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Derivative spectrophotometric, thin layer chromatographic-densitometric and high performance liquid chromatographic determination of trifluoperazine hydrochloride in presence of its hydrogen peroxide induced-degradation product

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Abstract

Three methods are presented for the determination of trifluoperazine HCl in presence of its hydrogen peroxide induced degradation product. The first method was based on measurement of first (^{1}D) and second (^{2}D) derivative amplitudes of trifluoperazine HCl in 0.1 N hydrochloric acid at the zero crossing point of its sulfoxide derivative, main degradation product, (at 268.4 and 262.5 nm for ^{1}D and ^{2}D, respectively). The second method was based on the separation of trifluoperazine HCl from its sulfoxide derivative followed by densitometric measurement of the intact drug spot at 255 nm. The separation was carried out on Merck aluminum sheet of silica gel 60 F_{254}, using chloroform–methanol (7:3 v/v) as mobile phase. The third method was based on high performance liquid chromatographic separation of trifluoperazine HCl from its sulfoxide derivative on reversed phase, ODS column, using a mobile phase of acetonitrile–phosphate buffer pH 4.2 (60:40 v/v) at ambient temperature. Quantitation was achieved with UV detection at 255 nm based on peak area. The first derivative spectrophotometric method was utilized to investigate the kinetics of the hydrogen peroxide degradation process at different temperatures. The apparent pseudo first-order rate constant, half life and activation energy were calculated.

Keywords: First and second derivative spectrophotometry; Thin layer chromatographic-densitometry; Reversed phase high performance liquid chromatographic; Trifluoperazine HCl; Stability

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1. Introduction

Trifluoperazine HCl, 10-[3-(4-methyl-1-piperazinyl)propyl]-2-(trifluoromethyl)-10H-phenothiazine dihydrochloride, (I) is a phenothiazine tranquilizer with anti-emetic effect. The official method for the determination of (I) is non-aqueous titration with perchloric acid, determining the end point potentiometrically [1] or using crystal violet indicator [2]. Various spectrophotometric methods have been reported for the determination of (I) by measurement the UV absorption at 256 nm [1] and using different reagents including bromocresol purple [3], potassium chlorate [4] and formaldehyde [5]. Other different methods have been reported for the determination of (I) including fluorimetry with eosin [6], spectrophotometric titration using 2,3-dichloro-5,6-dicyano-1,4-benzoquinone [7], conductimetric titration with $NaVO_3$ [8], potentiometric titration with tetrabutylammonium periodate [9], titrimetry with ferricyanide using methylene blue as indicator [10], flow injection spectrophotometry using analyte oxidation in manganese dioxide-packed reactors [11], or iron perchlorate [12], flow injection fluorimetry based on photo-oxidation of the drug [13] or oxidation by Ce (IV) [14], and high performance liquid chromatographic (HPLC) using ODS column and 0.01 M tetrabutyl ammonium sulfate–methanol (1:1) at pH 3.5 as mobile phase [15]. Simultaneous determination of (I) and tranylcypromine sulphate in tablets using first and fourth derivatives ultraviolet spectrophotometry [16] or (I) and chlorpromazine HCl in suspension using HPLC with Lichrosorb C_{18} column and acetonitrile–water (20:1) containing NH_4ClO_4 as mobile phase [17] have been reported. A photostability-indicating HPLC method for determination of (I) in bulk form and pharmaceutical formulations using μ-Bondapak C_{18} column with acetonitrile – 0.1% H_3PO_4 (23:17) as mobile phase has been reported [18].

The International Conference on Harmonization (ICH) guideline entitled 'stability testing of new drug substances and products' requires that stress testing be carried out to elucidate the inherent stability characteristics of the active substance [19]. Susceptibility to oxidation is one of the required tests. Also the hydrolytic and the photolytic stability are required. (I) is a phenothiazine derivative, which can be easily oxidized. The aim of this work was to develop stability indicating methods for determination of (I) in presence of its oxidative product. No derivative spectrophotometric and thin layer chromatographic (TLC) stability-indicating methods have been reported in the literature for the determination of (I) in the presence of its oxidative degradation product.

El-Gindy et al. previously introduced two spectrophotometric methods for simultaneous determination of trifluoperazine HCl and isopropamide iodide in tablets using second derivative and second derivative of the ratio spectra; while the present work presents three methods for determining (I) in the presence of its hydrogen peroxide induced – degradation product using first (^{1}D) and second (^{2}D) derivatives spectrophotometry, TLC-densitometry and HPLC. Furthermore, the developed first derivative spectrophotometric method was used to investigate the kinetics of the hydrogen peroxide degradation process at different temperatures. The three proposed methods are stability indicating methods and they are easier than the published methods for analysis of (I). The derivative spectrophotometric method has the advantages of the speed, low cost and environmental protecting. While, the two chromatographic methods are more specific than the derivative spectrophotometric method.

2. Experimental

2.1. Instrumentation

A double-beam Shimadzu (Japan) 1601 PC UV–Visible spectrophotometer connected to a computer fitted with UVPC personal spectroscopy software version 3.7 (Shimadzu) was used. The spectral bandwidth was 2 nm and the wavelength scanning speed was 2800 nm min^{-1}.

The absorbance spectra of test and reference solutions were recorded in 1 cm quartz cells over the range 190–400 nm. The first derivative ($dA/$

$d\lambda$) and second derivative ($d^2A/d\lambda^2$) of the measured spectra were obtained using the accompanying software with $\Delta\lambda = 4$ nm and scaling factor of 10.

The HPLC (Perkin-Elmer, Norwalk, CT) instrument was equipped with a model series 410 LC pump, Rheodyne 7125 injector with a 20 μl loop and a LC-235 photodiode array detector, Separation and quantitation were made on a 150×4.6 mm^2 (i.d.) PHENOMENEX®, PRODIGY 5 μ ODS, (5 μm particle size). The detector was set at $\lambda = 255$ nm. Data acquisition was performed on a model 1022 PE NELSON (Perkin-Elmer).

TLC plates (20×20 cm^2, aluminum plates precoated with 0.25 mm silica gel F_{254}) were purchased from E. Merck (Darmstadt, Germany). The samples were applied to the TLC plates using 10 μl Hamilton microsyringe. A Shimadzu dual wavelength flying spot densitometer Model CS-9000 was used. The experimental conditions of the measurement were: wavelength = 255 nm, photo mode = reflection, scan mode = zigzag, swing width = 16.

2.2. Materials and reagents

Pharmaceutical grade of trifluoperazine HCl was kindly supplied by Kahira Pharmaceutical and Chemical Industries Company (Cairo, Egypt) and certified to contain 100.0%. The water for HPLC was prepared by double glass distillation and filtrations through a 0.45 μm membrane filter. The acetonitrile used was HPLC grade (Honil, England). Other reagents were of analytical grade.

Phosphate buffer pH 4.2 was prepared by dissolving 1.15 g of monobasic ammonium phosphate in 1000 ml of water. The pH was adjusted to 4.2 using 10% phosphoric acid and 1 N sodium hydroxide.

The commercial Stelazine sugar coated tablets used (batch no. 410640) was manufactured by Kahira Pharmaceutical and Chemical Industries Company (Egypt), under license from Smith Klein and French Laboratories Limited. Each tablet contains 5 mg of trifluoperazine HCl, in addition to tablet excipients consisting of lactose, cellulose, crosscarmellose sodium, gelatin, magnesium stearate, F.D.&C. blue, titanium dioxide, and hydroxy propyl methyl cellulose.

2.3. Chromatographic conditions

The mobile phase of HPLC was prepared by mixing acetonitrile with phosphate buffer pH 4.2 in a ratio 60:40 v/v. the mobile phase was filtered using a 0.45 μm membrane filter (Millipore, Milford, MA) and degassed by ultrasonic vibration prior to use. The samples were also filtered using 0.45-μm disposable filters. The flow rate was 1 ml min^{-1}. All determinations were performed at ambient temperature. The injection volume was 20 μl.

The TLC plates were developed in chloroform–methanol (7:3 v/v) as a mobile phase. For detection and quantification, 10 μl of test and 10 μl of different concentrations of the standard solutions within the quantitation range were applied as separate compact spots 15 mm apart and 15 mm from the bottom of the TLC plate using 10 μl Hamilton microsyringe. The plate was developed up to the top (over a distance of 15 cm) in the usual ascending way. The chromatographic tank was saturated with mobile phase in the usual mode. After elution the plate was air dried and scanned for (I) at 255 nm as under the described instrumental parameters.

2.4. Preparation of the hydrogen peroxide induced-degradation product (II)

Accurately weighed 100 mg of (I) were dissolved in 50 ml distilled water. Subsequently, 1 ml of hydrogen peroxide 33.3% v/v was added and the solution was heated in boiling water bath for 30 min till the removal of excess hydrogen peroxide. The solution was quantitatively transferred to 100-ml volumetric flask and the volume was completed to 100 ml with distilled water. The material was tested for complete degradation using the HPLC system described above. A single peak at retention time = 1.51 min was observed. While no peak was observed at retention time = 2.84 min corresponding for (I).

2.5. Standard solutions and calibration graphs

2.5.1. For ^1D and ^2D methods

Standard solutions of (I) in the concentration range 2–16 μg ml^{-1} were prepared in 0.1 N hydrochloric acid. The values of ^1D and ^2D amplitudes were measured at 268.4 and 262.5 nm, respectively. The ^1D and ^2D amplitudes were plotted against the concentration of (I). Linear relationships were obtained.

2.5.2. For thin layer chromatographic method

Standard solutions of (I) in the concentration range 100–800 μg ml^{-1} were prepared in distilled water. Each standard solution of 10 μl were applied to the TLC plate. The plates were chromatographed and the peak areas were measured. Calibration graph was constructed by plotting peak areas versus concentrations of (I). Linear relationship was obtained.

2.5.3. For high performance liquid chromatographic method

Standard solutions of (I) in the concentration range 1.25–7.5 μg ml^{-1} were prepared in mobile phase. Triplicate 20 μl injections were made for each concentration and chromatographed. The average peak areas were calculated and plotted against concentrations. Linear relationship was obtained.

2.6. Sample preparation

Twenty tablets were weighed and powdered. An accurately weighed quantity of the powder tablets equivalent to 50 mg trifluoperazine HCl was extracted with 50 ml distilled water and filtered quantitatively into separating funnel. The filtrate was alkalinized by addition of 2 ml of 10% sodium hydroxide to liberate the drug free base, which was extracted four times each with 20 ml chloroform. The combined chloroformic extract was evaporated under nitrogen. The residue was dissolved by adding 2 ml of 0.01 N hydrochloric acid to regenerate the drug hydrochloride salt, which was extracted four times each with 10 ml distilled water. The combined aqueous extracts were collected in 50-ml volumetric flask and the volume was completed to 50 ml with distilled water. Further dilutions of the aqueous extract were made with 0.1 N hydrochloric acid (for ^1D and ^2D methods), distilled water (for TLC method) and mobile phase (for HPLC method) to suit each method. The general procedures for the three methods described under calibration were followed.

2.7. Percent recovery study

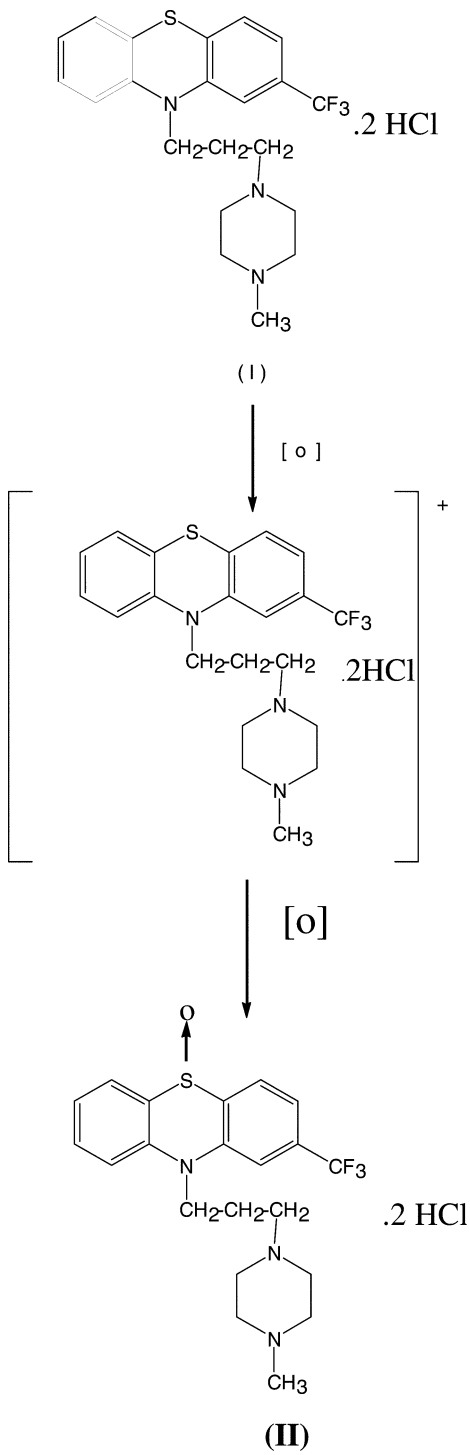
This study was performed by adding (I) and (II) to a known concentration of the commercial tablet (standard addition method). The resulting mixtures were assayed and the results obtained for (I) were compared with expected results.

2.8. Kinetic study

Accurately weighed 100 mg of (I) were dissolved in 100 ml distilled water. Ten milliliter of this solution were transferred into 250 ml volumetric flask, 1 ml of 33.3% hydrogen peroxide was added and the volume was completed to 250 ml with distilled water. For each temperature investigation, separate 25 ml aliquots of the above solution were transferred into separate covered conical flasks. The flasks were placed in thermostatic oven at different temperatures (90, 80, 70, 65, and 60 °C) for different time intervals. At specified time intervals, the contents of the flasks were quantitatively transferred to 100-ml volumetric flasks, 10 ml of 1 N hydrochloric acid was added and the volume was completed to 100 ml with distilled water. The ^1D amplitude was measured for the solution directly at 268.4 nm and the concentration of the remaining (I) was calculated for each temperature and time interval.

3. Results and discussion

(I) is subject to air and light induced oxidative degradation. The mechanism can be considered a two-step reaction involving the intermediate formation of a semiquinone free radicle, which is then oxidized to the sulfoxide [20] (Scheme 1). When (I) was heated with hydrogen peroxide in



Scheme 1. Mechanism of oxidation of trifluoperazine HCl.

boiling water bath for 30 min, a sulfoxide derivative (II), as a main degradation product, was formed.

3.1. D and ^{2}D methods

The main instrumental parameters that affect the shape of the derivative spectra are the wavelength scanning speed, the wavelength increment over which the derivative is obtained ($\Delta\lambda$) and the smoothing. These parameters need to be optimized to give a well-resolved large peak and to give good selectivity and larger sensitivity in the determination. Generally, the noise level decreases with an increase in $\Delta\lambda$ thus decreasing the fluctuation in the derivative spectrum. However if the value of $\Delta\lambda$ is too large, the spectral resolution is very poor. Therefore, the optimum value of $\Delta\lambda$ should be determined by taking into account the noise level and the resolution of the spectrum. Some values of $\Delta\lambda$ were tested. $\Delta\lambda = 4$ nm and wavelength scanning speed $= 2800$ nm min^{-1} were selected for the ^{1}D and ^{2}D method as the optimal conditions to give a satisfactory signal to noise ratio.

The UV absorption spectra of (I) and (II) in 0.1 N hydrochloric acid are overlapped (Fig. 1a), while their first (Fig. 1b) and second (Fig. 1c) derivative spectra showed significant differences in some areas that permits the determination of (I) in the presence of (II). (I) was determined by measurement of its first or second derivative amplitude at the zero – crossing point of II (at 268.4 or 262.5 nm, respectively).

In general, the characteristics profiles of the derivative spectra may constitute a specific fingerprint useful for the drug identification, in particular, the ratios between the amplitudes at selected wavelength can be regarded suitable parameters useful to confirm the drug identity, purity and stability [21]. The maxima ratio of the maximum value of $^1D_{250\ nm}/^1D_{265.8\ nm}$ of (I) was found to be 0.555 with relative standard deviation (SD) of 0.45% ($n = 7$) and the maxima ratio of the maximum value of $^2D_{268\ nm}/^2D_{261.8\ nm}$ of (I) was found to be 0.925 with relative SD of 0.39% ($n = 7$). These figures were altered in the presence of (II) in the sample.

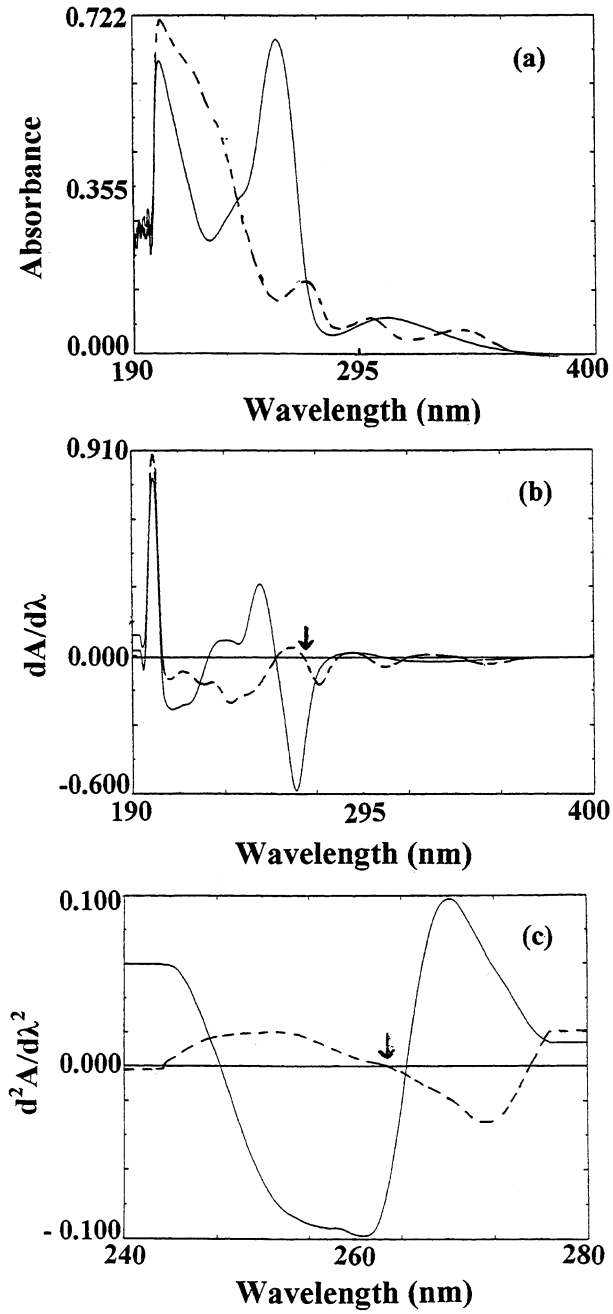


Fig. 1. (a) UV absorption, (b) first derivative and (c) second derivative spectra of 10 μg ml^{-1} of trifluoperazine HCl (– – – – –) and 10 μg ml^{-1} of its sulfoxide derivative (---------) in 0.1 N hydrochloric acid.

3.2. Thin layer chromatographic method

The experimental conditions for TLC method such as mobile phase composition, scan mode and wavelength of detection, were optimized to provide accurate, precise and reproducible results for determination of (I) in presence of (II). The chosen scan mode was the zigzag mode and the wavelength of scanning was chosen to be 255 nm. The greatest differences between the R_f values of the two compounds (0.77 and 0.00 for (I) and (II), respectively) were obtained by the system containing chloroform–methanol in ratio 7:3 v/v, respectively.

3.3. High performance liquid chromatographic method

The developed HPLC method has been applied for the determination of (I) in presence of (II). To optimize the HPLC assay parameters, the mobile phase composition and pH were studied. A satisfactory separation was obtained with a mobile phase consisting of acetonitrile–phosphate buffer pH 4.2 (60:40 v/v) at ambient temperature. Increasing acetonitrile concentration to more than 75% led to inadequate separation of (I) and (II). At lower acetonitrile concentration ($< 40\%$) separation occurred but with excessive tailing and increased retention time for (I) peak. Variation of apparent pH of the mobile phase resulted in maximum K value at apparent pH 7.7 with loss of peak symmetry for (I). At lower apparent pH value (2.5–3.3) bad resolution was observed for (I) and (II) peaks. At apparent pH 3.8–4.6 improved resolution was observed. At apparent pH 4.2 optimum resolution without peak tailing was observed. The effect of temperature on the separation of (I) and (II) was studied by changing the temperature in steps of 2 °C from 20 to 30 °C. Variation in temperature did not have a significant effect on separation and peak shape. Quantitation was achieved with UV detection at 255 nm based on peak area.

The specificity of the HPLC method is illustrated in Fig. 2 where complete separation of (I) and (II) was noticed. The average retention time $\pm$ SD for (I) and (II) were found to be

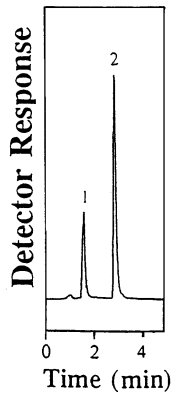


Fig. 2. HPLC chromatogram of 20 μl injection of synthetic mixture of 5 μg ml^{-1} of trifluoperazine HCl (2) and 10 μg ml^{-1} of it's sulfoxide derivative (1).

2.84 ± 0.009 and 1.51 ± 0.008 min, respectively, for ten replicates. The peaks obtained were sharp and have clear base line separation.

Under the described experimental conditions of the above mentioned methods, plots of the ^{1}D amplitudes, ^{2}D amplitudes, peak area of TLC method and peak area of HPLC method for the compound (I) versus the concentrations of the compound (I) within the range stated in the Table 1 show linear relationships. The regression analysis of these plots using the method of least squares was made (Table 1). The linearity of the calibration graphs was validated by the high values of correlation coefficients of the regression equations.

3.4. Accuracy of the proposed methods

The accuracy of the proposed methods was checked by analyzing different synthetic mixtures of (I) and (II) at various concentrations ranged from 6–14 μg ml^{-1} for (I) and 5–15 μg ml^{-1} for (II) [for ^{1}D method]; from 8–16 μg ml^{-1} for (I) and 5–30 μg ml^{-1} for (II) [for ^{2}D method]; from 1–6 μg per spot for (I) and 1–6 μg per spot for (II) (for TLC method); from 1.25–7.5 μg ml^{-1} for (I) and 5–15 μg ml^{-1} for (II) (for HPLC method). Satisfactory results were obtained (Table 2), indicating the high repeatability and accuracy of the proposed methods. The above mentioned methods were able to determined (I) in presence of (II) in ratios up to 1:2, 1:3, 1:6 and 1:12 for ^{1}D, ^{2}D, TLC, and HPLC methods, respectively. The proposed methods can be used as stability indicating methods for (I).

Table 1
Characteristic parameters for the regression equations of first derivative (^{1}D), second derivative (^{2}D) spectrophotometric, thin layer chromatographic (TLC) and high performance liquid chromatographic (HPLC) methods for determination of trifluoperazine HCl

Parameters	^{1}D	^{2}D	TLC	HPLC
Linearity	2–16	2–16	1–8	1.25–7.5
Regression equation (Y)[a]: slope (b)	46.55×10^{-3}	81.67×10^{-4}	36.70×10^{4}	2.83×10^{6}
SD of the slope (S_b)	2.32×10^{-4}	5.05×10^{-5}	2.35×10^{3}	8.4×10^{3}
Relative SD of the slope (%)	0.50	0.62	0.64	0.30
Confidence limit of the slope[b]	$46.05–47.05 \times 10^{-3}$	80.59×10^{-4} -82.75×10^{-3}	$36.19–37.21 \times 10^{4}$	$2.81–2.85 \times 10^{6}$
Intercept (a)	1.25×10^{-3}	7.5×10^{-4}	-19.00×10^{3}	-3.46×10^{3}
SD of the intercept (S_a)	2.34×10^{-3}	1.52×10^{-4}	1.3×10^{3}	4.34×10^{2}
Confidence limit of the intercept[b]	(-3.76×10^{-3}) $-(-6.26 \times 10^{-3})$	4.25×10^{-4} -1.07×10^{-3}	(-21.83×10^{3}) $-(-16.17 \times 10^{-3})$	(-4.41×10^{3}) $-(-2.51 \times 10^{3})$
Correlation coefficient (r)	0.9998	0.9998	0.9998	0.9998

[a] $Y = a + bC$, where C is the concentration of trifluoperazine HCl in μg ml^{-1} for ^{1}D, ^{2}D and HPLC methods; and μg per spot for TLC method; Y is the ^{1}D amplitude or ^{2}D amplitude or peak area for TLC and HPLC methods.
[b] 95% confidence limit.

Table 2
Determination of trifluoperazine HCl in synthetic mixtures and commercial tablets using the proposed first derivative (^{1}D), second derivative (^{2}D) spectrophotometric, thin layer chromatographic (TLC), high performance liquid chromatographic (HPLC) and published HPLC methods

	Mean found ± SD[a]				
	^{1}D	^{2}D	TLC	HPLC	Published HPLC
Synthetic mixtures	99.6 ± 0.31	99.9 ± 0.51	100.2 ± 0.89	99.7 ± 0.24	
Commercial tablets	100.0 ± 0.59	100.4 ± 0.60	99.9 ± 1.18	99.5 ± 0.89	99.8 ± 0.80
t	0.49	1.47	0.17	0.61	(2.23)[b]
F	1.84	1.77	2.18	1.24	(5.05)[b]
Recovery[c]	99.8 ± 0.67	99.6 ± 0.85	99.9 ± 0.89	99.8 ± 0.76	

[a] Mean and SD for six determinations, percentage recovery from the label claim amount.
[b] Theoretical values for *t* and *F*.
[c] For standard addition of 50% of the nominal content ($n = 6$).

3.5. Method validation

Spiked placebos were prepared according to the manufacturing formula. The spiked placebos were tested at five levels: 50, 75, 100, 125, and 150% of label claim for the drug. Assays were performed in duplicate on two samples at the five levels. This was repeated with a second instrument, standard and sample preparation and analyst on different days. The complete set of validation assays was performed for the drug, determined by the proposed methods. Spiked placebo assays were used to determine accuracy and precision of the proposed methods for determination of the drug. The recoveries ranging from 99.4 to 100.6% of the amount of active ingredient spiked into the placebo. The bias showed only minor variation in recovery at each level with 0.5% the maximum variation observed. The proposed methods were tested for repeatability, reproducibility, selectivity, specificity, robustness and ruggedness. Satisfactory results were obtained. The proposed methods complied with USP [2] validation guidelines.

The non-instrumental methods for determination of the detection limit and the quantitation limit were applied [2], the limit of detection is generally determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be reliably detected. While the limit of quantitation is generally determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be determined with acceptable accuracy and precision. The detection limits of the proposed methods were found to be 0.2, 0.3, 0.1 and 0.12 μg ml^{-1} for (I), detected by ^{1}D, ^{2}D, TLC, and HPLC methods, respectively. While the quantitation limits of the proposed methods were found to be 0.9, 1.0, 0.5, and 0.6 μg ml^{-1} for (I), determined by ^{1}D, ^{2}D, TLC, and HPLC methods, respectively.

The stability of (I) during the analytical procedures was studied and found to be stable. The analyte was stable for at least 10 h in solution, when it is protected from light.

3.6. Tablet analysis

The proposed methods were applied to the determination of (I) in commercial tablets after extracting the active ingredient to eliminate the interference resulted from colored sugar coat. Six replicates determination were made. Satisfactory results were obtained and were in a good agreement with the label claims (Table 2). Moreover, to check the validity of the proposed methods, the standard addition method was applied by adding (I) and (II) to the previously analyzed tablets. The recovery of (I) was calculated by comparing the concentration obtained from the spiked mixtures with those of the pure (I). The results of analysis

of the commercial tablets and the recovery study (standard addition method) of the (I) (Table 2) suggested that there is no interference from any excipients, which are normally present in tablets.

The results of determination of (I) in tablets obtained from the proposed ^{1}D, ^{2}D, TLC, and HPLC methods were compared with those of the published HPLC method using μ-Bondapak C_{18} column with acetonitrile–0.1% H_3PO_4 (23:17) as mobile phase [18]. Statistical comparison of the results was performed with regard 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 ^{1}D, ^{2}D, TLC, and HPLC methods and published HPLC method with regard to accuracy and precision.

Expired batch of Stelazine tablets stored at ambient temperature under normal conditions was analyzed by the proposed HPLC method, the degraded drug (sulfoxide derivative) was found.

3.7. Kinetic investigation

To assess the specificity and selectivity of the ^{1}D method for the assay of (I) without interference from (II), hydrogen peroxide induced degradation of (I) was carried out under the previously described experimental conditions. A regular decrease in the concentration of (I) with increasing time was observed. The influence of temperatures on the degradation process in hydrogen peroxide is shown in Fig. 3. At the selected temperatures (90, 80, 70, 65, and 60 °C), the degradation process followed pseudo first-order kinetics. 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, determined by ^{1}D method (Table 3). Plotting log K_{obs} values versus $1/T$, the Arrhenius plot (Fig. 4) was obtained, which was found to be linear in the temperature range 60–90 °C. The activation energy was calculated to be 16.27 K Cal mole^{-1}.

4. Conclusion

The proposed ^{1}D, ^{2}D, TLC, and HPLC methods provide simple, accurate and reproducible quantitative analysis for the determination of trifluoperazine HCl in pharmaceutical tablets and in the presence of its oxidative degradation product. The HPLC method was found to be more specific and selective than the derivative spectrophotometric methods. While the derivative spectrophotometric methods have the advantages of lower cost, rapid and environment protecting. The TLC method is simple and uses a minimal volume of solvents, compared to the HPLC method. The proposed methods were found to be easier than the published methods for determination of trifluoperazine HCl. The proposed methods complied with USP validation guidelines.

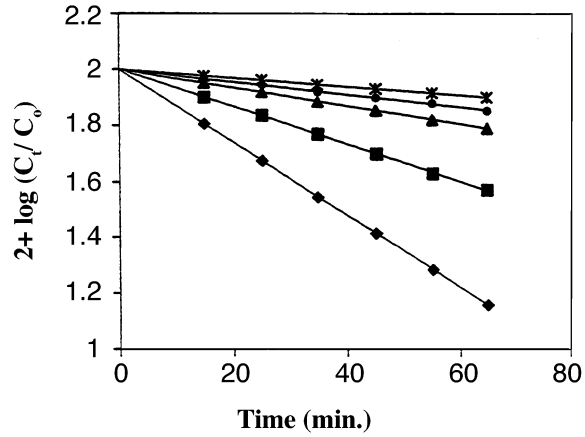


Fig. 3. Pseudo first-order plots for the degradation of trifluoperazine HCl with hydrogen peroxide at various temperature using first derivative spectrophotometric method. Key: 90 °C (◆), 80 °C (■), 70 °C (▲), 65 °C (●), 60 °C (∗); C_t, concentration at time t; and C_0, concentration at time zero.

Table 3
Degradation rate constant (K_{obs}) and half-life ($t_{1/2}$) for trifluoperazine HCl in presence of hydrogen peroxide (determined by first derivative spectrophotometric method)

Temperature (°C)	K_{obs}(h^{-1})	$t_{1/2}$ (h)
90	1.795	0.386
80	0.922	0.752
70	0.449	1.543
65	0.309	2.239
60	0.210	3.297

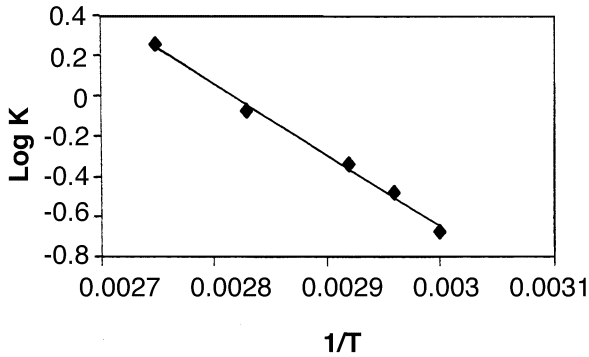


Fig. 4. Arrhenius plot for the degradation of trifluoperazine HCl in presence of hydrogen peroxide.

References

[1] British Pharmacopoeia. Her Majesty's Stationery Office, London, 1998, p. 1328, 1980.
[2] The United States Pharmacopeia, 24 revision, Asian Edition, United States Pharmacopeial Convention, Inc., Twinbrook Parkway, Rockville, MD, 2000, p.1703, 2149–2152.
[3] J.R. Waston, J. Pharm. Sci. 59 (1970) 391.
[4] C. Radhkrishnamutry, G.S. Rao, J. Inst. Chem. India 56 (2) (1984) 79 through Anal. Abst. (1985) 4E44.
[5] S.S. Zarapkar, K.R. Athalye, Indian Drugs 26 (1) (1988) 32–36.
[6] N.A. Zakhari, M. Rizk, M.I. Walash, F. Ibrahim, Anal. Lett. 18 (B11) (1985) 1405–1417.
[7] M. Rizk, N.A. Zakhari, F. Ibrahim, M.I. Walash, Mikrochim. Acta I (5–6) (1989) 355–363.
[8] K. Basavaiha, P.G. Ramappa, Indian J. Pharm. Sci. 51 (6) (1989) 231–233.
[9] M.H. Pournaghi-Azar, K. Farhadi, Talanta 44 (10) (1997) 1773–1781.
[10] A.S. Issa, M.S. Mahrous, Talanta 31 (4) (1984) 287–288.
[11] M. Polasek, J. Dolejsova, R. Karlicek, Pharmazie 53 (3) (1998) 168–172.
[12] M.A. Kouspparis, A. Barcuchova, Analyst 111 (3) (1986) 313–318.
[13] B. Laassis, J.J. Aaron, Analusis 25 (6) (1997) 183–188.
[14] T. Perez-Ruiz, C. Martinez-Lozano, V. Tomas, C. Sidrach de Cardona, Talanta 40 (9) (1993) 1361–1365.
[15] Y.P. Patela, U.J. Dhorda, M. Sundaresan, Talanta 47 (3) (1998) 625–630.
[16] M. Knochen, G. Altesor, I. Dol, Analyst 114 (1989) 1303–1305.
[17] S.M. Desai, J.M. Khatri, J.K. Lalla, Indian Drugs 27 (7) (1990) 389–391.
[18] E.M. Abdel-Moety, K.A. Al-Rashood, A. Rauf, N.A. Khattab, J. Pharm. Biomed. Anal. 14 (11) (1996) 1639–1644.
[19] ICH, Stability Testing of New Drug Substances and Products, International Conference on Harmonization, Geneva, October 1993.
[20] D.C. Borg, G.C. Cotzias, Proc. Nat. Acad. Sci. 48 (1962) 617 through Florey's 'Nowadays Brittan', Analytical profile 9 (1980) 561.
[21] D. Bonazzi, R. Gotti, V. Andrisano, V. Cavrini, J. Pharm. Biomed. Anal. 16 (1997) 431–438.