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Stability indicating spectrophotometric methods for the determination of Tiemonium Methylsulphate

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Abstract:
Two precise, accurate and sensitive UV-Spectrophotometric methods were developed and validated for the determination of Tiemonium methylsulphate in presence of its degradation product, first derivative spectrophotometric method (^{1}D) and first derivative of ratio spectrophotometry (^{1}DD). Forced degradation study was performed using 2N H_2SO_4 on cold. For the first method; upon examining the first derivative spectra of the drug and its degradation product, it was noticed that Tiemonium methylsulphate can be determined at 250 nm with zero contribution of its degradation product. The linearity range was 5.0 to 60.0 µg/mL. The mean percentage recovery was 100.17±0.622%. For the second method, zero order spectra of the drug were divided by the spectrum of 40 µg/mL of the degradation product as a divisor then the first order of the ratio spectra was obtained using $\Delta\lambda = 8$ and scaling factor 10. The peak amplitudes of the first derivative of the ratio spectra were measured at 250 nm. The linearity range was 5.0 to 60.0 µg/mL and the mean percentage recovery was 100.51±0.982%. Statistical comparison between the results obtained by these methods and those obtained by the manufacturer's method was done, and no significance difference was obtained.

Keywords: Tiemonium methylsulphate, stability studies, first derivative, derivative ratio, H_2SO_4

Introduction

Tiemonium methylsulphate (Fig.1) 4-(3-Hydroxy-3-phenyl-3-(2-thienyl) propyl)-4-methyl-morpholinium methyl sulphate is used as antimuscarinic with peripheral effects similar to those of atropine and is used in the relief of visceral spasms. It has been used as an antispasmodic [1].

Determination of Tiemonium methylsulphate is described in manufacturer's method by UV/VIS Spectrophotometry method; UV-Spectrum of an aqueous solution of Tiemonium methylsulphate was scanned between 200 – 400 nm, should show only one maximum peak at 235 nm.

Only one method was reported for the determination of Tiemonium methylsulphate, Swift Quantification of Fenofibrate and Tiemonium methylsulfate Active Ingredients in Solid Drugs Using Particle Induced X-Ray Emission [2].

This paper presented the study of the acid degradation of Tiemonium methylsulphate, followed by the development of two spectrophotometric stability-indicating methods for the determination of the drug in its pure powder form, in pharmaceutical dosage form and in laboratory prepared mixtures containing different percentages of the degradation product.

Experimental

Instruments

Shimadzu UV-2400 PC Series Spectrophotometer (Tokyo – Japan) with two matched 1cm quartz

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cells using the following spectral parameters; a single fast scan mode and a fixed slit width (2 nm). Connected to an IBM-PC computer loaded with Shimadzu UVPC software was equipped with HP desk jet printer and used for all the absorbance measurements and treatment of data. For isolation of the degradation product, the TLC plates (Merck, Germany) used were 10 x 20 cm, precoated with 0.25mm silica gel 60 F_{254} (Merck, Germany). The sample was applied to the plates using Hamilton micro syringe (10µl). The spots were visualized using UV lamp at 254 nm. For identification of the degradation product, IR Bruker Vector 22 8201 PC spectrometer (Bruker Instruments Ltd, Rheinstetten/ Karlsruhe, Germany) and Mass Spectrophotometer, Hewlett Packard Model 5988A GC/MS (Agilent Technologies, Wilmington, DE) were used.

Materials and Reagents

Tiemonium methylsulphate-Pure sample was kindly supplied by Centaur Pharmaceuticals PVT. LTD. India, B.N. 20094607. Its purity was found to be 99.73%±0.504 according to the manufacturer's method. Sulphuric acid was purchased from Sigma Chemical Co. (St. Louis - USA). While sodium hydroxide, methanol, glacial acetic acid were obtained from ADWIC (Cairo, Egypt). Spasmofree ampoule was supplied by Adwia Pharma, (Cairo, Egypt), B.N. 080904. Each ampoul is claimed to contain 5.0 mg of Tiemonium methylsulphate (5.0 mg/2.0mL). Spasmofree tablet was supplied by Adwia Pharma, (Cairo, Egypt), B.N. 031013. Each tablet is claimed to contain 50.0 mg of Tiemonium methylsulphate.

Preparation of the degradation product

Tiemonium methylsulphate (10.0 mg) was accurately transferred into a 100-mL volumetric flask then we add 2N H2SO4 to the mark. Leave it for 7 hours on cold and tested for complete degradation by TLC using water: methanol: glacial acetic acid (8: 4: 0.2 by volume) as the mobile phase. One spot was visualized under UV lamp at 254 not corresponding to Tiemonium methylsulphate. The degraded solution was then neutralized with 2N NaOH solution respectively till pH was approximately 7. The solution was nearly evaporated to dryness, cooled and transferred quantitatively with methanol to a volumetric flask 100-mL then the volume was completed to the mark to prepare solution of concentration (equivalent to 0.1 mg/mL of intact Tiemonium methylsulphate) in methanol and finally was filtered.

Standard solutions

Standard stock solutions of Tiemonium methylsulphate was prepared in a concentration of (0.1mg/ml) by transferring 10 mg of Tiemonium methylsulphate powder to a 100-ml volumetric flask and dissolved in 50.0 ml methanol, and then the volume was completed with methanol.

Laboratory prepared mixtures containing different ratios of Tiemonium methylsulphate and its degradation product

Aliquots (5.4 – 0.6 mL) of Tiemonium methylsulphate were accurately transferred from its stock standard solution (0.1mg/mL) equivalent to (540.0 – 60.0 µg) into a series of 10-mL volumetric flasks. Aliquots (0.6 – 5.4 mL) of degradation product solution (0.1 mg/mL) equivalent to (60.0 – 540.0 µg) were added, the volume was completed with methanol to prepare mixtures containing 10 – 90 % of the degradation product.

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Construction of Calibration Curves

For first derivative spectrophotometric method (^{1}D)

Aliquots (0.5, 1.0, 2.0, 3.0, 4.0, 5.0 and 6.0 mL) of Tiemonium methylsulphate were accurately transferred from its stock standard solution (0.1mg/mL) equivalent to (50.0, 100.0, 200.0, 300.0, 400.0, 500.0 and 600.0 µg) into a series of 10-mL volumetric flasks. The volume was completed to the mark with methanol. The spectra of the prepared solutions were scanned and stored in the computer. The derivative spectra were obtained using Δ λ=8 and scaling factor 10 and the peak amplitudes of the first derivative spectra of Tiemonium methylsulphate were measured at 250 nm. Linear calibration curve was constructed relating the peak amplitude at 250 nm to the corresponding concentration of Tiemonium methylsulphate and the regression equation was computed.

For the first derivative of ratio spectrophotometry (^{1}DD)

The zero order spectra obtained under (2.6.1) were divided by the spectrum of 40.0 µg/mL of the degradation product as a divisor then the first order of the ratio spectra was obtained using Δ λ = 8 and scaling factor 10. The peak amplitudes of the first derivative of the ratio spectra were measured at 250 nm. Linear calibration curves were constructed relating the peak amplitude of the first derivative of the ratio spectra at 250 nm ($^1DD_{250}$) to the corresponding concentration of Tiemonium methylsulphate and the regression equations were computed.

Application of the proposed methods for the analysis of laboratory prepared mixtures of Tiemonium methylsulphate and its degradation products

For first derivative spectrophotometric method (^{1}D)

The first derivative spectra of laboratory prepared mixtures containing different percentages of Tiemonium methylsulphate and its degradation product (2.5.) were recorded. Then the procedure was completed as described in subsection (.2.6.1.) of Linearity, and then the concentration was calculated from the regression equation.

For the first derivative of ratio spectra (^{1}DD)

The absorption spectra of laboratory prepared mixtures containing different percentages of Tiemonium methylsulphate and its degradation product (2.5.) were recorded. Then the procedure was completed as described in subsection (2.6.2.) of Linearity and then the concentration was calculated using the regression equations.

Application of the proposed methods for the analysis of Tiemonium methylsulphate in pharmaceutical preparation.

Spasmofree ampoule

Five ampoules were mixed carefully. A volume of solution equivalent to 10.0 mg Tiemonium methylsulphate was accurately transferred into a 100-mL volumetric flask, then the volume was completed to the mark with methanol, the flask was shacked well, to have stock solution (0.1 mg/mL). Further dilution with methanol was done to obtain solution of final concentration of (20.0 µg/mL). Then the proposed procedure was completed as described under subsection (2.6) of Linearity for both methods. The concentration of Tiemonium methylsulphate was calculated from the regression equation of the two methods.

Spasmoofree tablet

Five tablets of Spasmofree were weighed accurately and finely powdered in a small dish. An amount of powder equivalent to 10.0 mg Tiemonium methylsulphate was accurately transferred into a 100-mL volumetric flask, 50.0 mL

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of the methanol was added. The flask was sonicated for 30 minutes, then the volume was completed to the mark with the same solvent and finally was filtered to prepare solution of concentration equivalent to (0.1mg/mL). Further dilution with methanol was done to obtain solution of final concentration of (20.0 µg/mL).Then the proposed procedure was completed as described under subsection (2.6) of Linearity for both method. The concentration of Tiemonium methylsulphate was calculated from the regression equation of the two methods.

Result and discussion

Separation and identification of degradation products

The stability of the drug was studied according to ICH guidelines Q2 (R1) [3] for:

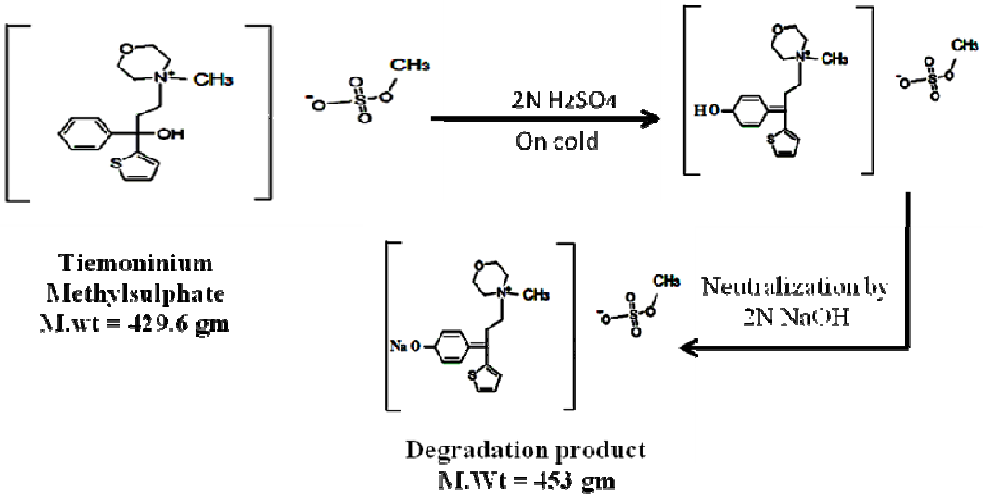
(a) Stress Acid and Alkaline: 7M HCl/7M NaOH for 5 hours, 6M HCl/6M NaOH for 7 hours and 2N H2SO4/2N NaOH 7hours.

(b) Oxidative Condition: 3% H_2O_2 for 2, 4, 6 and for 10 hours.

(c) Thermal Degradation: at 100°C in an oven for 2, 4 and for 6 hours.

The degradation process under the previously mentioned conditions was followed using TLC and the compound was found to be liable to acid degradations by using 2N H_2SO_4 on cold. There is one component which was confirmed by TLC.

The structure of the acidic-induced degradation product was confirmed using IR and mass spectroscopy.



Scheme 1: The degradation pathway of Tiemonium methylsulphate

In the present study, two stability-indicating methods for the simultaneous determination of Tiemonium methylsulphate, in presence of its acidic-induced degradation product were suggested.

For first derivative spectrophotometric method (^{1}D)

Derivative spectrophotometry is a useful technique for extracting qualitative and quantitative information from spectra composed of unresolved bands and for eliminating the effect of baseline shifts and baseline tilts [4,5] .

Zero-order absorption spectra of Tiemonium methylsulphate and its degradation product in methanol show great overlap which cannot

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permit direct measurement of the drug in the presence of its degradation product, Figure (2).

Upon examining the first derivative spectra of the drug and its degradation, Figure (3), it was noticed that Tiemonium methylsulphate can be determined at 250 nm with zero contribution of its degradation product. The main parameters that affect the shape of the derivative spectra such as scanning speed, the wavelength increment over which the derivative is obtained ($\Delta\lambda$) and the scaling factor were studied and it was found that fast scanning speed, $\Delta\lambda$=8 and scaling factor 10, gave best compromise in terms of signals to noise ratio, peak resolution and sensitivity throughout the determination.

For the first derivative of ratio spectra (^{1}DD)

The derivative ratio spectrophotomerty is one of the popular methods used in simultaneous determination of compounds in the presence of interfering substances or mixtures of drugs [6,7] .

The spectra of Tiemonium methylsulphate were divided by its degradation product spectrum as a divisor. The obtained ratio spectra are shown in Figure (4). Upon examining the first derivative of ratio spectrum of Tiemonium methylsulphate, Figure (5), it is noticed that Tiemonium methylsulphate can be determined at 250 nm.

The main parameters that affect the shape of the derivative ratio spectra such as wavelength, scanning speed and the wavelength increment over which the derivative is obtained ($\Delta\lambda$) were studied and it was found that fast scanning speed, $\Delta\lambda$=8 and scaling factor 10 gave best compromise in terms of signals to noise ratio, peak resolution and sensitivity throughout the determination.

Careful choice of the concentration of the divisor was of great importance, so different concentrations of the degradation product were tried as a divisor. It was found that upon division, by 10.0 and 50.0 µg/mL, noisy spectra were obtained.

Division by spectrum of 40.0 µg/mL gave best compromise in terms of sensitivity, repeatability and signals to noise ratio.

Method Validation

Validation of the proposed methods was made by measuring range, accuracy, precision, repeatabilities, interday precision, linearity and specificity. Results obtained are depicted in Table (1). This data render the applicability of the proposed methods for the quality control of the drug formulation.

Linearity

For first derivative spectrophotometric method (^{1}D)

The linear regression data for the calibration curves showed a good linear relationship over a concentration range of 5.0 – 60.0 µg/ml and the regression equation was computed and found to be:

$^1D = 0.01294\ C - 0.0137 \qquad r = 0.9996$

Where ^{1}D is the peak amplitude of first derivative spectra, C is the corresponding concentration (µg/mL) and r is the correlation coefficient.

For the first derivative of ratio spectra (^{1}DD)

A linear relationship between the concentration of Tiemonium methylsulphate and the peak amplitudes at 250 nm was existing. The proposed method was found to be valid in the range of 5.0 – 60.0 µg/mL and the regression equation was computed and found to be:

$^1DD_{250} = 0.0208\ C - 0.0484 \qquad r = 0.9998$

Where ^{1}DD is the peak amplitude for first derivative spectra of the ratio and C is the

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concentration (µg/mL) and r is the correlation coefficient.

Accuracy

The accuracy of two methods were assessed by the determination of pure Tiemonium methylsulphate samples within the linearity ranges, the mean accuracies are given in (Table 1).The recovery percentages (recovery %) and relative standard deviations (RSD) revealed excellent accuracy.

Repeatability and intermediate precision

The repeatability and interday precision were evaluated by assaying three freshly prepared solutions of the drug in triplicate on the same day and on three successive days respectively at concentrations within the linearity range for the two methods. RSD% shows the precision of the methods (Table 1).

The specificity

The specificity of the methods was proved by the analysis of laboratory prepared mixtures containing different percentages of the degradation products. The specificity of the two methods for Tiemonium methylsulphate was achieved in presence of its degradation up to 90% (Table 2).

Assay of pharmaceutical formulation

The usefulness of the proposed methods for the analysis of Tiemonium methylsulphate was studied by assaying Spasmofree ampoule and Spasmofree tablet (Table 3). Standard addition technique was also applied to assess the validity of the proposed method (Table 3).

Comparison with the manufacturer's method

Results obtained by the proposed method for the determination of pure samples of the drug were statistically [8] compared to those obtained by manufacturer's method and no significant differences were observed (Table 4).

Conclusion

The first derivative spectrophotometric (^{1}D) and the first derivative of ratio spectra (^{1}DD) methods proposed are accurate, precise and reproducible. They are stability-indicating methods, so can be used for stability studies to predict the expiry dates of pharmaceuticals. Both methods complied with the validation guidelines of the International Conference on Harmonization and could be used for purity testing, stability studies, quality control, and routine analysis of Tiemonium methylsulphate.

Table 1: Results of validation parameters of the responses and the regression equations obtained by the proposed methods

Parameter	Derivative spectrophotometric method(^{1}D)	Derivative ratio method (^{1}DD)
Validation of regression equation:		
Slope [a] S.E. of slope	0.01294 0.000149	0.0208 0.00015
Intercept [a] S.E. of intercept	-0.0137 0.005381	-0.0484 0.00499
Correlation coefficient	0.9996	0.9998
Validation of response:		
Concentration range	5.0 – 60.0	5.0 – 60.0
Average accuracy % S.D. R.S.D. %	100.17 0.623 0.622	100.51 0.987 0.982
Specificity± R.S.D. %	100.35±0.932	100.16±0.791
Repeatability*[b] %	100.94±0.469	100.38±0.790
Intermediate precision*[c] %	100.35±0.801	100.32±0.543

[a] Results of seven determinations
*[b] =3×3
*[c] =3×3

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Table 2: Results of analysis of Tiemonium methylsulphate in laboratory prepared mixtures containing different ratios of Tiemonium methylsulphate and its degradation product in pure powder form by the proposed methods

Degradation %	Derivative spectrophotometric method(^{1}D)			Derivative ratio method (^{1}DD)		
	Tiemonium methylsulphate (µg/mL)	Degradation (µg/mL)	Recovery %*	Tiemonium methylsulphate (µg /mL)	Degradation product (µg /mL)	Recovery %*
10	54.0	6.0	100.64	54.0	6.0	99.61
20	48.0	12.0	99.11	48.0	12.0	101.12
30	42.0	18.0	99.80	42.0	18.0	100.54
40	36.0	24.0	99.17	36.0	24.0	100.30
50	30.0	30.0	101.45	30.0	30.0	98.92
60	24.0	36.0	100.34	24.0	36.0	99.78
70	18.0	42.0	99.85	18.0	42.0	99.32
80	12.0	48.0	101.27	12.0	48.0	100.89
90	6.0	54.0	101.52	6.0	54.0	101.00
Mean			100.35			100.16
S.D.			0.935			0.792
R.S.D.%			0.932			0.791

* Average of three determinations

Table 3: Quantitative determination of Tiemonium methylsulphate in pharmaceutical formulation by the proposed methods and results of application of standard addition technique

Pharmaceutical formulation	Derivative spectrophotometric method(^{1}D)	Derivative ratio method (^{1}DD)
Spasmofree ampoule (5mg/2mL) B.N. 080904		
Found %[a]	100.11±0.795%	100.41 ± 1.025%
Recovery of standard added %[b]	100.40 ± 0.457%	100.71 ± 0.937%
Spasmofree tablet (50mg/tablet) B.N. 031013	Derivative spectrophotometric method(^{1}D)	Derivative ratio method (^{1}DD)
Found %[a]	99.78±0.651%	99.70±0.909%
Recovery of standard added %[b]	99.28±0.632%	99.80±0.595%

[a] Average of six determinations
[b] Average of six determinations

Table 4: Statistical analysis between the results obtained for the determination of Tiemonium methylsulphate in pure samples by the proposed methods and those obtained by the reported method

Parameters	Derivative spectrophotometric method(^{1}D)	Derivative ratio method (^{1}DD)	Manufacturer's method**
Mean	100.17	100.51	99.73
S.D	0.623	0.987	0.503
R.S.D%	0.622	0.982	0.504
Variance	0.388	0.974	0.253
n	6	6	6
Student's t	1.346 (2.228)*	1.725 (2.228)	
F test	1.534 (5.05)*	3.850 (5.05)	

*The values between parenthesis are the theoretical values of t and F at (p = 0.05).
** UV/VIS Spectrophotometry method; UV-Spectrum of an aqueous solution of Tiemonium methylsulphate was scan between (200 – 400 nm), should show only one maximum peak at 235 nm.

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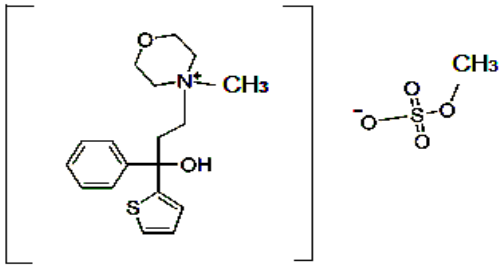
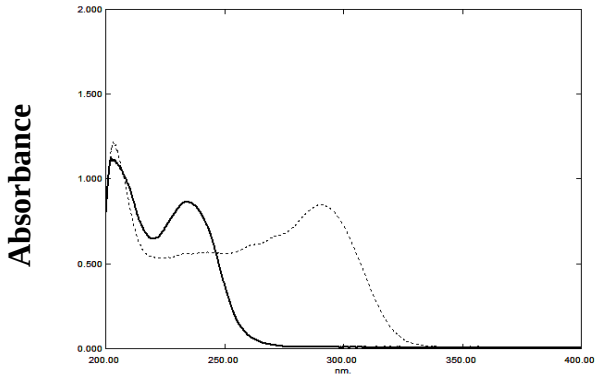
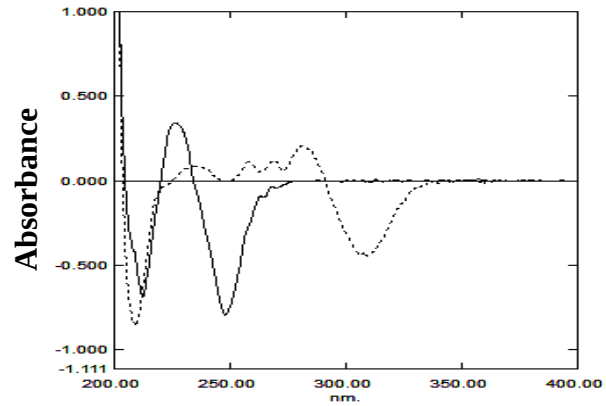


Fig. 1: Tiemonium methylsulphate Molecular formula = $C_{19}H_{27}NO_6S_2$; Molecular weight = 429.6



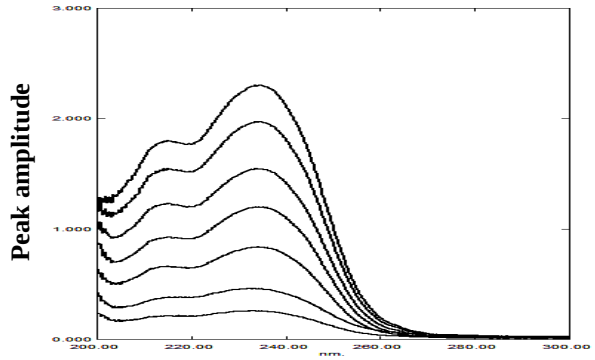
Wavelength (nm)

Figure 2: Zero–order absorption spectra of Tiemonium methylsulphate (50.0µg/mL) (——) and its acidic-induced degradation product (50.0 µg/mL) (…..) in methanol.



Wavelength (nm)

Figure 3: First derivative spectra of Tiemonium methylsulphate (50.0 µg/mL) (——) and its acidic-induced degradation product (50.0 µg/mL) (…..) in methanol.



Wavelength (nm)

Figure 4: Ratio spectra of Tiemonium methylsulphate (5.0 – 60.0 µg/mL) using the spectrum of (40.0 µg/mL) of the degradation product as a divisor.

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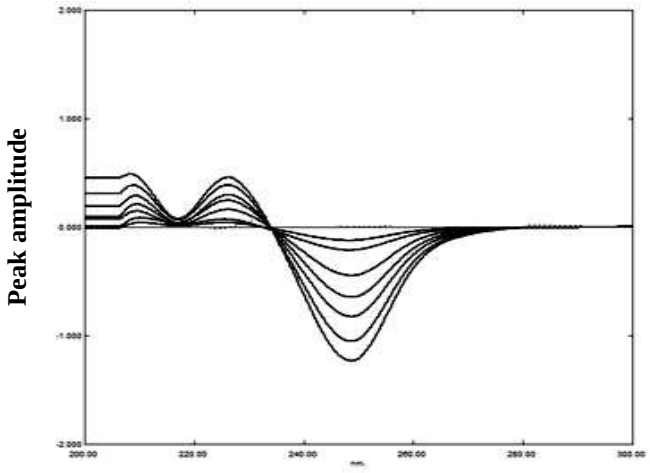

Figure 5: First derivative ratio spectra of Tiemonium methylsulphate (5.0 – 60.0 µg/mL) (——) and its degradation product (54.0 µg/mL) (------) using the spectrum of (40.0 µg/mL) of the degradation product as a divisor.

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