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Spectrophotometric and liquid chromatographic determination of trimebutine maleate in the presence of its degradation products

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Abstract

Three methods are presented for the determination of trimebutine maleate (TM) in the presence of its degradation products. The first method was based on a high performance liquid chromatographic (HPLC) separation of TM from its degradation products using an ODS column at ambient temperature with a mobile phase consisting of acetonitrile—5 mM heptane sulfonic acid disodium salt (45:55, v/v, pH 4) with UV detection at 215 nm. The second method depends on using first derivative spectrophotometry (^{1}D) by measurement of the amplitude at 252.2 nm. The third method depends on using first derivative of the ratio spectrophotometry (^{1}DD) by measurement of the amplitude at 282.4 nm where a normalized spectrum of 3,4,5-trimethoxy benzoic acid is used as divisor. The proposed HPLC and ^{1}D methods were used to investigate the kinetics of acidic and alkaline degradation processes. The pH-rate profile of degradation of TM in Britton–Robinson buffer solutions within the pH range 2–11.9 was studied.

Keywords: Trimebutine; HPLC; First derivative spectrophotometry; Kinetics of degradation; pH-rate profile

1. Introduction

Trimebutine maleate (TM), 2-dimethylamino-2-phenylbutyl-3,4,5-trimethoxybenzoate hydrogen maleate, is antispasmodic and is effective in the treatment of irritable bowel syndrome [1].

The literature survey reveals few spectrophotometric methods for determination of TM using ion-pair formation with bismuth(III)-iodide [2], bromocresol green, bromophenol blue and bromothymol blue; and through charge-transfer complexation with iodine [3]. High performance liquid chromatographic (HPLC) methods for determination of TM [4,5], its metabolite in human plasma [6–8] and related impurities [9] have been employed. Capillary electrophoresis methods have been reported for chiral separation [10] and determination of TM in capsules [11], rat plasma and tissues [12]. TM is an ester type antispasmodic, which can be easily degraded. However, no method has been reported for the assay of TM in the presence of its degradation products. The aim

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of this work was to develop stability indicating methods for determination of TM in presence of its degradation products using HPLC, first derivative spectrophotometry (^{1}D) and first derivative of the ratio spectrophotometry (^{1}DD). Furthermore, the proposed HPLC and ^{1}D methods were used to investigate the kinetics of the acidic and alkaline degradation processes and to calculate the activation energy for TM degradation.

2. Experimental

2.1. Instrumentation

A double-beam Shimadzu (Japan) UV–Visible spectrophotometer, model UV-1601 PC equipped with 1 cm quartz cells and connected to an IBM compatible computer and a HP 600 inkjet printer was used. The bundled software was UVPC personal spectroscopy software version 3.7 (Shimadzu). The spectral bandwidth was 2 nm and the wavelength scanning speed was 2800 nm min^{-1}.

The HPLC (Bischoff, Germany) instrument was equipped with model series 2250 LC pump, Rheodyne 7125 injector with a 20 μl loop and a LC lambda1010 variable-wavelength spectrophotometric detector (Bischoff). Separation and quantitation were made on a 150 × 4.6 mm (i.d) TSK-Gel 5 μm ODS-80 TM column (Tosoh, Japan). The detector was set at 215 nm. Data acquisition was performed on a model MCDACq data acquisition system (version 1.3x).

The IR spectrophotometer used was a Shimadzu IR-435.

^{1}H NMR spectra were recorded on a Varian Gemini 200 PMR spectrometer (200 mHz), USA.

2.2. Materials and reagents

Pharmaceutical grade of TM was kindly supplied by Hoechst Marion Roussel S.A.E. Company for pharmaceuticals and chemical industries, Cairo and certified as 99.99% pure. Acetonitrile used was HPLC grade. Sodium hydroxide, sodium dihydrogen phosphate, hydrochloric, phosphoric, boric and acetic acids were analytical grade.

5 mM phosphate buffer was prepared by dissolving 780 mg of $NaH_2PO_4 \cdot 2H_2O$ in 1 l of distilled water adjusted to pH 6.0 using 1 M NaOH.

The commercial Debridat tablets used was manufactured by Hoechst Marion Roussel S.A.E Cairo containing 100 mg TM per tablet.

2.3. HPLC conditions

The mobile phase was prepared by mixing acetonitrile and 5 mM heptane sulfonic acid disodium salt in a ratio 45:55 v/v, and the apparent pH was adjusted to 4 using acetic acid. The flow rate was 1.5 ml min^{-1}. The injection volume was 20 μl. All determinations were performed at room temperature.

2.4. Preparation of the alkali-induced degradation product

1 g of TM was refluxed with 100 ml 0.1 M sodium hydroxide at 100 °C. Thirty minutes were found to be sufficient for complete degradation. Subsequently the pH of the solution was adjusted to different values using 1 M hydrochloric acid to precipitate the degradation product. The optimum pH for precipitation of the degradation product was found to be 4.5. The precipitate was filtered and dried under vacuum. The dried precipitate was analyzed by IR and NMR and found to be 3,4,5-trimethoxy benzoic acid. The filtrate was washed three times, each with 10 ml chloroform. The washed aqueous extract was evaporated and dried under vacuum. The residue was analyzed by IR and NMR and found to be 2-(dimethyl amino)-2-phenylbutanol.

The stock solution of each of 3,4,5-trimethoxy benzoic acid and 2-(dimethyl amino)-2-phenylbutanol were prepared separately by dissolving 25 mg of each in 25 ml methanol.

2.5. Preparation of the acid-induced degradation product

1 g of TM was refluxed with 100 ml 1 M hydrochloric acid at 100 °C. Twelve hours were found to be sufficient for complete degradation.

Subsequently the pH of the solution was adjusted to different values using 2 M sodium hydroxide. The optimum pH for precipitation of the degradation product was found to be 4.5. The same procedure for separation of 3,4,5-trimethoxy benzoic acid and 2-(dimethyl amino)-2-phenylbutanol previously described under preparation of the alkali-induced degradation product was followed.

2.6. Standard solutions and calibration graphs

A stock solution was prepared by dissolving TM in methanol to obtain a concentration of 250 μg ml^{-1}. The standard solutions were prepared by diluting the stock solution with phosphate buffer pH 6.0 (for spectrophotometric methods) or mobile phase (for HPLC) to reach the concentration range of 5–35 μg ml^{-1} of TM.

2.6.1. For the first derivative method

The first derivative spectra (^{1}D) of the standard solutions were recorded in the range 200–330 nm against solvent blank using $\Delta\lambda = 8$ nm and scaling factor of 10. The values of ^{1}D amplitudes at 252.2 nm (zero-crossing of the degradation products) were measured for the determination of TM in presence of acid-induced or alkali-induced degradation products. The concentrations of TM versus their first derivative amplitudes at 252.2 nm were plotted in order to obtain the calibration graph.

2.6.2. For the first derivative of the ratio spectra method

The standard solution of the degradation product 3,4,5-trimethoxy benzoic acid was prepared by diluting the stock solution with phosphate buffer pH 6.0 to reach the concentration range of 5–35 μg ml^{-1}.

The UV absorption spectra of standard solutions of TM were recorded in the wavelength range 250–300 nm and divided by a normalized spectrum of 3,4,5-trimethoxy benzoic acid [a spectrum of unit concentration]. The first derivative was calculated for the obtained ratio spectra with $\Delta\lambda = 4$ nm. The first derivative of the ratio spectra obtained was smoothed with eight experimental points and scaling factor of 10. The amplitude at 282.4 nm was measured and found to be proportional to the concentration of TM.

2.6.3. For the HPLC method

Triplicate 20 μl injections were made for each concentration of TM and chromatographed under the conditions described above. The peak area of each concentration was plotted against the corresponding concentration to obtain the calibration graph of TM.

2.7. Sample preparation

Ten tablets were weighed and finely powdered. A portion of the powder equivalent to about 25 mg of TM was accurately weighed, dissolved and diluted to 100 ml with methanol. The sample solution was filtered. The filtrate was diluted with phosphate buffer pH 6.0 (for spectrophotometric methods) or mobile phase (for HPLC) to reach the concentration range of 5–35 μg ml^{-1} of TM.

2.7.1. For the ^{1}D and ^{1}DD methods

The ^{1}D and ^{1}DD amplitudes were measured for the sample solution at 252.2 and 282.4 nm, respectively, as described under the calibration graph. The concentration of TM in the sample was calculated from the regression equation.

2.7.2. For the HPLC method

A 20 μl volume of the sample solution was injected into the HPLC, in triplicate. The peak area was used for determination of TM concentration in the sample.

2.8. Kinetic investigation

Accurately weighed 80 mg of TM were dissolved in 100 ml distilled water. Separate 2 ml aliquots of this solution were transferred into separate stoppered conical flasks and mixed with 2 ml of 0.2 M sodium hydroxide or 2 M hydrochloric acid. The flasks were placed in a thermostatic oven at different temperatures (90, 85, 80, 70, 60 °C for acidic degradation and 50, 45, 40, 35, 30 °C for alkaline degradation) for different time intervals. At the specified time the contents of the flasks

were neutralized to pH 7.0 using predetermined volumes of 1 M sodium hydroxide and 0.1 M hydrochloric acid solutions. The contents of the flasks were transferred into 50 ml volumetric flasks and diluted to volume with phosphate buffer pH 6.0 (for the ^{1}D method) or mobile phase (for the HPLC method). Each experiment was repeated three times at each temperature and time interval.

For the ^{1}D method: The ^{1}D amplitude was measured for the solution directly as described under the calibration graph and the concentration of the remaining TM was calculated at each temperature and time interval for the three replicates.

For the HPLC method aliquots of 20 μl of each solution were chromatographed under the conditions described above and the concentration of the remaining TM was calculated at each temperature and at time interval for the three replicates.

2.9. pH-rate profile

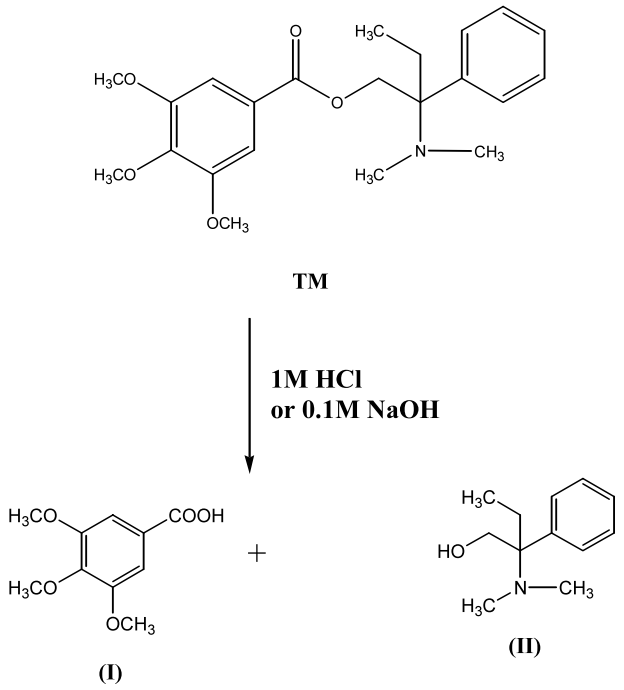
Accurately weighed 50 mg of TM were transferred into 100 ml volumetric flask and diluted to volume with Britton–Robinson buffer solutions [13]. The pH-values of Britton–Robinson buffer solutions used for the measurement of the pH-rate profile of the degradation of TM were as follows; pH 2, 2.7, 3.3, 4.6, 5.8, 6.8, 8, 9.2 and 11.9. The pH values of these buffer solutions were checked before and after the reaction, and were unchanged. Separate 3 ml aliquots of the buffer solution containing TM were transferred into stoppered conical flasks. The flasks were placed in a thermostatic oven at 80 °C for different time intervals. At the specified time interval the contents of flasks were neutralized to pH 7.0 using 1 M sodium hydroxide or 1 M hydrochloric acid solutions. The contents of these flasks were transferred into 50 ml volumetric flasks and diluted to volume with mobile phase. Aliquots of 20 μl of each solution were chromatographed under the conditions described above and the concentration of the remaining TM was calculated at each pH value and time interval.

3. Results and discussion

3.1. Identification of the degradation products

When TM was boiled with 0.1 M sodium hydroxide for 30 min or 1 M hydrochloric acid for 12 h, 3,4,5-trimethoxy benzoic acid (I) and 2-(dimethyl amino)-2-phenylbutanol (II) were formed. The two degradation products occurred in the same ratio in the acid or alkaline degradation processes. The suggested pathway for the degradation in 0.1 M sodium hydroxide and 1 M hydrochloric acid is presented in Scheme 1.

The assignments of the degradation products (I) and (II) as 3,4,5-trimethoxy benzoic acid and 2-(dimethyl amino)-2-phenylbutanol, respectively, were based on the comparison of the IR and PMR spectral data of the purified specimens, separated from the degradation reaction, with those of the intact TM. The spectra obtained were entirely consistent with the proposed structures.



Scheme 1.

3.2. Assay parameters

3.2.1. ^1D method

The UV absorption spectra of TM and its two degradation products (I) and (II) in phosphate buffer pH 6.0 are overlapped (Fig. 1a), while their first derivative spectra (Fig. 1b) showed significant differences in some areas that permit the determination of TM in presence of (I) and (II) by measurement of its first derivative amplitude at the zero-crossing point of its degradation products at 252.2 nm.

3.2.2. ^1DD method

The main advantage of the derivative ratio spectra method (^{1}DD) may be that the measurements are carried out at peaks, hence there is a potential for greater sensitivity and accuracy. The main disadvantage of the zero crossing method in derivative spectrophotometry is the risk of small drifts of the working wavelengths, which may not fall in correspondence of peaks of the derivative spectrum. This may be particularly dangerous when the slope of the spectrum is very high with consequent loss of accuracy and precision [14]. In the present case, the above circumstances did not occur.

To optimize the ^{1}DD method for the determination of TM in presence of its degradation products, it is necessary to test the influence of the variables: divisor concentration, $\Delta\lambda$ and smoothing function. All these variables were studied and $\Delta\lambda = 4$ nm was selected as optimum value. The best results in terms of signal to noise ratio, sensitivity and repeatability followed using a normalized spectrum of (I) as divisor. Due to the extent of the noise levels on the ratio spectra, a smoothing function was used and eight experimental points were considered as suitable.

The UV absorption spectra of TM and its mixture with the two degradation products (I) and (II) in the wavelength range 250–330 nm were divided by the normalized spectrum of (I), where the degradation product (II) has negligible absorbance in this wavelength range. The first derivative was calculated for the obtained ratio spectra with

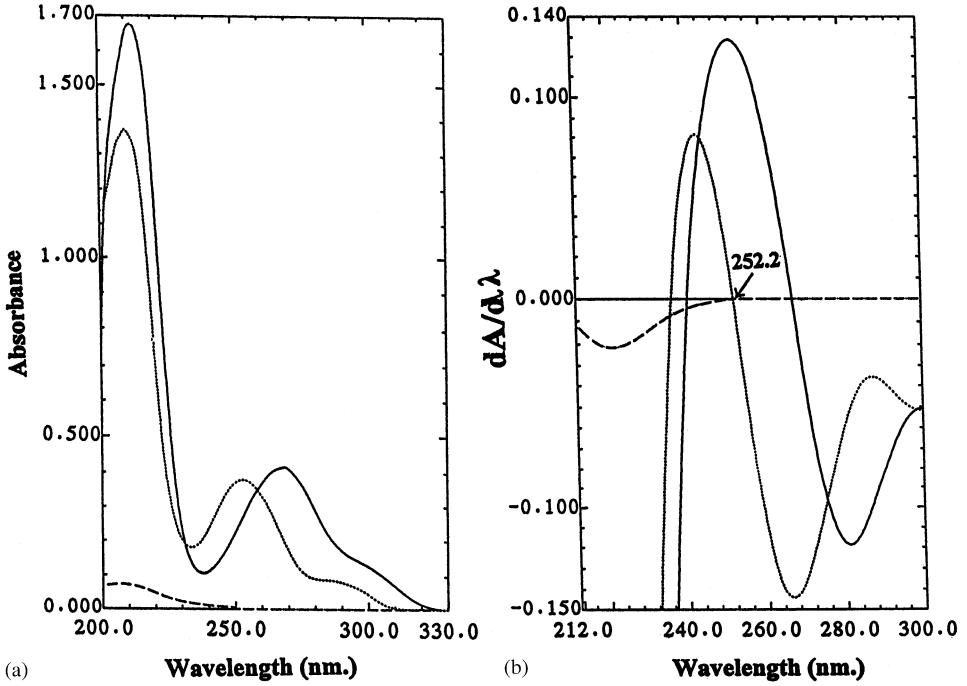


Fig. 1. UV absorption spectra (a) and first derivative spectra (b) of 20 μg ml^{-1} of TM (—), 12.5 μg ml^{-1} of 3,4,5-trimethoxy benzoic acid (------) and 12.5 μg ml^{-1} of 2-(dimethyl amino)-2-phenylbutanol (– – – –) in 5 mM phosphate buffer pH 6.

$\Delta\lambda = 4$ nm. These spectra were smoothed with eight experimental points and scaling factor of 10. The amplitude at 282.4 nm was measured and found to be proportional to the concentration of TM (Fig. 2). The characteristic parameters for regression equation of ^{1}DD method are given in Table 1.

3.2.3. HPLC method

The developed HPLC method has been applied for the determination of TM in presence of its acidic and alkaline degradation products.

To optimize the HPLC assay parameters, the effect of acetonitrile composition and the apparent pH of the mobile phase on the capacity factor (K′) were studied. A satisfactory separation was obtained with a mobile phase consisting of acetonitrile—5 mM heptane sulfonic acid disodium salt (45:55 v/v) at ambient temperature. Increasing acetonitrile concentration to 60% led to inadequate separation of TM and its degradation products (I) and (II). At lower acetonitrile concentration, separation occurred but with excessive tailing and increased retention time for TM. Variation of apparent pH of the mobile phase resulted in maximum K′ value at pH 6.0 with loss of peak symmetry for TM. At lower apparent pH values (2–3) bad resolution for TM, (I) and (II) peaks was observed. At apparent pH 3.5–4.5 improved resolution of the three peaks was observed, however, at apparent pH 4.0 optimum resolution with reasonable retention time was affected.

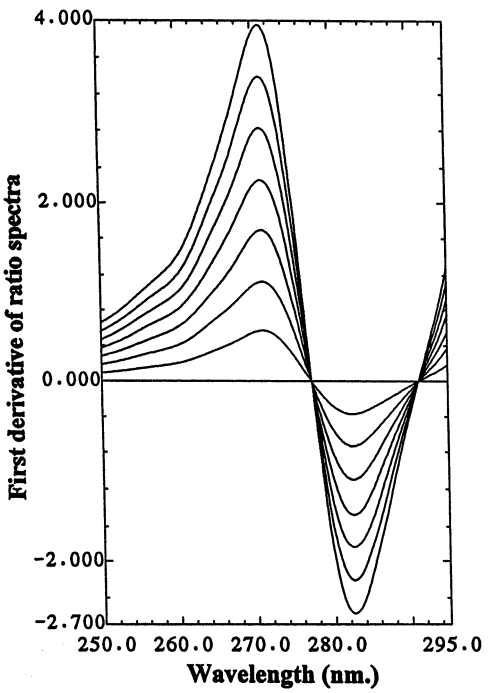


Fig. 2. First derivative of the ratio spectra for different concentrations of TM (5, 10, 15, 20, 25, 30, 35 μg ml^{-1}) using normalized spectrum of 3,4,5-trimethoxy benzoic acid as divisor.

The average retention time ± standard deviation (S.D.) for TM and its degradation products (I) and (II) were found to be 4.51 ± 0.007, 2.06 ± 0.004 and 1.00 ± 0.006 min, respectively, for ten replicates. The peaks obtained were sharp and have clear base line separation.

3.3. Tablet analysis

The three proposed methods were applied to the determination of TM in commercial tablets. Seven replicates determinations were made. Satisfactory results were obtained in a good agreement with the label claims (Table 2). These results were compared with those of the published HPLC method for determination of TM using verapamil as internal standard. The separations were performed on C_{18}-bonded silica column with mixture (82:18, v/v) of acetonitrile and 0.05 M acetate buffer containing 5 mM heptane sulfonic acid adjusted to pH 5.5 with acetic acid as the mobile phase at a flow rate of 1 ml min^{-1} at ambient temperature and UV detection at 267 nm [7]. Statistical comparison of the results was performed with regards to accuracy and precision using Student's *t*-test and the F-ratio at 95% confidence level (Table 2). There is no significant difference between the proposed and the published methods with regard to accuracy and precision.

The proposed methods were found to be easier than the published methods for the determination of TM, whereas there is no need neither for pre-derivatization nor using internal standard while the other published spectrophotometric methods need pre-derivatization with bismuth(III)-iodide [2], bromocresol green, bromophenol blue, bro-

Table 1
Characteristic parameters for the regression equations of HPLC, first derivative (^{1}D) and first derivative of the ratio (^{1}DD) spectrophotometric methods for determination of TM

Parameters	HPLC	^{1}D	^{1}DD
Calibration range (μg ml^{-1})	5–35	5–35	5–35
Detection limit (μg ml^{-1})	2.28×10^{-2}	1.07×10^{-2}	1.05×10^{-3}
Quantitation limit (μg ml^{-1})	7.61×10^{-2}	3.57×10^{-2}	3.48×10^{-3}
Regression equation (Y)[a]: Slope (b)	14.79	6.44×10^{-3}	7.363×10^{-2}
S.D. of the slope (S_b)	1.44×10^{-1}	2.94×10^{-5}	3.28×10^{-5}
Relative standard deviation of the slope (%)	9.71×10^{-1}	4.56×10^{-1}	4.46×10^{-2}
Confidence limit of the slope[b]	14.65–14.93	6.41×10^{-3}–6.47×10^{-3}	7.360×10^{-2}–7.367×10^{-2}
Intercept (a)	−2.12	-2.90×10^{-4}	1.22×10^{-15}
S.D. of the intercept (S_a)	3.20	6.54×10^{-4}	7.33×10^{-4}
Confidence limit of the intercept[b]	(−5.24)–1.00	(-9.2×10^{-4})–3.5×10^{-4}	(-7.1×10^{-4})–7.1×10^{-4}
Correlation coefficient (r)	0.9999	0.9999	0.9999
Standard error of estimation	1.44	2.93×10^{-4}	3.27×10^{-4}

[a] $Y = a + bC$,where C is the concentration of TM in μg ml^{-1} and Y is the peak area for HPLC method and the amplitude at the specified wavelength for ^{1}D and ^{1}DD methods.
[b] 95% confidence limit.

mothymol blue and iodine [3]. The other published HPLC and capillary electrophoresis methods used internal standard such as propyl para-hydroxybenzoate sodium salt [5], procaine [6], verapamil [7], bifendate [9], caffeine [11] and ephedrine hydrochloride [12]. Moreover, the three proposed methods are the first publication for determination of TM in presence of its degradation products.

Expired commercial tablets stored at ambient temperature under normal conditions was analyzed by the three proposed methods and the mean percentage of TM ± S.D. ($n = 7$) were found to be 85.0 ± 0.95, 84.5 ± 0.94 and 85.4 ± 0.92, determined by ^{1}D, ^{1}DD and HPLC methods, respectively. The HPLC chromatogram of expired commercial tablet showed the peaks of the two degradation products of TM (Fig. 3).

3.4. Kinetic investigation

The kinetic of degradation of TM was investigated in 0.1 M sodium hydroxide and 1 M hydrochloric acid, since the decomposition rate of TM at lower strength of hydrochloric acid was too slow to obtain reliable kinetic data. Each experiment was repeated three times at each temperature and time interval. The mean concentration of intact TM ± S.D. was calculated for each experiment. A regular decrease in the concentration of intact TM with increasing time intervals

Table 2
Determination of TM in synthetic mixtures and commercial tablets using the proposed methods

	Mean found ± S.D.[a]			
	HPLC	^{1}D	^{1}DD	Published HPLC [7]
Synthetic mixtures	100.0 ± 0.25	99.9 ± 0.36	99.9 ± 0.64	
Commercial tablets	99.0 ± 0.45	98.9 ± 0.36	99.4 ± 0.76	98.7 ± 0.52
t	1.15	0.84	2.01	(2.18)[b]
F	1.34	2.09	2.13	(4.28)[b]
Recovery[c]	99.9 ± 0.19	99.9 ± 0.23	100.0 ± 0.48	

[a] Mean and S.D. for seven determinations; percentage recovery from the label claim amount.
[b] Theoretical values for t and F.
[c] For standard addition of different concentrations of TM; 3,4,5-trimethoxy benzoic acid and 2-(dimethyl amino)-2-phenylbutanol.

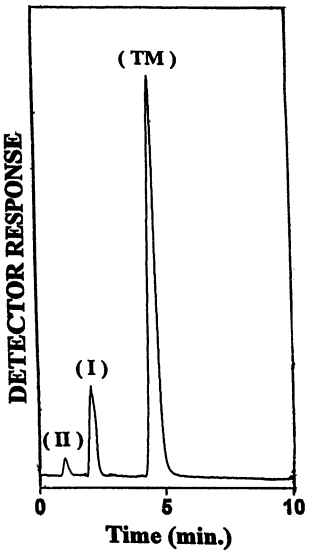


Fig. 3. HPLC chromatogram of expired Debridat tablets containing TM and its two degradation products 3,4,5-trimethoxy benzoic acid (I) and 2-(dimethyl amino)-2-phenyl butanol (II).

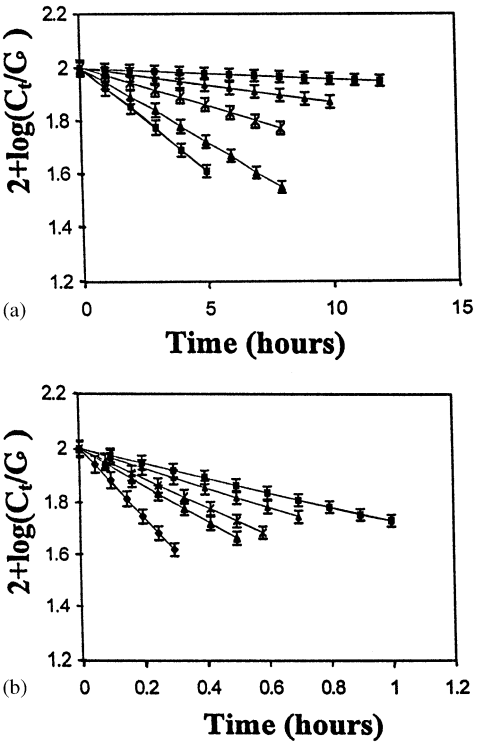


Fig. 4. Pseudo first-order plots for the degradation of TM in (a) 1 M hydrochloric acid and (b) 0.1 M sodium hydroxide at various temperatures using HPLC method. Key, 60 (■); 70 (▲); 80 (×); 85 (△) and 90 (□); for acidic degradation, 30 (■); 35 (▲); 40 (×); 45 (△); and 50 (□) for alkaline degradation; C_t, concentration at time t, and C_0, concentration at zero time. Vertical bar represents the S.D. of the mean.

was observed. At the selected temperature (60, 70, 80, 85 and 90 °C for the acidic degradation; and 30, 35, 40, 45 and 50 °C for alkaline degradation) the degradation process followed pseudo first order kinetic (Fig. 4). From the slopes of the straight lines it was possible to calculate the apparent first order degradation rate constant and the half-life at each temperature for both acidic and alkaline degradation processes determined by ^{1}D and HPLC methods (Table 3). Plotting log K_{obs} values versus 1/T, the Arrhenius plots (Fig. 5) were obtained, which were found to be linear in the temperature range 60–90 °C for acidic degradation and 30–50 °C for alkaline degradation of TM. The activation energy was calculated to be 23.35 and 23.91 kcal mol^{-1} for acidic degradation process, and 13.70 and 13.75 kcal mol^{-1} for alkaline degradation process, determined by the proposed HPLC and ^{1}D methods, respectively.

The pH-rate profile of degradation of TM in Britton–Robinson buffer solutions was studied at 80 °C using the HPLC method (Fig. 6). Britton–Robinson buffer solutions were used throughout the entire pH range in order to avoid possible effects of different buffer species. The apparent first order degradation rate constant and the half-life were calculated for each pH value (Table 4). TM was found to be most stable at a pH of 3.3.

3.5. Validation

3.5.1. Linearity

The linearity of the proposed methods was evaluated by analyzing seven concentrations of TM. ranging between 5 and 35 μg ml^{-1}. Each concentration was repeated three times. The assay was performed according to experimental conditions previously established. The linearity of the calibration graphs and adherence of the system to Beer's law were validated by the high value of the correlation coefficient and the intercept value,

Table 3
Degradation rate constant (K_{obs}) and half-life ($t_{1/2}$) for TM in 1 M hydrochloric acid and 0.1 M sodium hydroxide (determined by HPLC and ^{1}D methods)

Temperature (°C)	K_{obs} (h^{-1})		$t_{1/2}$ (h)	
	HPLC	^{1}D	HPLC	^{1}D
In 1 M hydrochloric acid				
60	0.010	0.010	69.300	69.300
70	0.029	0.028	23.896	24.750
80	0.072	0.071	9.625	9.761
85	0.126	0.122	5.500	5.680
90	0.186	0.180	3.726	3.850
In 0.1 M sodium hydroxide				
30	0.617	0.617	1.123	1.123
35	0.871	0.861	0.796	0.805
40	1.243	1.245	0.558	0.557
45	1.780	1.778	0.389	0.390
50	2.510	2.512	0.276	0.276

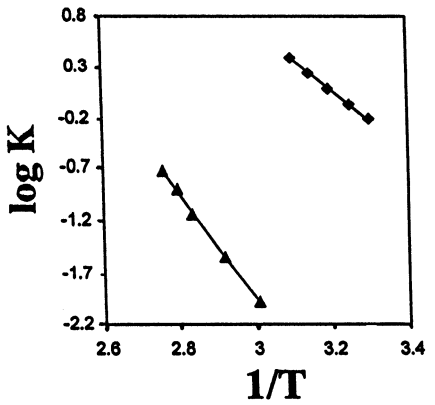


Fig. 5. Arrhenius plots for the degradation of TM in 1 M hydrochloric acid (▲) and 0.1 M sodium hydroxide (■) using HPLC method.

which was not statistically ($P < 0.05$) different from zero (Table 1).

In order to test the mutual independence of the ^{1}D value for TM, ten calibration graphs were constructed from the ^{1}D signals at 252.2 nm for standard solutions containing 5–35 μg ml^{-1} of TM in absence or presence of different concentrations of degradation products (I) and (II) ranging from 5 to 35 μg ml^{-1}. This experiment showed that the amplitude height of ^{1}D at 252.2 nm was proportional to the concentration of TM in

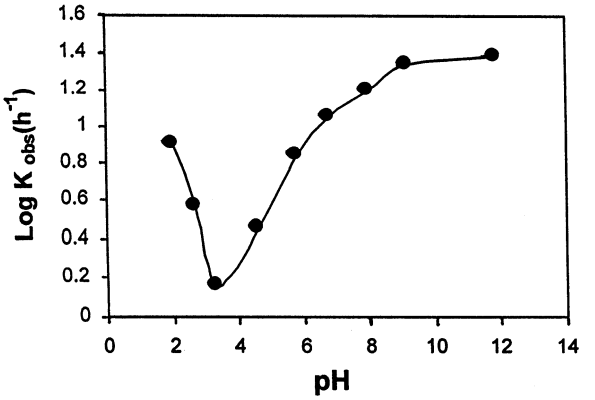


Fig. 6. pH-rate profile for the decomposition of TM in Britton–Robinson buffer at 80 °C.

Table 4
Degradation rate constant (k_{obs}) and half-life ($t_{1/2}$) for TM in Britton–Robinson buffer at different pH values and a temperature of 80 °C

PH	k_{obs} (h^{-1})	$t_{1/2}$ (h)
2.0	0.900	0.770
2.7	0.565	1.226
3.3	0.158	4.386
4.6	0.453	1.530
5.8	0.837	0.828
6.8	1.050	0.660
8.0	1.200	0.578
9.2	1.341	0.517
11.9	1.380	0.502

presence or absence of its degradation products. The slopes of the calibration graphs of TM were virtually independent of degradation products. Therefore, it can be deduced that amplitude of the ^{1}D signal measured at 252.2 nm was functionally of TM.

3.5.2. Precision

For evaluation of the precision estimates, repeatability and intermediate precision were performed at three concentration levels for TM. The data for each concentration level were evaluated by one-way analysis of variance (ANOVA). An 8 days × 2 replicates design was performed. Statistical comparison of the results was performed using the P-value of the F-test. Three univariate analyses of variance for each concentration level

were made. Since the P-value of the F-test is always greater than 0.05, there is no statistically significant difference between the mean results obtained from one level of day to another at the 95% confidence level.

3.5.3. Range

The calibration range was established through consideration of the practical range necessary, according to TM concentration present in pharmaceutical product, to give accurate, precise and linear results. The calibration ranges of the proposed methods are given in Table 1.

3.5.4. Detection and quantitation limits

According to the ICH recommendations [15] the approach based on the S.D. of the response and the slope was used for determining the detection and quantitation limits. The theoretical values were assessed practically and given in Table 1.

3.5.5. Selectivity

Methods selectivity was achieved by preparing different mixtures of TM at various concentrations within the linearity range and its degradation products (I) and (II) within ratio ranged from 1:0.4:0.2 to 1:3.5:2 for TM:(I):(II), respectively. The synthetic mixtures were analyzed according to the previous procedures described under the proposed methods. Satisfactory results were obtained (Table 2), indicating the high selectivity of the proposed methods for determination of TM in presence of its degradation products.

3.5.6. Accuracy

This study was performed by addition of known amounts of TM and its degradation products (I) and (II) to a known concentration of the commercial tablets (standard addition method). The resulting mixtures were assayed and the results obtained for TM were compared with the expected results. The excellent recoveries of standard addition method (Table 2) suggested good accuracy of the proposed methods.

The influence of the commonly used tablet excipients (lactose, starch, magnesium stearate, talc and microcrystalline cellulose) was investigated before the determination of TM in tablets. No interference could be observed with the proposed methods.

3.5.7. Robustness

Variation of the pH of the HPLC mobile phase by ± 0.1 and its organic strength by $\pm 1.5\%$ did not have a significant effect on HPLC chromatographic resolution. Variation of pH of the phosphate buffer by ± 0.1 did not have a significant effect on ^{1}D and ^{1}DD amplitude in spectrophotometric methods.

3.5.8. Stability

The TM solution in mobile phase or phosphate buffer pH 6.0 exhibited no chromatographic or spectrophotometric changes for 5 h when kept at room temperature, and for 2 days when stored refrigerated at 5 °C.

4. Conclusion

The proposed HPLC, ^{1}D and ^{1}DD methods provide simple, accurate and reproducible quantitative analysis for the determination of TM in pharmaceutical tablets, without any interference from the excipients and degradation products. The HPLC method was found to be more specific and selective than the ^{1}D and ^{1}DD methods. While the ^{1}D and ^{1}DD methods have the advantages of low cost and speed. The proposed methods were found to be easier than published methods for the determination of TM. It was found that TM is rapidly degraded in alkaline medium, while it is more stable in acidic medium. The most stability of TM was found to be at pH 3.3.

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