ISSN : 0974-7419 Volume 10 Issue 7

Trade Science Inc.

Analytical CHEMISTRY

An Indian Journal

Full Paper

ACAIJ, 10(7) 2011 [470-480]

Novel spectrophotometric techniques for determination of trazodone hydrochloride in bulk powder and pharmaceutical formulation

A.E.El-Gindy[1], M.Farouk[2], O.Abd El-Aziz[2]*, E.A.Abdullah[1]

[1]Pharmaceutical Chemistry Department, Faculty of Pharmacy, Misr International University, KM 28 Cairo-Ismailia Road (ahmed Orabi District), Cairo, (EGYPT)

[2]Analytical Pharmaceutical Chemistry Department, Faculty of Pharmacy, Ain Shams University, African Union Authority St. Abbassia, Cairo, (EGYPT)

E-mail : dr_omarghonim@hotmail.com

Received: 12th October, 2010 ; Accepted: 22nd October, 2010

ABSTRACT

Three simple novel spectrophotometric methods were established for the analysis of Trazodone (TRZ). The proposed methods are stability indicating for determination of Trazodone in presence of its degradation products, obtained by stressing Trazodone by alkali and hydrogen peroxide in presence of heat, respectively. The adopted zero-crossing derivative, derivative ratio and pH-induced difference spectrophotometric techniques were proved to be sensitive and accurate, where the accuracy of the assay was evaluated using the standard deviation coefficient of variation which were found to be within the acceptable limit of 2% and were specifically 0.761 & 0.598%, 0.389 & 0.341% and 0.738 & 0.406% for intraday and interday samples, respectively. All the proposed methods were validated according to the International Conference on Harmonization (ICH) guidelines reference and successfully applied for determination of Trazodone Hydrochloride in pure form, in laboratory prepared mixtures and in pharmaceutical formulation. The obtained results were statistically compared to the reference method of analysis for Trazodone Hydrochloride and no significant differences were found.

KEYWORDS

Trazodone hydrochloride;
Derivative spectrophotometry;
Ratio spectra;
Difference spectrophotometry;
Stability indicating methods.

INTRODUCTION

Trazodone hydrochloride (TRZ), 2-[3-(4-m-Chlorophenylpiperazin-1-yl)propyl]-1,2,4-triazolo[4,3-a]pyridin-3(2H)-one hydrochloride is a white or almost white crystalline powder, soluble in water; sparingly soluble in alcohol; practically insoluble in ether, should be stored in airtight containers and protected from light, having a chemical formula $C_{19}H_{22}ClN_5O$,HCl with molecular weight 408.3[1].

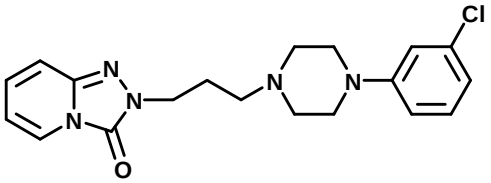


Figure 1 : Chemical structure of trazodone

It is an antidepressant drug indicated in symptomatic treatment of moderate to severe depression. Its major advantages include a low incidence of anticholinergic and cardiovascular side effects along with mini-

mal stimulatory effects upon dopamine and nor epinephrine receptors and considered particularly useful in geriatric population[2]. Trazodone is SSRIs which are a new group of chemically unique antidepressant drugs that specifically inhibit serotonin reuptake[3,4], commercially available as Trittico®.

The ICH-guidelines[5] requires performing stress-testing of the drug substance that can help in identifying the likely degradation-products, also can be useful in establishing the degradation-pathways and validating the stability-indicating power of the analytical procedures used. Moreover, validated stability-indicating method should be applied in the stability study[6]. Stability-indicating methods can be specific one that evaluates the drug in the presence of its-degradation products, excipients and additives[7]. In this perspective, several methods have been reported for the analysis of Trazodone in pure form and pharmaceutical preparations, the official methods are potentiometric non-aqueous titration with perchloric acid[8] and chromatographic technique using octadecyl silane column and methanol-0.01 M ammonium phosphate buffer pH 6.0 (60:40) as mobile phase[9], also various methods have been reported for determination of Trazodone including UV absorption measurement at 246nm[10], ion-selective electrode[11,12], voltametry[13] and capillary gas chromatography, gas chromatography-mass spectrometry and instrumental thin-layer chromatography[14,15]. But, till now neither derivative, derivative-ratio nor pH-induced difference spectrophotometric have been reported for determination of Trazodone HCl in pharmaceutical formulation.

The present work, establishes new simple, accurate, rapid and reproducible stability indicating zero-crossing technique, utilizing three different ways of analysis including derivative, derivative-ratio and pH-induced difference spectrophotometry for the determination of Trazodone Hydrochloride in pharmaceutical formulation, which can be used for the routine quality control analysis of these drugs in raw material and pharmaceutical formulations and for stability studies.

MATERIALS AND METHODS

Chemicals and reagents

Trazodone Hydrochloride was kindly supplied by Egyptian International Pharmaceutical Industrial Company (E.I.P.I.Co), Egypt, and certified to contain 100.0%. Trittico® tablets: Batch number 051427 were manufactured by Egyptian International Pharmaceutical Industrial Company (E.I.P.I.Co), Egypt, under license from F. Angelini, Italy. Each tablet contains 100 mg Trazodone Hydrochloride in addition to some excipients consisting of starch, sodium glycolate, povidone, lactose, microcrystalline cellulose, magnesium stearate, dibasic calcium phosphate and F.D. & C. yellow lack.

Bi-distilled water (Riedel-dehaen, Sigma-Aldrich, Germany), Hydrochloric acid (Adwic), aqueous (0.01M and 2M), Sodium Hydroxide (Adwic), aqueous (2M), Hydrogen Peroxide 30% (E. Merck, Germany) and TLC aluminium plates pre-coated with silica gel 60 F_{254} (E.Merck).

All chemical and reagents used through this work are of spectroscopic analytical grade. Bi-distilled water is used throughout the whole work and is indicated by the word "water".

Instruments

A double-beam Shimadzu (Japan) UV-VIS Spectrophotometer (UV-1601 PC), model TCC-240 A; connected to an IBM compatible computer and HP 695 C DeskJet printer is used. The bundled software is UVPC personal spectroscopy software version 3.7 (Shimadzu). The spectral bandwidth is 2 nm and the wavelength scanning speed was 2800.0 $nmmin^{-1}$. The absorption spectra of the reference and the test solutions are recorded in1.0-ml quartz cells at 25.0 ^{0}C, using '$\Delta\lambda = 4$ nm and scaling factor of 10 for computing first derivative (D^1)' and '$\Delta\lambda = 8$ nm and scaling factor of 100 for second (D^2) third (D^3) derivatives'.

A (Jenway 3510, UK) pH-meter, equipped with combined glass electrode for pH adjustment.

Standard solutions

Standard solution of the studied drug

Stock standard solutions of Trazodone Hydrochloride having concentration of (0.1 $mg.ml^{-1}$) were prepared in 0.01 M HCl and water, for the 'derivative and derivative-ratio' and the pH-induced difference spectrophotometry, respectively. These solutions were used as standard working solutions.

Full Paper

Standard solution of trazodone hydrochloride alkaline and oxidative-degradates

Stock standard solutions of Trazodone Hydrochloride alkaline and oxidative-degradates, having concentration of (0.1 mg.ml^{-1}) were prepared by "adding 10 ml of 2M NaOH to 10 mg of (TRZ), left in a water bath for 17 hours and the neutralized using 2M HCl" and "adding 10 ml of 30%v/v hydrogen peroxide to 10 mg of (TRZ) for 5 hours" and then the volumes were made up to the mark with 0.01 M HCl and water respectively, as discussed under (2.3.1.). Those prepared degradated solutions were used as working standard solutions.

Complete degradation was checked by TLC using silica gel F254 plates using chloroform: methanol: ammonia (50:30:0.5v/v) as a mobile phase.

Procedures

Spectrophotometric methods

Derivative spectrophotometric method (D^n)

From stock standard solution of Trazodone Hydrochloride, aliquots were transferred into a series of 50 ml volumetric flasks, and diluted to volume with 0.01 M HCl to obtain a concentration range of '4.0-38.0, 2.0-34.0 and 2.0-40.0 μg.ml^{-1}' and '2.0-40.0 μg.ml^{-1}', respectively. The values of 'first (D^1), second (D^2) and third (D^3)' derivative spectrophotometry amplitudes at 257.90, 245.50 and 234.0 0 nm (Zero-crossing of alkaline-degradate) and 'first (D^1) and third (D^3)' derivative spectrophotometry amplitudes at 290.30 and 231.80 nm (Zero-crossing of oxidative-degradate) were then computed, plotted versus corresponding concentrations; and the regression equations were then computed, respectively.

Derivative ratio spectrophotometric method (DR^n)

Calibration curve was performed by transferring aliquots from stock standard solution of the analyzed drug into a series of 50 ml volumetric flasks, and diluting to volume with 0.01 M HCl to obtain a concentration range of '2.0–36.0 μg.ml^{-1}' and '2.0–40.0 μg.ml^{-1}', respectively. The spectra of alkaline and oxidative-degradate solutions, having concentration 12.00 and 14.00 μg.ml^{-1} were scanned and stored in the instrument PC as devisors. The spectra of Trazodone Hydrochloride were divided by each devisor's spectrum, then the first derivative of the ratio spectra (DR^1) were computed at 276.40 and 320.40 nm, plotted versus concentrations, and the regression equations were computed, respectively.

pH-induced difference spectrophotometric (DD^n) method

From standard working solutions, aliquots were transferred into two sets of 50 ml volumetric flasks which were then diluted with 0.1M [HCl and NaOH], respectively. ΔA spectra were computed by placing the acid solution in the reference beam and the alkaline solution in the sample beam. (TRZ) can be determined in a concentration range of 2-36 μg.ml^{-1} in presence of its alkaline and oxidative-degradates, where the values of the first derivative of ΔA spectra (DD^1) were computed at 275.20 and 289.00 nm, respectively. The calibration curves were constructed and the regression equation was then computed.

Assay of the pharmaceutical formulation Trittico® tablets

Twenty tablets were accurately weighed and finely powdered. A portion of the powder equivalent to 10 mg of Trazodone Hydrochloride was accurately weighed, transferred to beaker with 70.0 ml of 0.01 M HCl, shaken, sonicated, filtered, then completed to the volume with '0.01 M HCl and water, separately' to obtain a concentration of 0.1 mg.ml^{-1} and the procedures mentioned under (2-4) were adopted.

RESULTS AND DISCUSSION

Method development 'Spectrophotometric method'

The stability of Trazodone Hydrochloride was studied until complete degradation was achieved by using 2M sodium hydroxide for 17 hours and 30%v/v hydrogen peroxide for 5 hours, where different concentrations of sodium hydroxide and hydrogen peroxide and at different time intervals were studied until reaching these optimum conditions which accomplish complete degradation. The degradation process under the previously mentioned conditions was followed by using TLC, where there was a single component indicating to the presence of one spot of either alkaline or oxidative-degradate after complete degradation. Also, complete degradation

process was conformed by adopting IR and mass spectrometry, where the suggested degradates were produced as shown in Figure 2a-2f. All the degradation products did not interfere with (TRZ) quantification.

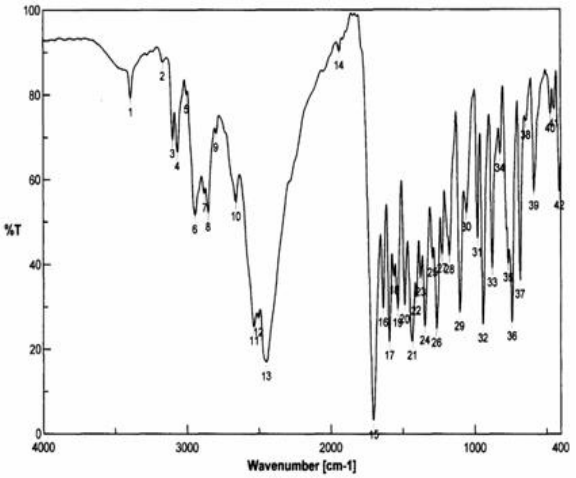


Figure 2a : IR spectrum of intact trazodone hydrochloride.

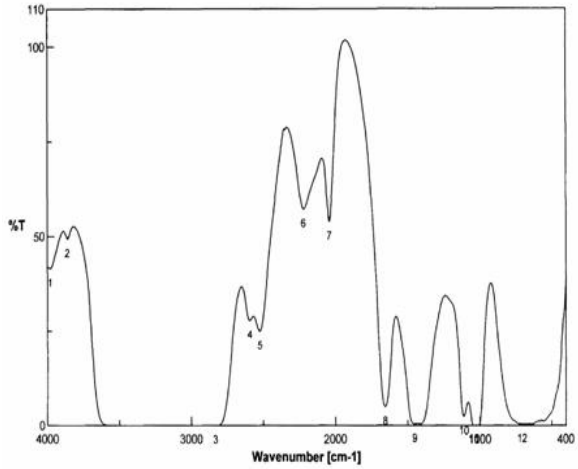


Figure 2b : IR spectrum of trazodone hydrochloride alkaline-degradate.

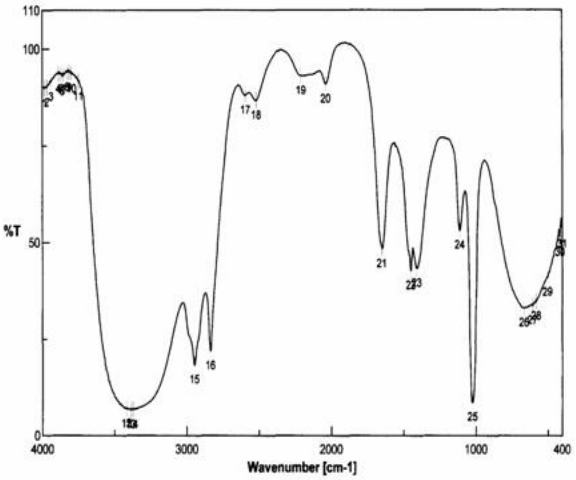


Figure 2c : Mass spectrum of trazodone hydrochloride oxidative-degradate.

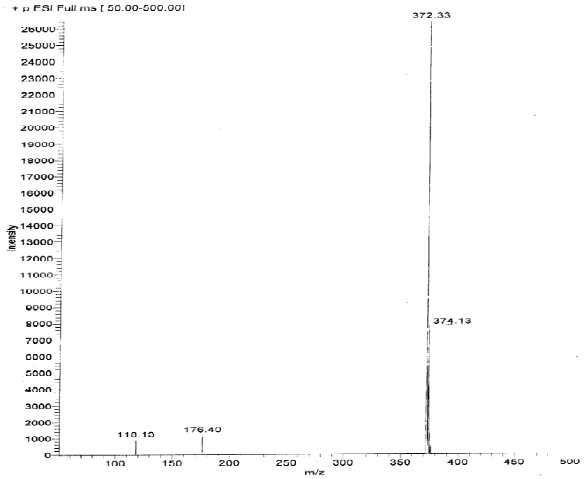


Figure 2d : Mass spectrum of the intact trazodone hydrochloride.

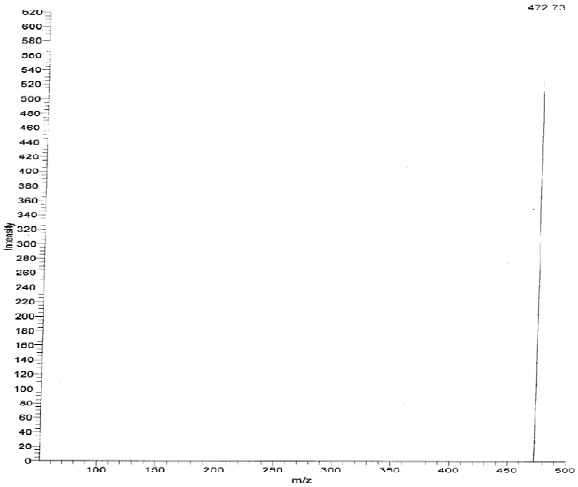


Figure 2e : Mass spectrum of trazodone hydrochloride alkaline-degradate.

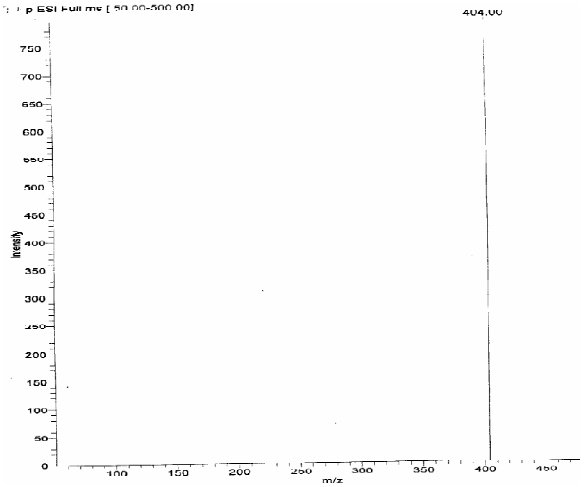


Figure 2f : Mass spectrum of trazodone hydrochloride oxidative-degradate.

Full Paper

The present work is concerned with determination of Trazodone Hydrochloride in presence of its alkaline or oxidative-degradates, where three simple, sensitive and rapid zero-crossing techniques were described. These adopted methods were derivative (D^n), derivative ratio (DR^n) and pH-induced difference (DD^n) spectrophotometric techniques, where the investigated drug could be determined in presence of its both-degradates.

Derivative spectrophotometric method (D^n)

In the derivative spectrophotometric technique, Trazodone Hydrochloride could be determined a concentration range of 4.0-38.0, 2.0-34.0 and 2.0-40.0 $\mu g.ml^{-1}$ in presence of its alakline-degradate by computing first (D^1), second (D^2) and third (D^3) derivative spectrophotometry, while in presence of oxidative-degradate it could be determined a concentration range of 2.0-40.0 $\mu g.ml^{-1}$ by utilizing second (D^1) and third (D^3) derivative spectrophotometry, where the amplitudes were measured at 257.90 nm, 245.50 nm and 234.0 0 nm (Zero-crossing of alkaline-degradate) and at 290.30 nm and 231.80 nm (Zero-crossing of oxidative-degradate), as shown in Figure 3a-3c and 4a-4b, respectively.

Derivative of ratio spectrophotometric method (DR^n)

The advantage of the derivative ratio spectral method may be the chance of doing measurement in correspondence of peaks, so there is a potential for greater sensitivity and accuracy. While the main disadvantages of zero-crossing method for resolving a mixture of components with overlapped spectra are the risk of small drifts of the working wavelengths and the circumstance that the working wavelengths generally do not fall in correspondence of peaks of the spectrum. This particularly pronounced disadvantage when the slope of the spectrum is very high with consequent loss of accuracy and precision and the working wavelength is in proximity of the base of the spectrum, which causes poor sensitivity[16].

The main instrumental parameter conditions were optimized for a reliable determination of the investigated drug. Different divisor concentrations of either alkaline or oxidative-degradate was examined to select an appropriate concentration, which is very important factor

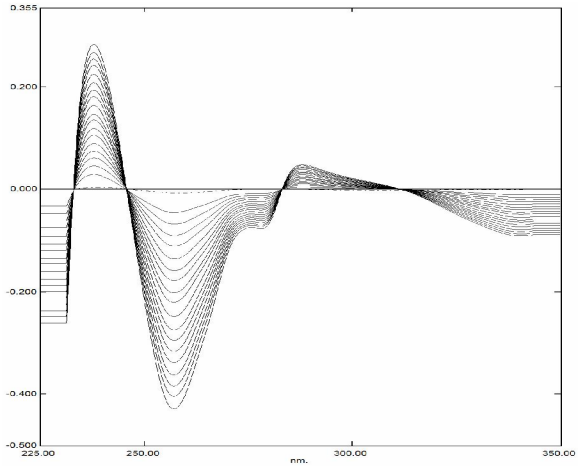


Figure 3a : First derivative (D^1) for different concentrations (4.0-38.0μgml^{-1}) trazodone hydrochloride (-) in presence of its alkaline–degradate (…).

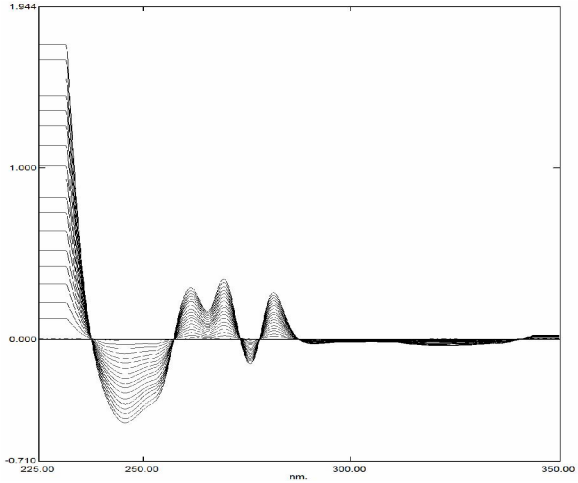


Figure 3b : Second derivative (D^2) for different concentrations (2.0-34.0μg ml^{-1}) of trazodone hydrochloride (-) in presence of its alkaline- degradate (…).

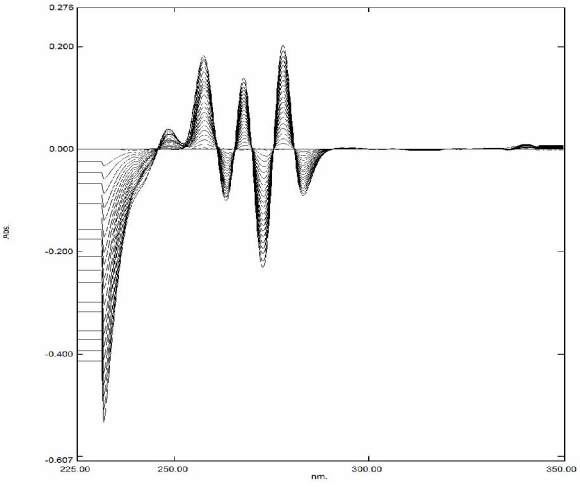


Figure 3c : Third derivative (D^3) for different concentrations (2.0-40.0μg ml^{-1}) of trazodone hydrochloride (-) in presence of its alkaline degradate (…).

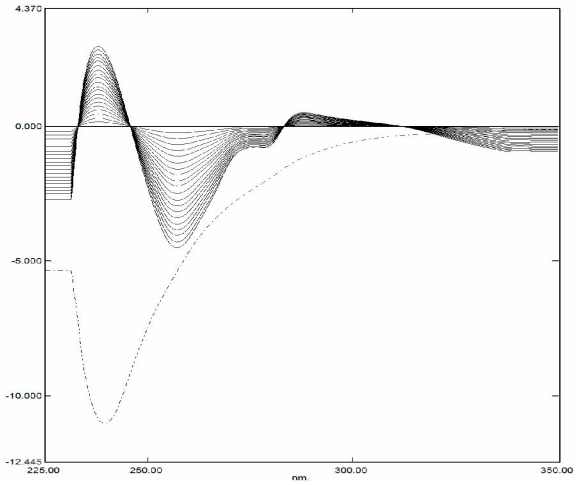


Figure 4a : First derivative (D^1) for different concentrations (2.0-40.0μgml^{-1}) of trazodone hydrochloride (-) in presence of its oxidative –degradate (…).

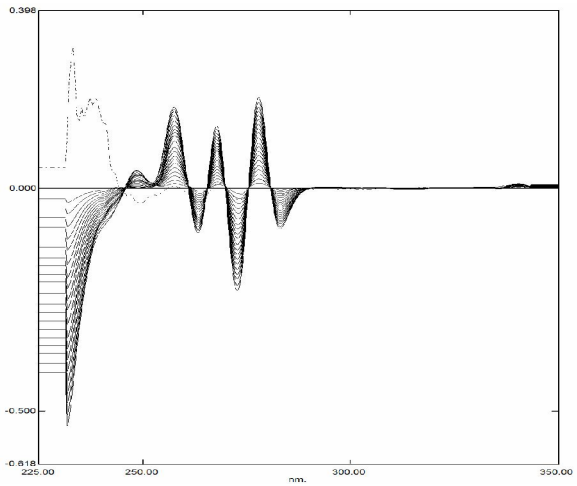


Figure 4b : Third derivative (D^3) for different concentrations (2.0-40.0μg ml^{-1}) of trazodone hydrochloride (-) in presence of its oxidative - degradate (…).

in practice, where the best results were obtained by using 12.00 and 14.00 μg.ml^{-1} concentration of either alkaline and oxidative-degradate stock standard as devisors. The first derivative of the ratio spectra (DR^1) at 276.40 and 320.40 nm permitted a selective determination of Trazodone Hydrochloride in a concentration range of '2.0–36.0 μg.ml^{-1}' and '2.0–40.0 μg.ml^{-1}', respectively, in the presence of its alkaline or oxidative-degradates as shown in Figure 5a-5b, where no noise was observed from the divisor.

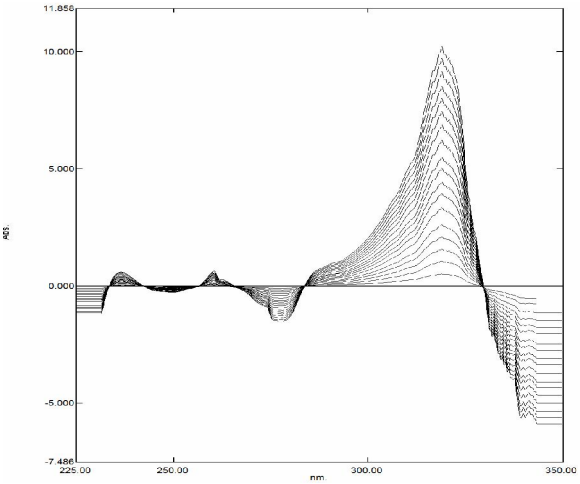


Figure 5a : First – derivative of the ratio- spectra for different concentrations (DR^1) (2.0-36.0μgml^{-1}) of trazodone hydrochloride, using 12.0 μgml^{-1} of its alkaline-degradate as a divisor.

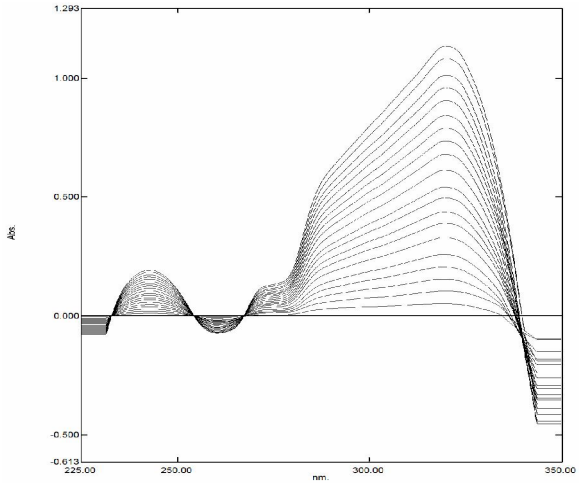


Figure 5b : First – derivative of the ratio- spectra for different concentrations (DR^1) (2.0-40.0μgml^{-1}) of trazodone hydrochloride, using 14.0 μgml^{-1} of its oxidative – degradate as a divisor.

pH-induced difference spectrophotometric (DD^n) method

The change in the absorption spectrum of Trazodone Hydrochloride, by using acid and alkaline media could be used as a stability-indicating study. The direct UV measurement of ΔA spectra was not suitable for assaying (TRZ) in presence of its alkaline and oxidative-degradates due to severe overlapping, but computing the first derivative of ΔA spectra (DD^1) at 275.20 and 289.00 nm, allowing its determination as shown in Figure 6a-6b, where zero-crossing point for its alkaline and oxidative-degradates is indicated.

Method validation

ICH guidelines[6] for validation method were followed, where all validation parameters were shown in TABLES 1 and 2. All the obtained results were statistically compared to the reference method[17] and no significant differences were found TABLES 3a and 3b, respectively.

Full Paper

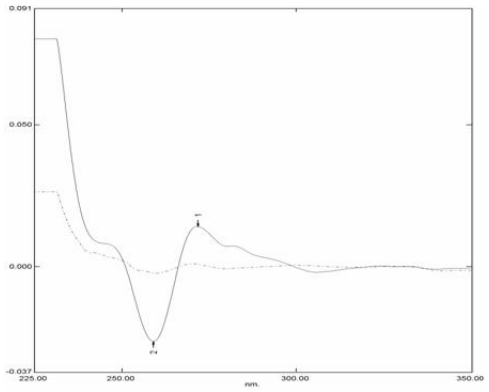


Figure 6a : First derivative of ΔA spectra of trazodone hydrochloride (-) and it's alkaline- degradates (….) (each of 10.0μgml^{-1}).

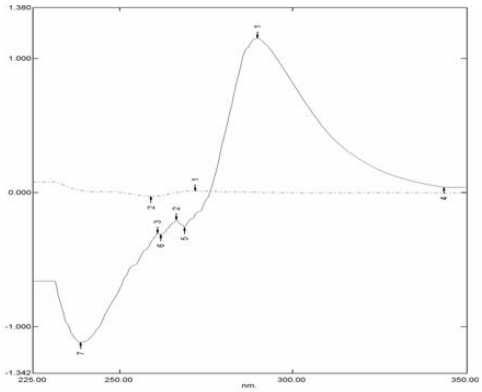


Figure 6b : First derivative of ΔA spectra of trazodone hydrochloride (-) and it's oxidative-degradate (…) (each of 10.0μgml^{-1}).

TABLE 1 : Validation of the proposed methods for determination of trazodone hydrochloride in presence of its alkaline-degradate

Parameters	D^1 at 257.90 nm	D^2 at 245.50 nm	D^3 at 234.00 nm	DR^1 at 276.40 nm	DD^1 at 275.20 nm
Linearity (μgml^{-1})	4.0-38.0	2.0-34.0	2.0-40.0	2.0-36.0	2.0-40.0
Slope	11 x 10^{-4}	-162 x 10^{-4}	-92 x 10^{-4}	19 x 10^{-4}	0.13 x 10^{-2}
Standard error of slope	8.74 x 10^{-6}	122 x 10^{-6}	5 x 10^{-5}	2.74 x 10^{-5}	1.21 x 10^{-5}
R.S.E of slope	7.73 x 10^{-3}	7.65 x 10^{-3}	9.89 x 10^{-1}	0.152 x 10^{-1}	9.37 x 10^{-3}
Confidence limit of slope	109 x 10^{-5}- 113 x 10^{-5}	1593 x 10^{-5}- 1645 x 10^{-5}	933 x 10^{-5}- 912 x 10^{-5}	18 x 10^{-4}- 19 x 10^{-4}	0.12 x 10^{-2}- 0.13 x 10^{-2}
Intercept	-1 x 10^{-4}	-83 x 10^{-4}	-61 x 10^{-4}	25 x 10^{-4}	-3 x 10^{-5}
Standard error of intercept	205 x 10^{-6}	2497 x 10^{-6}	1197 x 10^{-6}	62 x 10^{-5}	3 x 10^{-4}
Confidence limit of intercept	-33 x 10^{-5}- -54 x 10^{-5}	-1363 x 10^{-5}- 299 x 10^{-6}	866 x 10^{-5}- 363 x 10^{-5}	114 x 10^{-5}- 38 x 10^{-4}	-64 x 10^{-4}- 58 x 10^{-4}
Correlation coefficient (r)	0.9995	0.9995	0.9997	0.9993	0.9991
Standard error of estimation	385 x 10^{-6}	4921 x 10^{-6}	2577 x 10^{-6}	13 x 10^{-4}	63 x 10^{-4}
LOD	0.615	0.509	0.429	1.077	0.762
LOQ	1.864	1.541	1.301	3.263	2.308
S.D*^{a}	0.885	0.886	0.900	0.365	0.931
S.D*^{b}	0.769	0.357	0.675	0.548	0.780

***^{a}The intraday (n=5) *^{b}the interday (n=9) are the standard deviations of different samples concentrations of trazodone hydrochloride.**

TABLE 2 : Validation of the proposed methods for determination of trazodone hydrochloride in presence of its oxidative-degradate

Parameters	D^1 at 290.30 nm	D^3 at 231.80 nm	DR^1 at 320.40 nm	DD^1 at 289.00 nm
Linearity (μgml^{-1})	2.0-40.0	2.0-40.0	2.0-40.0	2.0-36.0
Slope	12 x 10^{-4}	-133 x 10^{-4}	288 x 10^{-4}	0.17 x 10^{-1}
Standard error of slope	1189 x 10^{-6}	6.42 x 10^{-5}	101 x 10^{-6}	18 x 10^{-4}
R.S.E of slope	1.015	4.78 x 10^{-3}	3.53 x 10^{-3}	0.105 x 10^{-1}
Confidence limit of slope	1171 x 10^{-6}- 1207 x 10^{-6}	1343 x 10^{-5}- 1315 x 10^{-5}	0.285 x 10^{-1}- 0.289 x 10^{-1}	0.166 x 10^{-1}- 0.173x 10^{-1}
Intercept	2 x 10^{-4}	99 x 10^{-4}	-22 x 10^{-4}	0.131 x 10^{-1}
Standard error of intercept	203 x 10^{-6}	1537 x 10^{-6}	242 x 10^{-5}	38 x 10^{-4}
Confidence limit of intercept	-25 x 10^{-5}- 605 x 10^{-5}	67 x 10^{-4}- 1316 x 10^{-5}	-724 x 10^{-5}- 292 x 10^{-5}	0.51 x 10^{-2}- 0.21 x 10^{-1}
Correlation coefficient (r)	0.9995	0.9997	0.9998	0.9991
Standard error of estimation	437 x 10^{-6}	331 x 10^{-5}	52 x 10^{-4}	0.8 x 10^{-2}
LOD	0.558	0.381	0.277	0.738
LOQ	1.692	1.156	0.840	2.235
S.D*^{a}	0.465	0.668	0.413	0.545
S.D*^{b}	0.492	0.699	0.134	0.428

***^{a}The intraday (n=5) *^{b}the interday (n=9) are the standard deviations of different samples concentrations of trazodone hydrochloride.**

TABLE 3a : Determination of trazodone hydrochloride in commercial tablets using the proposed methods and reference method in presence of alkaline-degradate

Items	D^1	D^2	D^3	DR^1	DD^1	Ref. method
Commercial* tablets	99.80 ±0.436 t =- 0.607 (2.160)** F = 4.866 (5.998)**	99.98±0.114 t = 0.706 (2.160)** F = 3.024 (3.633)**	99.67±0.196 t = 2.105 (2.160)** F = 1.011 (3.633)**	100.19±0.277 t =-1.917 (2.160)** F = 1.967 (5.998)**	99.99±0.250 t = 0.109 (2.310)** F = 1.605 (5.998)**	99.93±0.198
Recovery***	99.90±0.754	99.39±0.650	100.27±0.645	99.15±0.723	101.60±0.730	----------

***Mean and S.D for five determinations, percentage recovery from the label claim amount. **Theoretical values for t and F at (p = 0.05). ***For standard addition (n=5).**

TABLE 3b : Determination of trazodone hydrochloride in commercial tablets using the proposed methods and reference method in presence of oxidative-degradate

Items	D^1	D^3	DR^1	DD^1	Ref. method
Commercial* tablets	99.67±0.305 t = -1.669 (2.160)** F = 2.323 (5.998)**	99.78±0.313 t = -1.001 (2.160)** F = 2.323 (5.998)**	100.06±0.155 t = -1.399 (2.160)** F = 1.455 (3.633)**	100.27±0.257 t = -0.619 (2.310)** F = 1.939 (6.388)**	99.93±0.198
Recovery***	99.97±0.690	99.92±0.369	99.80 ±0.653	99.25±0.975	----------

***Mean and S.D for five determinations, percentage recovery from the label claim amount. **Theoretical values for t and F at (p = 0.05). ***For standard addition (n=5).**

TABLE 4a : Determination of trazodone hydrochloride in laboratory prepared mixtures with its alkaline-degradate by the proposed methods

Sample Number	Intact in µgml^{-1}	Degradate inµgml^{-1}	*%Recovery				
			D^1	D^2	D^3	DR^1	DD^1
1	18.00	2.00	100.40	99.28	99.42	99.00	100.00
2	18.00	4.00	100.90	100.00	100.00	99.37	100.00
3	18.00	8.00	101.40	98.28	101.11	99.68	100.40
4	18.00	12.00	101.90	99.70	99.44	99.37	100.00
5	18.00	16.00	102.00	98.92	101.59	99.68	101.40
6	18.00	18.00	102.00	98.20	99.72	99.68	100.909
	Mean		101.40	99.06	100.21	99.46	100.45
	±S.D.		0.665	0.736	0.918	0.273	0.585

***The average recovery of 5-separate determinations of the intact drug.**

TABLE 4b : Determination of trazodone hydrochloride in laboratory prepared mixtures with its oxidative-degradate by the proposed methods

Sample Number	Intact in µgml^{-1}	Degradate in µgml^{-1}	*% Recovery			
			D^2	D^3	DR^1	DD^1
1	18.00	2.00	100.00	99.95	99.80	99.87
2	18.00	4.00	100.40	99.87	99.61	99.93
3	18.00	8.00	100.40	100.40	99.80	99.90
4	18.00	12.00	100.40	99.79	100.57	100.60
5	18.00	16.00	100.90	99.70	100.00	99.81
6	18.00	18.00	101.40	99.83	99.79	99.71
	Mean		100.58	99.92	99.93	99.97
	±S.D.		0.491	0.247	0.337	0.318

***The average recovery of 5-separate determinations of the intact drug.**

Specificity

Degradation behavior of Trazodone Hydrochloride was investigated by the proposed methods, where it was determined in solutions containing different amounts (up to 100%) of its alkaline and oxidative-degradates each separately, by the proposed spectrophotometric methods, as shown in TABLE 4a and 4b where, the recovery % and S.D. proved high specificity of the adopted method.

Standard addition technique

To check the validity of the proposed methods, the standard addition method was applied by adding Trazodone Hydrochloride to the previously analyzed tablets. The recovery of it was calculated by comparing the concentration obtained from the spiked samples with that of the pure drug. The results of analysis of the commercial tablets and the standard addition method (recovery study) of the studied drug are shown in

Full Paper

TABLES 5a-5e and 6a-6d suggested that there is no interference from any excipients, which are normally present in tablets and capsules.

TABLE 5a : Quantitative determination of trazodone hydrochloride in the pharmaceutical formulation and applications of standard addition technique by the proposed first derivative spectrophotometric method.

Pharmaceutical Preparation	Found		Standard addition techniqu e (D^1)			
	Mg	%	Claimed (μgml^{-1})	Pure added (μgml^{-1})	Pure Found (μgml^{-1})	%Recovery*
Trittico® Tablets 100 mg B.N. 051427	99.80	99.80 ± 0.436	10.0	6.00	6.00	100.00
				8.00	8.00	100.00
				10.0	10.00	100.00
				12.0	12.09	100.80
				14.0	13.81	98.70
	Mean ± S.D				99.90±0.754	

TABLE 5b : Quantitative determination of trazodone hydrochloride in the pharmaceutical formulation and applications of standard addition technique by the proposed second derivative spectrophotometric method.

Pharmaceutical Preparation	Found		Standard addition technique (D^2)			
	Mg	%	Claimed (μgml^{-1})	Pure added (μgml^{-1})	Pure Found (μgml^{-1})	%Recovery*
Trittico® Tablets 100 mg B.N. 051427	99.98	99.98 ±0.114	10.0	6.00	6.00	100.00
				8.00	7.95	99.35
				10.0	9.98	99.85
				12.0	11.80	98.34
				14.0	13.92	99.45
	Mean ± S.D				99.39 ±0.650	

TABLE 5c : Quantitative determination of trazodone hydrochloride in the pharmaceutical formulation and applications of standard addition technique by the proposed third derivative spectrophotometric method.

Pharmaceutical Preparation	Found		Standard addition technique (D^3)			
	mg	%	Claimed (μgml^{-1})	Pure added (μgml^{-1})	Pure Found (μgml^{-1})	%Recovery*
Trittico® Tablets 100 mg B.N. 051427	99.67	99.67 ±0.196	10.0	6.00	6.05	100.99
				8.00	7.99	99.87
				10.0	10.09	100.97
				12.0	11.98	99.83
				14.0	13.96	99.72
	Mean ± S.D				100.27±0.645	

TABLE 5d : Quantitative determination of trazodone hydrochloride in the pharmaceutical formulation and applications of standard addition technique by the proposed first derivative ratio spectrophotometric method.

Pharmaceutical Preparation	Found		Standard addition technique (DR^1)			
	mg	%	Claimed (μgml^{-1})	Pure added (μgml^{-1})	Pure Found (μgml^{-1})	%Recovery*
Trittico® Tablets 100 mg B.N. 051427	100.19	100.19±0.277	10.0	6.00	5.94	99.10
				8.00	8.00	100.00
				10.0	9.90	99.05
				12.0	11.94	99.53
				14.0	13.72	98.05
	Mean ± S.D				99.15±0.723	

TABLE 5e : Quantitative determination of trazodone hydrochloride in the pharmaceutical formulation and applications of standard addition technique by the proposed pH-induced difference spectrophotometric method.

Pharmaceutical Preparation	Found		Standard addition technique (DR^1)			
	mg	%	Claimed (μgml^{-1})	Pure added (μgml^{-1})	Pure Found (μgml^{-1})	%Recovery*
Trittico® Tablets 100 mg B.N. 051427	99.99	99.99±0.250	10.0	6.00	6.00	100.00
				8.00	8.07	100.90
				10.0	10.09	100.90
				12.0	12.20	101.70
				14.0	14.25	101.80
	Mean ± S.D				101.60±0.730	

***The average recovery of 4-separate determinations for pharmaceutical preparation.**

TABLE 6a : Quantitative determination of trazodone hydrochloride in the pharmaceutical formulation and applications of standard addition technique by the proposed First derivative spectrophotometric method.

Pharmaceutical Preparation	Found		Standard addition technique (D^1)			
	mg	%	Claimed (μgml^{-1})	Pure added (μgml^{-1})	Pure Found (μgml^{-1})	%Recovery*
Trittico® Tablets 100 mg B.N. 051427	99.67	99.67±0.305	10.0	6.00	5.99	99.90
				8.00	7.92	99.01
				10.0	10.00	100.00
				12.0	12.11	100.96
				14.0	14.00	100.00
	Mean ± S.D				99.97±0.690	

TABLE 6b : Quantitative determination of trazodone hydrochloride in the pharmaceutical formulation and applications of standard addition technique by the proposed third derivative spectrophotometric method.

Pharmaceutical Preparation	Found		Standard addition technique (D^3)			
	mg	%	Claimed (μgml^{-1})	Pure added (μgml^{-1})	Pure Found (μgml^{-1})	%Recovery*
Trittico® Tablets 100 mg B.N. 051427	99.78	99.78 ±0.313	10.0	6.00	5.97	99.50
				8.00	8.00	100.00
				10.0	9.95	99.57
				12.0	12.02	100.23
				14.0	14.04	100.30
	Mean ± S.D				99.92±0.369	

TABLE 6c : Quantitative determination of trazodone hydrochloride in the pharmaceutical formulation and applications of standard addition technique by the proposed first derivative ratio spectrophotometric method.

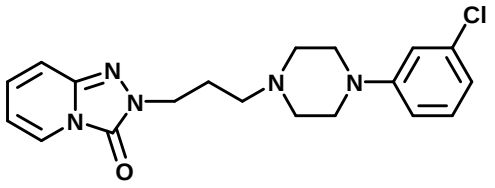
Pharmaceutical Preparation	Found		Standard addition technique (DR^1)			
	mg	%	Claimed (μgml^{-1})	Pure added (μgml^{-1})	Pure Found (μgml^{-1})	%Recovery*
Trittico® Tablets 100 mg B.N. 051427	100.06	100.06 ±0.155	10.0	6.00	6.01	100.29
				8.00	7.89	98.70
				10.0	9.99	99.92
				12.0	12.03	100.29
				14.0	13.97	99.84
	Mean ± S.D				99.80±0.653	

TABLE 6d : Quantitative determination of trazodone hydrochloride in the pharmaceutical formulation and applications of standard addition technique by the proposed pH-induced difference spectrophotometric method.

Pharmaceutical Preparation	Found		Standard addition technique (DR^1)			
	mg	%	Claimed (μgml^{-1})	Pure added (μgml^{-1})	Pure Found (μgml^{-1})	%Recovery*
Trittico® Tablets 100 mg B.N. 051427	99.98	99.98 ±0.129	10.0	6.00	6.02	100.33
				8.00	7.99	99.94
				10.0	9.99	99.92
				12.0	12.00	100.00
				14.0	14.20	101.43
	Mean ± S.D				100.32 ±0.640	

***The average recovery of 4-separate determinations for pharmaceutical preparation.**

Identification of acid-degradates of (TRZ) by structure elucidation



Trazodone hydrochloride M.wt.=408.3

(TRZ) was influenced by the reaction with 2M NaOH for 17 hrs at 100°C and 30%v/v hydrogen peroxide for 5 hours, giving one degradate for each alkaline and oxidative-degradation pathway. This was then explained by structural elucidation, utilizing IR and mass spectroscopy, where a complete apparent change was obtained between the spectra of the intact Trazodone Hydrochloride and its alkaline and oxidative-degradates. In the IR spectra, the peak corresponding to carbonyl group [C=O] in the intact (TRZ) appearing at 1704 cm^{-1}, was appeared at 1655 cm^{-1} and 1650 cm^{-1} in the spectrum of alkaline and oxidative-degradates, respectively. Also, in the spectra for alkaline and oxidative-degradates, new peaks were obtained corresponding to –N=C=O- and C=C at 2221 cm^{-1} and 1650 cm^{-1}, as shown in Figures 2a-2c, respectively.

Figures 2d-2f show the electron impact mass ion peak at m/z 372, 472 and 404 for the intact (TRZ), its alkaline-degradate and its oxidative-degradate, respectively.

CONCLUSION

The proposed methods are accurate, precise and specific ones, where Trazodone Hydrochloride can be determined in bulk powder, in laboratory prepared mixtures with different ratios of its both-degradates, separately and in pharmaceutical formulation without any interference from common excipients present. ICH guidelines were followed throughout the study for method validation and stress testing, and the suggested methods can be applied for routine quality control analysis and stability studies.

REFERENCES

[1] Martindale, The Extra Pharmacopoeia, 35th Edn., The Complete Drug Reference.

[2] Dennis K.J.Gorecki, Roger K.Verbeeck; Analytical Profiles of Drug Substances. Academic Press, Canada, **16**, 693-730 **(1987)**.

[3] C.S.Sean; Martindale, The Complete Drug Reference, Third Edn., Pharmaceutical Press, U.K., **(2002)**.

[4] E.F.James; Reynolds, Martindale, The Extra Pharmacopeia, Thirty One Edn., Royal Pharmaceutical Society, **(1996)**.

[5] ICH [Stability Testing of New Drug Substances and Products (Q1AR2)]; International Conference on Harmonization, Food and Drug Administration, USA, November 1996 and February **(2003)**.

[6] ICH [Validation of Analytical Procedures: Methodology (Q2AR1)]; International Conference on Harmonization, Food and Drug Adminstration, USA, November 1996 and November **(2005)**.

[7] M.Bakshi, S.Singh; J.Pharm.Biomed.Anal., **28**, 1011-1040 **(2002)**.

Full Paper

[8] British Pharmacopoeia; Her Majesty's Stationary Office, London, 1318 **(1998)**.
[9] The United States Pharmacopeia; 24 Revision, Asian Edn., United States Pharmacopeial Convention, Inc., Twinbrook Parkway, Rockville, M.D., 1681-1682, 2149-2152 **(2000)**.
[10] Mascarenhas, C.D.Gaitonde; Indian Drugs, **28(12)**, 565-567 **(1999)**.
[11] S.Khalil; Analyst, **124(2)**, 139-142 **(1999)**.
[12] H.Suzuki, K.Akimoto, H.Nakagawa, I.Sugimoto; J.Pharmacol.Sci., **78(1)**, 62-65 **(1989)**.
[13] J.M.Kauffmann, J.C.Vire, G.J.Patriarche, L.J.Nunez-Vergara, J.A.Squella; Electrochim.Acta, **32(8)**, 1159-1162 **(1987)**.
[14] T.J.Siek; J.Anal.Toxicol., **11(5)**, 225-227 **(1987)**.
[15] A.El Gindy, B.El Zeany, T.Awad, M.M.Shabana; J.Pharm.Biomed.Anal., **26(2)**, 211-217 **(2001)**.
[16] Z.Pawlak, B.J.Clark; J.Pharm.Biomed.Anal., **7**, 1907 **(1989)**.
[17] British Pharmacopoeia Printed in the U.K., by the Stationary Office Limited under the Authority and Superintendence of Her Majesty's Stationary Office and the Queen's Printer of Acts of Parliament, London, 3rd Edn., European Pharmacopoeia, B.P., **(2011)**.