5 μg per spot	1.11 μg per spot	1.25	99.82 ± 1.084
Derivative ratio				
At 210.2 nm	1.00–10.00	0.144	1.00	100.17 ± 0.551
At 247 nm	1.00–10.00	0.141	1.00	100.20 ± 0.517
At 259.8 nm	1.00–10.00	0.137	1.00	100.05 ± 0.508
First derivative at 290 nm	1.00–10.00	0.139	1.00	98.95 ± 1.226
MBTH at 685 nm	1.00–16.00	0.199	1.00	99.96 ± 0.396
Gibbs reagent at 405 nm	50.00–600.00	28.22	50.00	100.21 ± 0.918

Average of at least three separate determinations.

* $\text{L.O.D.} = \dfrac{3.3 \times \text{S.D. for single results}}{\text{Slope of regression equation}}$.

Applying the above experimentally approached optimum values, standard calibration graph was constructed. Beer–Lambert law was obeyed in the concentration ranges of 50.00–600.00 μg/ml.

The regression equation was calculated as mentioned in Section 2.4.7.

Regression equations and S.E. of slopes and intercepts are shown in Table 1.

3.7. Separation and identification of the degradation product

Degradation of mequitazine was influenced by reaction with 30% hydrogen peroxide and reflux for 4 h, then extraction with ether. Etherial extract was evaporated under vacuum and kept in a desiccator. Its m.p. was determined and found to be 148 °C (m.p. of pure mequitazine is 130–131 °C). The degradate was expected to be the corresponding sulphone. This was confirmed by mass spectroscopy, as shown in Fig. 13. It shows the parent peak of the degraded mequitazine at m/z 355, while the whole molecular weight of mequitazine is 322.47. This indicates that the sulfur atom of phenothiazine ring undergoes oxidation with hydrogen peroxide leading to the formation of the corresponding sulphone m/z 355, as shown below.

Table 3
Determination of mequitazine in primalan* tablets by applying the proposed procedures and results of standard addition

Procedure	Claimed	Found	% Found	Mean % recovery**	S.D.
HPLC	2 μg/ml	2	100	99.76	0.607
Densitometry	1.25 μg per spot	1.25	100	99.83	0.470
Derivative ratio					
At 210.2 nm	2 μg/ml	2.01	100.5	99.84	1.457
At 247 nm	2 μg/ml	2.02	101	100.12	0.927
At 259.8 nm	2 μg/ml	2	100	99.76	0.607
First derivative at 290 nm	2 μg/ml	2.07	103.5	101.05	1.996
MBTH at 685 nm	2 μg/ml	2	100	100.06	0.427
Gibbs reagent at 405 nm	100 μg/ml	100	100	100.17	0.625

* Primalan tablets claimed to contain 5 mg of mequitazine per tablet.
** These results are the average of three experiments.

Table 4
Determination of mequitazine in primalan* syrup by applying the proposed procedures and results of standard addition

Procedure	Claimed	Found	% Found	Mean % recovery**	S.D.
HPLC	2 μg/ml	2.01	100.5	99.96	0.342
Densitometry	1.25 μg per spot	1.25	100	100.20	0.396
Derivative ratio					
At 210.2 nm	2 μg/ml	2.01	100.5	100.40	0.710
At 247 nm	2 μg/ml	2	100	100.54	0.615
At 259.8 nm	2 μg/ml	2	100	100.30	0.528
First derivative at 290 nm	2 μg/ml	2.07	103.5	100.65	2.418
MBTH at 685 nm	2 μg/ml	2	100	99.77	0.308
Gibbs reagent at 405 nm	100 μg/ml	100	100	99.90	0.609

* Primalan syrup claimed to contain 1.25 mg of mequitazine per 2.5 ml syrup.
** These results are the average of three experiments.

Table 5
Statistical comparison of the results obtained by adopting the proposed procedures compared to the reference method* for analysis of authentic mequitazine

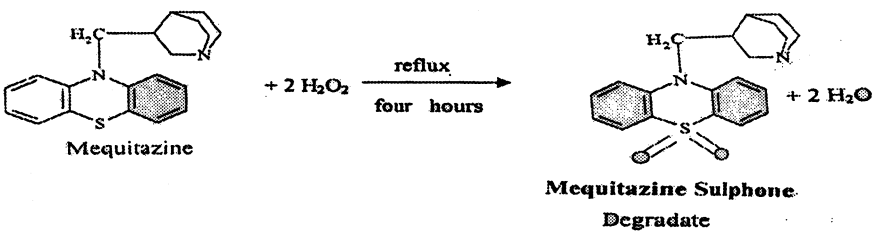
Procedure	Mean ± S.D.	*n*	Variance	Student (*t*-test)	*F*-ratio
Reference method*	99.90 ± 0.728	5	0.530	–	–
HPLC	100.15 ± 0.581	7	0.338	0.533 (2.228)**	1.568 (4.53)**
Densitometry	99.82 ± 1.084	6	1.175	0.137 (2.262)**	2.217 (6.26)**
Derivative ratio					
At 210.2 nm	100.17 ± 0.551	10	0.304	0.634 (2.160)**	1.743 (3.63)**
At 247 nm	100.20 ± 0.517	10	0.267	0.718 (2.160)**	1.985 (3.63)**
At 259.8 nm	100.05 ± 0.508	10	0.258	0.361 (2.160)**	2.054 (3.63)**
First derivative at 290 nm	98.95 ± 1.226	7	1.503	1.823 (2.228)**	2.836 (6.16)**
MBTH at 685 nm	99.96 ± 0.396	7	0.157	0.141 (2.228)**	3.376 (4.53)**
Gibbs reagent at 405 nm	100.21 ± 0.918	7	0.843	0.576 (2.228)**	1.591 (6.16)**

* Quantative UV spectrophotometry in 0.1 N HCl using A (1%–1 cm) at 258 nm.
** Figures in parentheses are the theoretical *t* and *F* values at ($P = 0.05$).

Table 6
Percentage recoveries of mequitazine in the presence of its degradate

Procedure	Wavelength of measurements (nm)	Degradate added					Mean ± S.D.
		10%	20%	40%	60%	80%	
HPLC ($n = 5$)	At 256	100.14	100.16	100.83	101.25	100.63	100.60 ± 0.469
Densitometry ($n = 5$)	At 256	100.44	99.33	100.00	102.00	100.67	100.49 ± 0.988
Derivative ratio ($n = 5$)	At 210.2	101.44	100.63	100.67	100.75	100.50	100.80 ± 0.370
	At 247	100.56	100.00	100.83	100.00	101.00	100.48 ± 0.464
	At 259.8	100.44	100.00	100.00	99.75	100.00	100.04 ± 0.249
First derivative ($n = 5$)	At 290	98.78	99.75	98.83	102.75	103.50	100.72 ± 2.243
MBTH ($n = 5$)	At 685	99.84	99.82	100.36	100.00	100.36	100.08 ± 0.269
Gibbs reagent ($n = 5$)	At 405	99.40	100.21	100.83	100.00	100.83	100.25 ± 0.604

These results are the average of three experiments.



It was further confirmed by IR spectroscopy, as shown in Figs. 14 and 15. IR spectroscopy of the degraded mequitazine Fig. 14 shows a new band at wavenumber 1800–1700 cm^{-1} related to the corresponding sulphone [17]. This band is not found in the pure mequitazine, as shown in Fig. 15.

The proposed methods were successfully applied for the analysis of mequitazine in pure and dosage forms; tablets and syrup, as shown in Tables 2–4, respectively. L.O.D. and L.O.Q. are shown in Table 2. The validity of the procedures was further assessed by applying the standard addition technique. Results obtained are presented in Tables 3 and 4 proving no interference from additives or excipients.

For the purpose of comparison, the results of the suggested procedures were statistically analyzed upon carrying out the reference method for analysis of mequitazine. Results are represented in Table 5, which shows comparable accuracy (t-test) and precision (F-ratio) since the calculated values of (t) and F are less than the theoretical values [18].

To assess the stability-indicating efficiency of the proposed methods, the degradation product of mequitazine was mixed with its intact sample in different ratios (10–80%) and mixtures were analyzed by the proposed methods. The results obtained are shown in Table 6. It is clear that the accuracy of the proposed methods is not affected by up to 80% of the degradation product.

Thus, it could be concluded that the suggested procedures are simple, sensitive and stability indicating. They can be recommended for routine analysis of mequitazine, both in pure and dosage forms, without interference of the degraded mequitazine.

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