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Stability indicating methods for determination of ziprasidone hydrochloride

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ABSTRACT

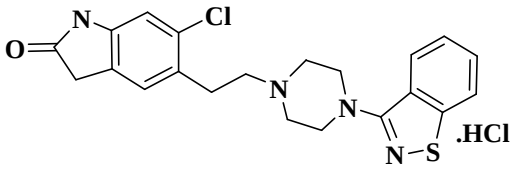
This manuscript describes the development and validation of a quantitative analytical method for determination of Ziprasidone Hydrochloride (ZIP) in pure form and pharmaceutical product using ratio subtraction, first derivative ratio, TLC-densitometry and multivariate calibration techniques. The proposed methods are accurate, precise, sensitive, and selective and can be used in quality control laboratories.

KEYWORDS

Ziprasidone hyydrochloride;
Ratio subtraction;
First derivative ratio spectrophotometry;
Thin layer chromatography-densitometry;
Chemometric technique.

1. INTRODUCTION

Ziprasidone Hydrochloride monohydrate is 5-[2-[4-(1, 2-benzisothiazol-3-yl)-1-piperazinyl] ethyl] -6-chloro-1, 3-di-hydro-2H-indol-2- one Hydrochloride as shown in figure 1[1].



$C_{21}H_{20}N_4OS$ Mol.Wt = 467.4

Figure 1: Structure of Ziprasidone hydrochloride

Ziprasidone Hydrochloride is the suggested drug to be used for treatment of schizophrenia[1].

Several chromatographic methods have been recommended for the determination of ZIP; these include HPLC[2-9] and TLC[10].

ZIP is not official in any pharmacopoeia, so it is desirable to develop a simple and fast procedure that could be applied in quality control laboratories for the selective determination of ZIP in presence of its degradates. The utility of the developed methods to determine the concentration of drug in its pharmaceutical dosage form is also demonstrated.

Theory of ratio subtraction method

The method depends on that, when a mixture of ZIP (X) and I (Y) where the spectrum of (Y) is extended than (X), the determination of (X) can be done by dividing the spectrum of the mixture by certain concentration of (Y) as a divisor (Y'). Producing a new ratio spectrum that represents X/Y' + constant. Subtraction of this constant, followed by multiplication of given spectra by (Y') spectrum will give the original spectrum of (X). This can be summarized as follow:

$$(X+Y)/Y' = X/Y' + Y/Y' = X/Y' + \text{constant}$$

$$X/Y' + \text{constant} - \text{constant} = X/Y'$$

$$X/Y' \times Y' = X$$

The constant can be determined directly from the curve X+Y/Y' by the straight line which is parallel to the wavelength axis in the region where (Y) is extended. The correct choice of the divisor is fundamental as if the concentration of the divisor increased or decreased; the resulting constant value will be proportionally de-

creased or increased[11].

Derivative ratio spectrophotometry is an analytical technique of good utility and better selectivity than normal spectrophotometry for resolving binary mixtures and some ternary mixtures without previous separation which was developed by Salinas et al.[12].

In multivariate methods, calibration sets of standard samples composed of different mixtures of ZIP and its degradates have been designed.

2. EXPERIMENTAL

2.1. Instruments

1. A double beam UV-Visible spectrophotometer (SHIMADZU, Japan) model UV-1601 PC with quartz cell of 1cm path length, connected to IBM compatible computer and HP 680 inkjet printer. The bundled software was UVPC personal spectroscopy software version 3.7. The spectra bandwidth was 2nm and wavelength scanning speed 2800 nm/min.
2. CAMAG TLC Scanner "Scanner 3_130319 (1.14.26).
3. TLC glass plates precoated with silica gel 60 F_{254} (20 × 20), 0.2 mm thickness (E.Merck), Darmstadt, Germany.
4. 25- µl Hamilton syringe.
5. Chromatographic tank 20 × 21 ×9 cm (Desaga)
6. UV short wavelength (254 nm) lamp. (Desaga, Germany)
7. Ultrasonic, Bandelin electronic, Sonorex RK51OS, HF-Frequency 35 KHz (10 liter capacity), Germany.
8. HPLC, RP-C_{18} column, UV-Visible detector, model 1310A, Agilent, 1100 series.
9. All data analysis was performed using PLS-Toolbox 2.0 running under MATLAB®, version 6.5[13].

2.2. Reagents and solvents

All reagents used throughout this work were of analytical pure grade, and solvents were of spectroscopic grade.

1. Methanol, trifluoroacetic acid, triethylamine, chloroform, glacial acetic acid and acetonitrile (HPLC grade).
2. NaOH (ADWIC) - 1N NaOH,aqueous solution.
3. Water for injection.
4. Hydrogen peroxide, 30% (ADWIC).

2.3. Samples

(a) Pure standard

Ziprasidone Hydrochloride was kindly supplied by Pfizer-Egypt S.A.E Cairo, Egypt and having a purity of 99.95% according to the reported HPLC[2].

(b) Pharmaceutical dosage form

Zeldox® capsule (Pfizer-Egypt S.A.E Cairo, Egypt under authority of Pfizer INC., U.S.A) labeled to contain 40mg of Ziprasidone Hydrochloride per capsule, batch number 610142134.

(c) Degraded sample

1. Preparation of alkaline degradates of Ziprasidone hydrochloride

It was prepared by dissolving 25mg of ZIP in 50ml methanol then adding 20 ml of 1N NaOH and refluxing for 7 hours. The solution was neutralized using 1N HCl, evaporated to dryness on hot plate and then the residue was dissolved in 20ml methanol. The obtained solution was filtered into 25-ml volumetric flask and the volume was completed with methanol to have a concentration of 1 mg mL^{-1}.

2. Preparation of oxidative degradates of Ziprasidone hydrochloride

It was prepared by dissolving 25mg of ZIP in 20mL methanol then adding 2.5ml of 30% H_2O_2 ,complete to the mark with methanol and leave for 24 hours. The solution was evaporated to dryness on hot plate and then the residue was dissolved in 20mL methanol. The obtained solution was filtered into 25-mL volumetric flask and the volume was completed with methanol to have a concentration of 1 mg/mL.

2.4. Standard stock and working solutions

a. Standard stock solution of Ziprasidone Hydrochloride, alkaline degradates and oxidative degradate (1 mg mL^{-1}) of each.
b. Working standard solution of Ziprasidone Hydrochloride, alkaline degradates and oxidative degradate (100 µg mL^{-1}) of each.
c. Working standard solution of Ziprasidone Hydrochloride, alkaline degradates and oxidative degradate

(200 μg mL^{-1}) of each.

d. Laboratory prepared mixtures of drug and its degradates in different ratios from 10 to 90% .

2.5. Procedures

2.5.1. Construction of calibration graph for determination of ZIP by ratio subtraction method

Aliquots of ZIP working standard solution equivalent to 100-900 μg were accurately transferred into a series of 10-mL volumetric flasks, the volume was completed to the mark with methanol. The spectra were scanned in the range 270-390 nm. A calibration curve was constructed representing the absorbance versus concentration and the regression equation was computed.

2.5.2. Construction of calibration graph for determination of ZIP by first derivative ratio method

Aliquots of ZIP working standard solution equivalent to 100-900 μg were accurately transferred into a series of 10-mL volumetric flasks, the volume was completed to the mark with methanol.

Aliquots of 9.0ml of I and II working standard solution (100μg mL^{-1}) were accurately transferred, separately into 10-ml volumetric flask and the volume was completed to the mark with methanol. The absorbance of each solution was measured in the range of 270-390 nm in presence of I and II, respectively. For the determination of ZIP in presence of I and II, the stored spectra of ZIP were divided separately by the spectrum of I and II. The first derivative corresponding to each ratio spectrum ^{1}DD was recorded using Δλ 4nm and scaling factor 10.

A calibration curve was constructed representing the peak amplitude of ^{1}DD versus concentration and the regression equations were computed.

2.5.3. Construction of calibration graph for determination of ZIP by TLC-densitometric method

Aliquots equivalent to 0.3-2 mg ZIP from stock standard solution (1mg mL^{-1}) was transferred into 10-ml volumetric flasks and the volume was completed with methanol. 20 μl was applied to thin layer chromatographic plates (20×20) using 25μl Hamilton syringe. Spots were spaced 2 cm apart from each other and 1.5cm from the bottom edge of the plate. The plates were developed in the chromatographic tank previously saturated with the developing mobile phase, chloroform: methanol: glacial acetic acid (80:20:0.1 by volume), for at least 20 minutes. The plates were developed by ascending technique to a distance of about 8 cm, dried at room temperature. The spots were detected under UV-lamp and scanned, at 317nm for ZIP. The peak areas were recorded for the drug. The calibration curve was constructed by plotting the area under the peak versus the corresponding concentrations of ZIP. The corresponding regression equation was computed.

2.5.4. Multivariate calibration methods

Calibration solutions (training set) of ZIP, I and II were prepared by diluting different volumes of the drug and its degradates working solutions into 25-mL volumetric flasks. The volume was completed to the mark with methanol. The absorbances of these mixtures were measured between 270 and 350 nm at 0.5 nm intervals with respect to a blank of methanol .Several multivariate calibration models were constructed using the data obtained. The concentration of ZIP was calculated by application of the developed models and to analyze the spectra of unknown samples.

2.5.5. Determination of ZIP in presence of its degradates in laboratory prepared mixtures by the proposed methods

a. Ratio subtraction method

Aliquots of 9 to 1 mL were separately transferred from ZIP working standard solution (100 μg mL^{-1}) into 10-mL volumetric flasks. To the previous solutions, aliquots of 1 to 9 ml of I and II working standard solution (100 μg mL^{-1}) were added separately and the volume was completed to the mark with methanol. Mixtures of different ratios were obtained. The absorbance of the D at 317nm was scanned for the samples of the laboratory prepared mixtures from 270-390 nm and stored in the computer. For the determination of ZIP in the presence of I and II, the spectra of the laboratory prepared mixtures were divided separately by the spectrum of 90 μg mL^{-1} of I and II (divisor), respectively to obtain division spectra.

The absorbance in the plateau at λ above 364nm was subtracted, multiplied by the corresponding spectrum of I and II (divisors) (90 μg mL^{-1}).

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The obtained curve was used for direct determination of ZIP at 317 nm and concentration of the intact drug was calculated from its corresponding regression equation.

b. First derivative ratio method

Aliquots of 9 to 1 mL were separately transferred from ZIP working standard solution (100μg mL^{-1}) into 10-mL volumetric flasks. To the previous solutions, aliquots of 1 to 9 mL of I and II working standard solutions (100 μg mL^{-1}) were added separately and the volume was completed to the mark with methanol. Mixtures of different ratios were obtained and the peak amplitudes of ^{1}DD at 268.3 nm and 332nm were measured in presence of I and II, respectively. Using the procedure described under 2.5.2, the concentration of the intact drug was calculated from its corresponding regression equation.

c. TLC-densitometric method

Aliquots of 0.3 to 1.7 mL were separately transferred from ZIP standard stock solution (1 mg mL^{-1}) into 10-mL volumetric flasks. To the previous solutions, aliquots of 1.7 to 0.3 ml of I and II working standard solutions (1mg mL^{-1}) were added and the volume was completed to the mark with methanol. Mixtures of different ratios were obtained. The peak areas of the obtained chromatogram were measured for the samples of the laboratory prepared mixtures using the procedure described under 2.5.3. The concentration of the intact drug was calculated from its corresponding regression equation.

2.5.6. Determination of ZIP in pharmaceutical dosage form by the proposed methods

Content of ten capsules were evacuated, mixed well and an accurate weight equivalent to 100mg of ZIP dissolved in 20 mL methanol, sonicated for 5 min, mixed well and then filtered. The volume was completed to 100 mL using methanol, 10 milliliters of the filtrate were transferred into 100-mL volumetric flask and the volume was completed with methanol to obtain a concentration of 100μg mL^{-1}. The general procedures were followed.

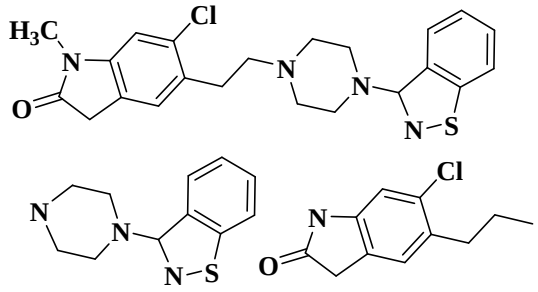

Figure 2: Suggested structures for alkaline degradates

3. RESULTS AND DISCUSSION

The stability of ZIP was studied according to the ICH guidelines for:

a. stress, acid and alkaline

Reflux with 0.1N HCl /0.1N NaOH for 8 hours, 1N HCl/ 1N NaOH for 12 hours, 2N HCl for 24 hours finally 6N HCl for 24hours.

b. Oxidative condition

Keep with 3% H_2O_2 for 24 hours and 10% for 24 hours at room temperature. The degradation process under the previously mentioned conditions was followed using TLC and the compound was found to be stable under acidic condition but it is liable to degradation in alkaline condition giving three components which is confirmed with a previous study on stability of ZIP[6] and with hydrogen peroxide giving one component.

This work is concerned with determination of ZIP in presence of its alkaline and oxidative degradate.

To detect the complete degradation of ZIP, a TLC procedure was suggested. Different systems were tried, where complete separation of ZIP from I and II was achieved using methanol-chloroform- glacial acetic acid (20:80:0.1 by volume) as the mobile phase. The R_f values were 0.95 for ZIP, 0.87 , 0.52 and 0.15 for I and 0.76 for II. Using other systems such as methanol-chloroform-ammonia and butanol-chloroform-glacial acetic acid in different ratios were not successful for separating ZIP from I and II. Spotting of 5μg at different successive times of reflux and after evaporation, showed complete alkaline degradation after seven hours and the obtained degradates unaffected during evaporation. In case of oxidative degradate, left for 24 hours at room temperature with 10% hydrogen peroxide. They were three components in case of alkaline degradates and one component as indicated by the appearance of three

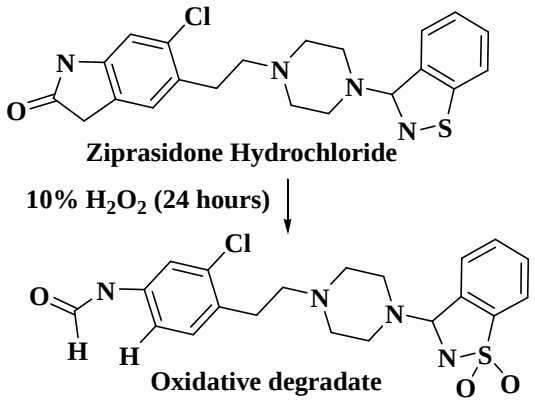

Figure 3: Suggested mechanism for oxidative degradate

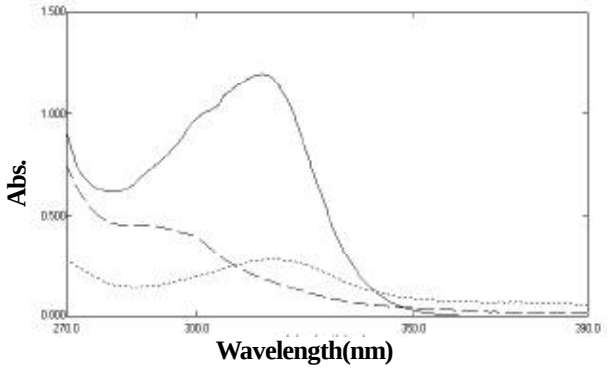

Figure 4: Absorption spectra of a solution of Ziprasidone Hydrochloride 90µg/ml (—), its alkaline degradates 90 µg/ml(…...) and its oxidative degradate 90 µg/ml (- - - - -) in methanol

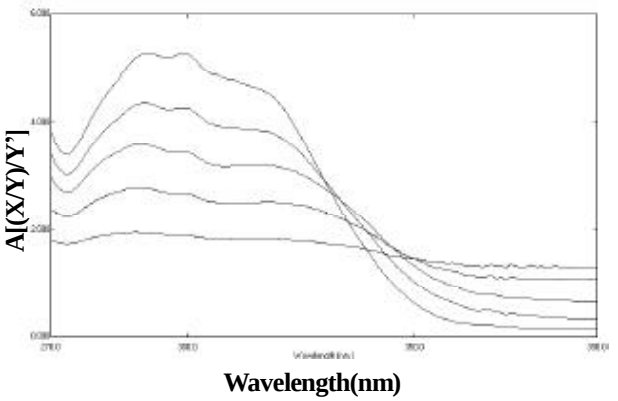

Figure 5: Division spectra of laboratory prepared mixtures of Ziprasidone Hydrochloride (X) and its alkaline degradates (Y) using 90µg/ml of degradation products (Y') as a divisor and methanol as a blank

spots of alkaline degradates and one spot of oxidative degradate after complete degradation and also confirmed by IR.

Suggested structures for alkaline degradates are

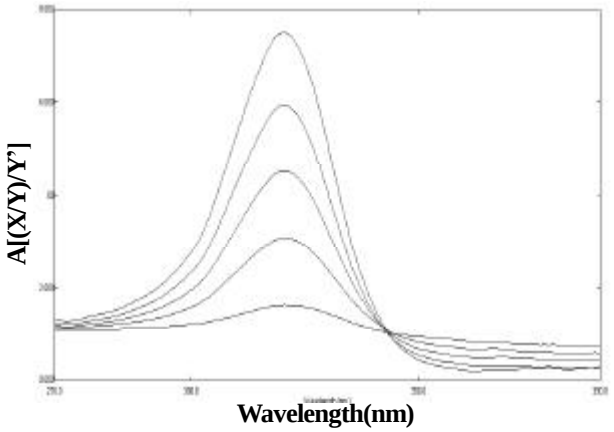

Figure 6: Division spectra of laboratory prepared mixtures of Ziprasidone Hydrochloride (X) and its oxidative degradates (Y) using 90µg/ml of degradation products (Y') as a divisor and methanol as a blank

shown in figure 2 and a suggested mechanism for oxidative degradate is shown in figure 3.

Its structure was confirmed by IR spectrometry where (C=O) of aldehyde appears at 1713.15 cm^{-1}, and sulphone appears at 1048.07 cm^{-1} were assigned and the expected molecular weight was found to be 501.4 which is confirmed with mass spectrometry.

The high solvolytic instability of ZIP, even in aqueous solution requires great care in the formulation of liquid and solid dosage formulations so that the environment should be acidic as possible, alkaline lubricant and excipients shouldn't be used and the granulation should be kept as free of water as possible. Even in refrigerator, ZIP aqueous solution, is predicted to be only stable for one week[6].

Upon scanning the absorption spectra of each of ZIP, I and II, it was observed that ZIP has a λ_{max} at 317nm, at this wavelength, I and II show complete overlapping as shown in figure 4.

Trials to use zero order absorption at 317nm for determination of ZIP in presence of I and II was failed.

This work concerned with the determination of ZIP in presence of its alkaline (I) and oxidative degradate (II). For ratio subtraction method, the absorbance spectrum at 317nm was selected for selective determination of ZIP in presence of I and II, where calibration graph was linear in the range of 10-90 µg mL^{-1}.

For the determination of ZIP in the presence of I and II, the spectra of the laboratory prepared mixtures were divided by the spectrum of 90 µg mL^{-1} of I and II

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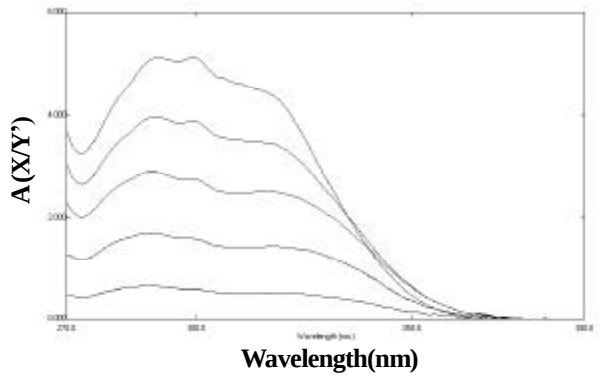

Figure 7: Division spectra of laboratory prepared mixtures of Ziprasidone hydrochloride (X) and its alkaline degradates (Y) using 90µg/ml of degradation products (Y') as a divisor and methanol as a blank after subtraction of the constant

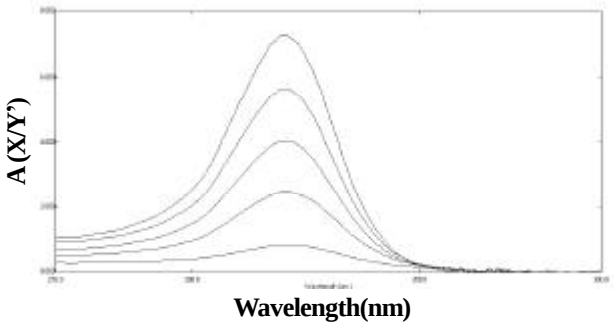

Figure 8: Division spectra of laboratory prepared mixtures of Ziprasidone hydrochloride (X) and its oxidative degradates (Y) using 90µg/ml of degradation products (Y') as a divisor and methanol as a blank after subtraction of the constant

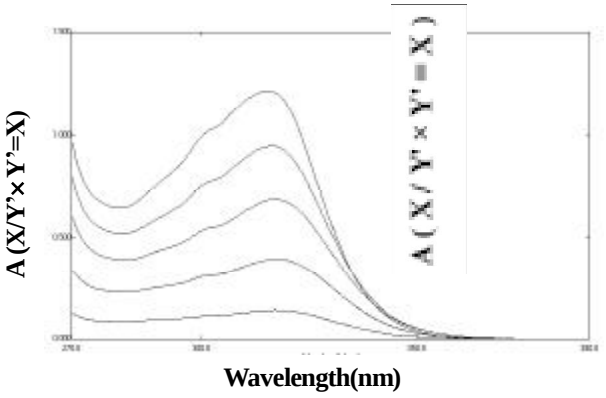

Figure 9: The zero order absorption spectra of Ziprasidone hydrochloride obtained by the proposed method for the analysis of laboratory prepared mixtures after multiplication by the divisor I (Y')

(divisor), respectively to obtain division spectra as in figures (5 and 6).

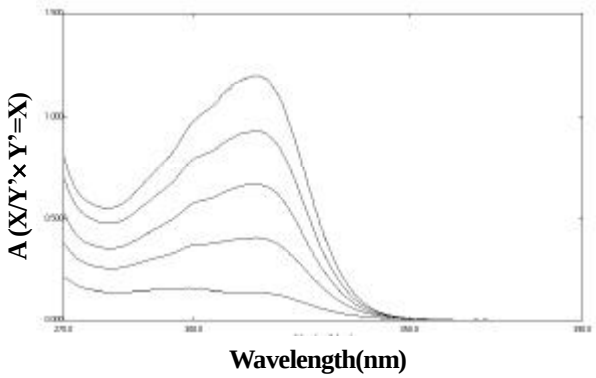

Figure 10: The zero order absorption spectra of Ziprasi done Hydrochloride obtained by the proposed method for the analysis of laboratory prepared mixtures after multiplication by the divisor II (Y')

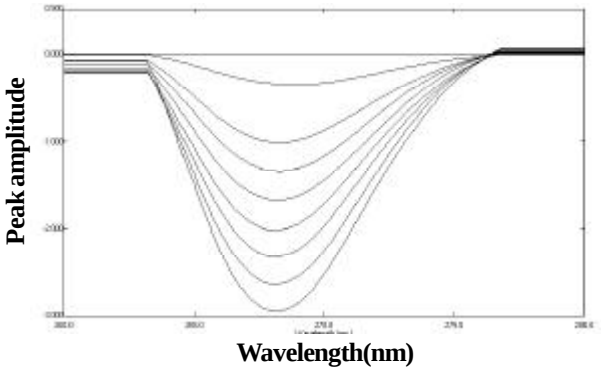

Figure 11: First derivative of ratio spectra of Ziprasidone Hydrochloride 20-90 µg/ml using 90 µg/ml of its alkaline degradates as a divisor

Different divisor concentrations (10, 20,30,40,50, 60,70,80 and 90 µg mL^{-1}) of I and II were tried. The divisor concentration 90 µg mL^{-1} was found the best regarding average recovery percent when the model was used for the calculation of ZIP in its laboratory prepared mixtures. The absorbance in the plateau at λ above 364nm was subtracted as in figures (7 and 8)

The obtained spectra were multiplied by the spectrum of the divisor 90 µg mL^{-1} as in figures (9 and 10).

The obtained curve was used for direct determination of ZIP at 317 nm and concentration of the intact drug was calculated from its corresponding regression equation.

Applying first derivative ratio, the peak amplitudes at 268.3 nm was used for selective determination of ZIP in presence of I in the range of 20-90 µg mL^{-1} as in figure 11, and at 332 nm for selective determination of ZIP in presence of II in the range of 10-90 µg mL^{-1}, as

in figure 12.

Careful choice of the divisor and the working wavelength were of great importance, so different concentrations of I and II (10,20,30,….90) μg mL^{-1} were tried as divisors. It was found that the best one was 90 μg mL^{-1} of I and II as they produce minimum noise and give better results in agreement with selectivity.

TLC-Densitometric technique allows selective determination of ZIP in presence of I and II in range of 0.6-4 μg/spot.

The regression equations were calculated and found to be:

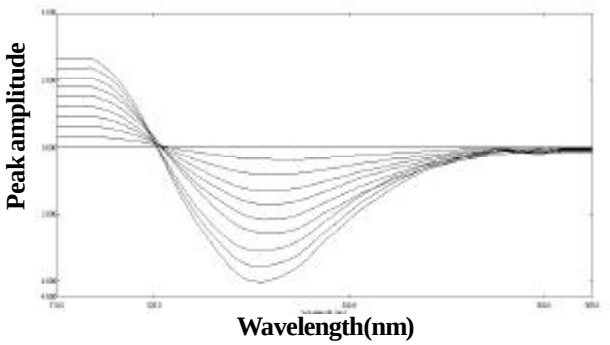

Figure 12: First derivative of ratio spectra of Ziprasidone Hydrochloride 10-90 μg/ml using 90 g/ml of its oxidative degradate as a divisor μg/ml

A=0.0134 C - 0.0197 r = 0.9999 …. Ratio subtraction method at 317nm

A=0.0327 C - 0.0690 r = 0.9990 …..^{1}DD in presence of I at 268.3 nm

A=0.0433 C - 0.0263 r = 0.9999 …..^{1}DD in presence of II at 332.6 nm

A=2679.15 C + 2070.71 r = 0.9999 ……TLC-densitometry

Where, "A" is the absorbance at 317 nm in ratio subtraction, peak amplitude in ^{1}DD and peak area in densitometry, respectively. "C" is the concentration in μg mL^{-1} in ratio subtraction and ^{1}DD and μg/spot in TLC-densitometry and "r" is the regression coefficient.

The mean percentage recoveries and standard deviations of the pure drug were calculated as shown in TABLE 1.

The selectivity of the proposed method was assessed by the analysis of laboratory prepared mixtures containing different ratios of ZIP and its degradates. The results shown in TABLE 2 contributed to the good performance of the methods with high selectivity to determine the studied drug in presence of up to 70% and 90% in presence of I and II, respectively, in ratio subtraction method and 90% and 70% in presence of I and II, respectively in ^{1}DD method and to more than

TABLE 1 : Determination of pure Ziprasidone hydrochloride by the proposed methods

Ratio subtraction at 317nm			^{1}DD at 268.3nm In presence of I		^{1}DD at 332.6nm In presence of II		TLC-Densitometric method		
Taken (μg/ml)	Found (μg/ml)	Recovery%	Found (μg/ml)	Recovery%	Found (μg/ml)	Recovery%	Taken (μg /spot)	Found (μg /spot)	Recovery%
10.00	10.05	100.50	-	-	9.52	95.20	0.60	0.61	101.67
20.00	20.17	100.85	19.81	99.05	20.26	101.30	1.00	0.99	99.00
30.00	29.86	99.53	29.81	99.37	30.17	100.57	1.50	1.48	98.67
40.00	39.66	99.15	40.32	100.80	40.01	100.03	2.00	2.03	101.50
50.00	50.20	100.40	48.88	97.76	50.38	100.76	2.50	2.54	101.60
60.00	59.98	99.97	61.07	101.78	60.08	100.13	3.00	2.92	97.33
70.00	70.65	100.93	71.00	101.43	70.10	100.14	3.50	3.53	100.86
80.00	80.32	100.40	79.44	99.30	79.58	99.48	4.00	4.00	100.00
90.00	89.65	99.61	89.40	99.33	90.12	100.13			
Mean ± S.D		100.15±0.618		99.85±1.359		99.75±1.781			100.08± 1.612

TABLE 2 : Results of laboratory prepared mixtures of Ziprasidone hydrochloride in presence of its alkaline and oxidative degradates by the proposed methods

Degradates	Ratio subtraction at 317nm		^{1}DD at 268.3nm	^{1}DD at 332 nm	TLC-Densitometry	
%	In presence of I	In presence of II	In presence of I	In presence of II	In presence of I	In presence of II
10%	101.93	99.38	100.09	99.23	-	-
30%	100.9	99.19	98.66	97.08	100.23	99.32
50%	102.81	99.15	100.55	95.51	103.29	101.68
70%	101.26	100.79	101.3	95.34	102.43	100.43
85%	-	-	-	-	103.73	98.29
90%	106.28*	103.77	101.64	87.30*	-	-
	101.73 ± 0.840	100.46 ± 1.972	100.45 ± 1.171	96.79 ± 1.805	102.42 ± 1.557	99.93 ± 1.458

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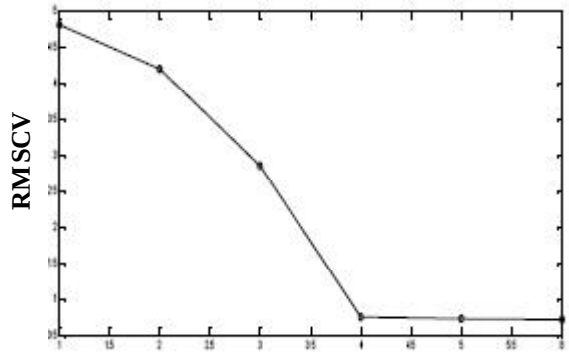

Figure 13: RMSECV plot of the cross validation results of the training set as a function of the number of principle component used to construct the PLS calibration for ZIP

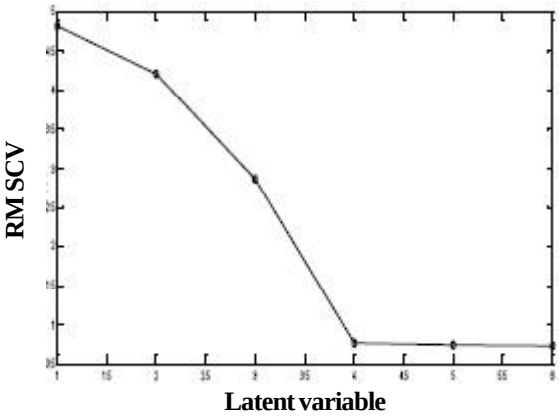

Figure 14: RMSECV plot of the cross validation results of the training set as a function of the number of principle component used to construct the PCR calibration for ZIP

TABLE 3: Determination of pure authentic sample of Ziprasidone hydrochloride by the chemometric methods

Sample no.	ZIP	I	II	Recovery of ZIP	
				PLS	PCR
1	50	45.2	45.2	100.65	100.65
2	45.2	45.2	45.2	99.62	99.62
3	54.8	60	54.8	99.87	99.87
4	60	60	40	99.86	99.86
5	40	54.8	40	99.39	99.39
6	54.8	40	50	100.29	100.29
7	54.8	45.2	40	99.89	99.89
8	45.2	40	45.2	98.85	98.85
9	40	45.2	50	101.15	101.15
10	45.2	50	40	100.56	100.56
Mean ± S.D				100.00 ± 0.658	100.00 ± 0.658

85% in TLC-densitometry.

Chemometric method

In this work, mixtures of different concentrations of ZIP, I and II were used as a calibration samples to construct the models, the spectra of these mixtures were collected and examined, the noisy region from 200-270 nm and the absorbance after 350nm accounted for these parts from the spectra.

The selection of the optimum number of factors for the PLS and PCR techniques was a very important step before constructing the models because if the number of factors retained was more than the required, more noise will be added to the data. On the other hand, if the number retained was too small meaningful data was that could be necessary for the calibration might be discarded.

Selection of the optimum number of factors to build the PLS and PCR models

Use the cross validation method, leaving out one sample at a time, to select the optimum number of factors[14]. Given a set of ten calibration samples, the PLS and PCR calibrations were performed on concentrations were then compared with the known concentrations and the root mean square error of calibration (RMSECV) was calculated. The RMSECV was calculated in the same manner each time; a new factor was added to the model. The maximum number of factors used to calculate the optimum RMSECV was selected to be 6 (half the number of samples+1). The method described by Haland and Thomas[15-8] was used for selecting the optimum number of factors. Four factors were found suitable for both PLS and PCR methods as in figures (13 and 14).

The results predicted by the multivariate methods for the validation model are summarized in TABLE 3.

The evaluation of the predictive abilities of the models was performed by plotting the actual known concentrations against the predicted concentrations.

Another diagnostic test was carried out by plotting the concentration residuals against the predicted concentrations. The residuals appear randomly distributed around zero, indicating adequate models as shown in figures 15.

The RMSECV was used as a diagnostic test for examining the error in the predicted concentrations, another parameter Q^2, was calculated which determined the variation in the prediction samples as shown in TABLE 4.

The proposed methods were also applied for the

TABLE 4: RMSECV and Q2 values of the validation set analysis of ZIP by the chemometric methods

Item	PLS	PCR
RMSECV	0.27364	0.27364
Q^2	1	1

TABLE 5 : Determination of Ziprasidone Hydrochloride in Zeldox® capsules by the proposed methods and application of standard addition technique

Method	Zeldox® 40 mg ZIP/capsule (B.N: 610142134) Recovery%*± S.D	Standard addition technique Pure added (µg/ml)	Recovery %
Ratio subtraction	99.44 ± 1.129	20	101.54
		40	101.92
		50	100.62
		Mean ± S.D	101.36±0.668
^{1}DD in presence of I	98.58± 0.587	30	96.11
		40	94.65
		50	93.38
		Mean ±S.D	94.46 ± 1.457
^{1}DD in presence of II	95.46 ± 0.099	30	98.97
		40	96.03
		50	101.18
		Mean ±S.D	98.73 ± 2.584
TLC-densitometry	101.94± 0.700	1.0	97.62
		1.6	98.17
		2.0	99.24
		Mean ±S.D	98.34 ± 0.824
Chemometric methods	98.18 ± 0.498	10	100.17
		20	101.41
		30	98.93
		Mean ±S.D	100.17±1.240

*Average of three different determinations

TABLE 6: Validation results of the proposed methods for the determination of ZIP

Parameters	Ratio subtraction	^{1}DD in presence of I	^{1}DD in presence of II	TLC-densitometry
Linearity				
Slope	0.0134	0.0327	0.0433	2679.16
Intercept	-0.0197	-0.069	-0.0263	2070.72
Correlation coefficient	0.9999	0.9990	0.9999	0.9990
Range	10-90 µg/ml	20-90 µg/ml	10-90 µg/ml	0.6-4 µg/spot
Accuracy (Mean±S.D)	100.15± 0.618	99.85± 1.359	99.75± 1.781	100.08± 1.612
Precision (RSD%)				
Repeatability[(a)]	1.914	2.33	0.466	0.921
Intermediate precision[(b)]	0.511	2.176	2.723	3.96

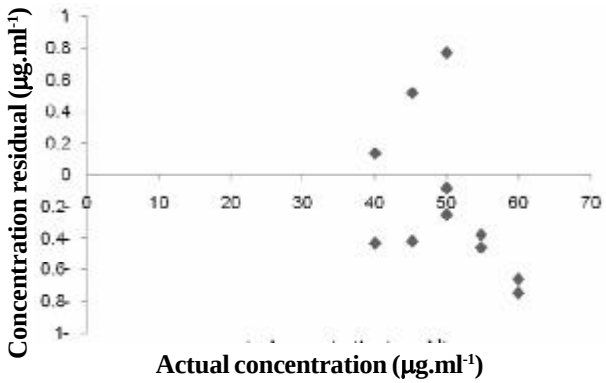

Figure 15: Concentration residuals versus actual concentration of ZIP in the validation set using PLS

TABLE 7 : Statistical analysis of the results of the proposed methods and the reported method for determination of pure Ziprasidone hydrochloride

Items	Ratio subtraction	^{1}DD in presence of I	^{1}DD in presence of II	TLC-densitometry	Chemometric methods	Reported method*
Mean ± S.D	100.15 ± 0.618	99.85 ± 1.359	99.75 ± 1.781	100.08 ± 1.612	100.00 ± 0.658	99.95 ± 0.786
RSD%	0.617	1.361	1.785	1.611	0.658	0.786
n	9	8	9	8	10	5
Variance	0.381	1.847	3.172	2.598	0.433	0.618
Student's t-test	0.529(2.179)	0.280(2.201)	0.241(2.179)	0.196(2.201)	0.138(2.160)**	
F value	1.62(3.84)	2.99(6.09)	5.13(6.04)	4.20(6.09)	1.43(3.63)**	

*Reported HPLC procedure, using acetonitrile –water - trifluoro acetic acid- triethylamine (300-600-0.45-0.72 by volum - Figures in the parenthesis are the corresponding theoretical values of t and F at 0.05 level of significance

determination of ZIP in its dosage form. Furthermore, the validity of the methods were assessed by applying the standard addition technique, as in TABLE 5.

Mean percentage recovery revealed that there was no interference from any excipients ,that may be found in the pharmaceutical dosage forms. Method validation was performed according to USP 2007[19] for all the proposed methods as in TABLE 6.

The results obtained by applying the proposed methods were statistically compared with the reported HPLC method[2] and no significant difference was found regarding accuracy and precision as in TABLE 7.

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CONCLUSION

- In conclusion, the described methods give accurate and precise results for the determination of ZIP. The most striking feature of the ratio subtraction and first derivative ratio method is its simplicity and rapidity over the reported HPLC method, as it does not need filtration or degassing.
- It is the first time to study the oxidative degradate.
- Ratio subtraction is considered to be from the recently applied spectrophotometric methods.
- In this part, chemometric methods present simple, selective, accurate and economical procedures for the simultaneous determination of ZIP and would be useful for the stability investigation of ZIP in pharmaceutical analysis and quality control for medicinal manufacturer.

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