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IODIMETRIC ASSAY OF OLANZAPINE IN PHARMACEUTICALS USING IODATE AND NILE BLUE AS REAGENTS

*A simple, selective and cost effective spectrophotometric method has been described for the determination of olanzapine (OLP) in bulk drug and in tablets. The method involves treating OLP with an excess of iodate in acid medium followed by the determination of liberated iodine by reacting with a fixed amount of Nile blue and measuring the absorbance at 400 nm. In this method, the amount of iodine reacted corresponds to the OLP concentration. The experimental conditions for the assay have been optimized and the absorbance is found to increase linearly with the concentration of OLP ($r = 0.997$). Beer's law is obeyed over the range 15-120 $\mu\text{g mL}^{-1}$. The calculated molar absorptivity and Sandell sensitivity values are $0.657 \times 10^3 \text{ l mol}^{-1} \text{ cm}^{-1}$ and $0.475 \mu\text{g cm}^{-2}$, respectively. The limits of detection (LOD) and quantification (LOQ) are 3.93 and $11.90 \mu\text{g ml}^{-1}$. The performance of the method was validated according to the present ICH guidelines. The repeatability and intermediate precision, expressed by the RSD was better than 3%. The accuracy of the method expressed as relative error was satisfactory. The proposed method was applied to the analysis of tablet form of OLP and the results tallied well with the label claim. No interference was observed from concomitant substances normally added to tablets. The results were statistically compared with those of a literature method by applying the Student's *t*-test and *F*-test. The accuracy and validity of the method were further ascertained by performing recovery studies via spike method.*

Key words: olanzapine; assay; spectrophotometry; iodate; pharmaceuticals; Nile blue.

Olanzapine (OLP), chemically known as 2-methyl-4-(4-methyl-1-piperazinyl)-10 β -thieno-[2,3b] [1,5] benzodiazepine (Fig 1), is an atypical antipsychotic drug used in the treatment of Schizophrenia and other psychotic syndromes [1]. Since its introduction in 1996 in over 84 countries, several workers have reported HPLC methods for the determination of OLP in human plasma, serum, urine, breast milk and rat brain [2-12]. HPLC has also been used for the assay of OLP in pharmaceutical formulations when present either alone [13,14] or in combination with fluoxetine [15,16]. Various other techniques including HPTLC [16], non-aqueous titrimetry and UV-spectrophotometry [17], derivative spectrophotometry, capillary zone electrophoresis and linear voltammetry [13] have also been

reported for the assay of OLP in pharmaceuticals. Biryol and Erk [18] have applied voltametric, spectrophotometric and HPLC techniques for the analysis of OLP. The reported HPLC methods [13,14] require expensive experimental set up and costly solvents besides involving time-consuming start-up and clean-up procedures. In both the methods, the separation and detection is crucially dependant on the pH of the mobile phase. Moreover, the method of Xuejun [14] is less sensitive with a linear range of 10-1000 $\mu\text{g ml}^{-1}$. The currently available voltammetric techniques [13,18] require sophisticated instrumentation and normally lack robustness and ruggedness because of the involvement of scrupulous experimental conditions. The UV-spectrophotometric methods reported by Firdous *et al.* [17] and Biryol and Erk [18] suffer from the disadvantage of interference from the inactive ingredients which absorb in the UV-region. Further, spectrophotometry and voltammetry [13] are reported to be less precise ($RSD \approx 3\%$) and less accurate ($RE \approx 2.6\%$).

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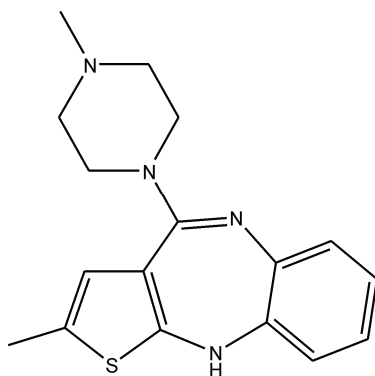


Fig. 1. Structure of olanzapine.

In contrast, a visible spectrophotometry needs a simple and inexpensive instrumental set up and often provides high sensitivity, fair accuracy and precision and requires cheap and easily available chemicals. There are only three reports on the use of the visible spectrophotometry in the assay of OLP. Jasinska and Nalewajko [19] have developed one indirect and two direct flow-injection spectrophotometric methods using hexacyanoferrate (III) and cerium (IV) sulphate as reagents. *N*-Bromosuccinimide (NBS) and cerium (IV) sulphate have been suggested as the oxidimetric reagents for the sensitive determination of OLP by direct and indirect methods in conjunction with Celestine blue [20]. Mohamed [21] has reported two kinetic

spectrophotometric methods for the determination of OLP in its dosage forms and spiked serum samples. However, the reported methods suffer from such disadvantages as drastic experimental conditions, color instability and a meticulous control of experimental variables (Table 1).

Organic sulphides are prone to easy oxidation to form sulphoxides (R_2SO) [22]. The presence of the heterocyclic sulphur atom in OLP makes it susceptible to oxidation by iodate which in recent years has widely been used in the analysis of pharmaceutical substances such as clindamycin [23], novalgin [24], ascorbic acid [25], primaquine phosphate [26], famotidine [27], captopril [28], ranitidine [29], methdilazine [30] and phenothiazines [31] to name a few. In the present method, OLP was oxidized to its sulphoxide by iodate in H_2SO_4 medium and the iodine released was used to convert the blue colored Nile blue to a yellow product ($\lambda_{max} = 400$ nm). The absorbance at 400 nm was found to be linearly dependent on the OLP concentration which served as the basis of quantification of the drug. The method was found to possess the adequate accuracy and precision, sensitivity and selectivity to determine OLP in tablets.

Table 1. Performance characteristic of the existing spectrophotometric methods and the proposed method

Sl. No.	Reagents used	Methodology	λ_{max} nm	Linear range $\mu g\ ml^{-1}$	LOQ $\mu g\ ml^{-1}$	Remarks	Ref.
1	Hexacyano ferrate (III)	Unreacted oxidant measured	425	2.5-40.0	-	Reaction requires 1:1 mixture of H_2SO_4 and H_3PO_4 , FIA assembly required	19
	Hexacyano ferrate (III)	Radical cation measured	540	0.5-250		Contact time 60 min required	
	Cerium (IV) sulphate	-do-	540	0.05-300		-do-	
2	NBS	Radical cation measured	532	10-120 ($\epsilon = 4.2 \times 10^4\ l\ mol^{-1}\ cm^{-1}$)	7.0	Uses 1:1 mixture of H_2SO_4 and H_3PO_4 as the reaction medium, color stable for only 30 S.	20
	NBS-Celestine blue	Unbleached dye color measured	538	0.5-6.0 ($\epsilon = 6.4 \times 10^4\ l\ mol^{-1}\ cm^{-1}$)	0.30	High acidic conditions required	
	Cerium(IV)-Celestine blue	-do-	-do-	0.6-3.0 ($\epsilon = 1.5 \times 10^5\ l\ mol^{-1}\ cm^{-1}$)	0.37		
3	KIO_3	Initial rate of formation of radical cation measured	537	0.4-7.0		Scrupulous control of experimental variables and special equipment for kinetic measurement required.	21
	KIO_3	Maximum absorbance measured	537	0.4-7.0			
4	KIO_3 -Nile blue	Colored product formed between I_2 and dye measured	400	15-120 ($\epsilon = 0.66 \times 10^3\ l\ mol^{-1}\ cm^{-1}$)	11.90	Wide linear dynamic range, Present moderate sensitivity and high accuracy and precision	method

EXPERIMENTAL

Apparatus

A Systronic model 106 digital spectrophotometer equipped with 1-cm matched quartz cells was used for absorbance measurements.

Reagents and standards

All chemicals used were of analytical reagent grade and distilled water was used to prepare the solutions.

Potassium iodate (5%) was prepared by dissolving 12.5 g of the chemical (Qualigens Fine Chemicals, Mumbai, India) in water and diluted to volume in a 250 ml calibrated flask. A stock solution equivalent to 1000 $\mu\text{g ml}^{-1}$ Nile blue was prepared by dissolving 111 mg of the dye (Loba Chemie, Mumbai, India, dye content: 90%) in alcohol and diluting to the mark with the same solvent in a 100 ml standard flask. The stock solution was then diluted appropriately with water to obtain a working concentration of 100 $\mu\text{g ml}^{-1}$. Sulphuric acid (0.1 and 2 M) was prepared by diluting the appropriately concentrated acid (Merck, Mumbai, India, Sp. gr. 1.81) with water.

Standard solution of olanzapine

Pharmaceutical grade OLP certified to be 99.85% pure was obtained as a gift from Cipla India Ltd., Mumbai, India, and used as received. A stock standard solution equivalent to 1000 $\mu\text{g ml}^{-1}$ OLP was prepared by dissolving 100 mg of pure drug in 0.1 M H_2SO_4 and diluting to the mark with the same acid in a 100 ml calibrated flask. The solution was diluted to obtain 300 $\mu\text{g ml}^{-1}$ OLP with the same acid.

General procedure

Different aliquots (0.0, 1.0, 2.0, 3.0 and 4.0 ml) of the standard 300 $\mu\text{g ml}^{-1}$ OLP solution were measured accurately and transferred into a series of 10 ml calibrated flasks and the total volume was adjusted to 4 ml with 0.1 M H_2SO_4 . Two ml of 2 M H_2SO_4 and 1 ml of 5% KIO_3 were added and the content was mixed and let stand for 15 min with occasional shaking. Finally, 0.5 ml of 100 $\mu\text{g ml}^{-1}$ Nile blue was added to each flask and diluted to the mark with water and mixed well. The absorbance of the resulting solution was measured at 400 nm against the reagent blank.

A calibration curve was prepared by plotting the measured absorbance *vs.* concentration of OLP, and the concentration of the unknown was read from the calibration graph or deduced from the regression equation derived using the Beer's law data.

Procedure for tablets

Twenty to forty tablets (depending on the number of mg per tablet) were weighed accurately and

ground into a fine powder. A quantity of the powder containing 100 mg of OLP was accurately weighed into a 100 ml calibrated flask and 60 ml of 0.1 M H_2SO_4 was added. The content was shaken for about 20 min to extract the drug into the liquid phase; the volume was finally diluted to the mark with the same acid; mixed well and filtered using a Whatman No. 42 filter paper. First 10 ml portion of the filtrate was discarded, and the subsequent portion was appropriately diluted with 0.1 M H_2SO_4 to obtain a working concentration of 300 $\mu\text{g ml}^{-1}$ OLP and the assay completed according to the procedure described earlier.

Method validation procedures

Linear range and sensitivity

A pure drug solution in the concentration range 15–120 $\mu\text{g ml}^{-1}$ was treated as described under the "General procedure" and the resulting yellow color was measured at 400 nm *vs.* reagent blank to determine the linear range of applicability.

Selectivity

In order to determine the selectivity of the method, a placebo blank consisting of starch (20 mg), sodium alginate (15 mg), lactose (10 mg), methyl cellulose (20 mg), talc (10 mg), acacia (15 mg) and magnesium stearate (10 mg) was prepared, extracted with 0.1 M H_2SO_4 and the extract was subjected to analysis by applying the procedure described earlier. To further investigate the influence of the tablet excipients on the assay of OLP, a synthetic mixture was prepared separately by adding 20 mg of OLP to the placebo blank cited above, and the steps involved in the tablet assay were repeated.

Assay of precision and accuracy

To evaluate the method for its precision and accuracy, three different concentrations of OLP were analyzed in seven replicates during the same day (intra-day) and on five consecutive days (inter-day). The results of this study are presented in Table 3.

Robustness and ruggedness

To determine the robustness, two experimental variables, the acid concentration and the reaction time, were altered deliberately in small increments. The method ruggedness was determined by having four analysts to perform the assay, and also a single analyst doing analysis on three different instruments.

Application to analysis of tablets

The method was applied to the determination of OLP in three brands of tablets containing different amounts (mg), and the results are summarized Table 4.

Recovery study

A pre-analyzed tablet powder was spiked with pure OLP at three different concentration levels (50, 100 and 150% of that in the tablet powder) and the total concentration was found by the proposed method. Each determination was repeated three times.

RESULTS AND DISCUSSION

The proposed method is based on the oxidation of OLP by an unmeasured excess of iodate and the resulting iodine (the reduced form of iodate) was used

to iodinate the dye, Nile blue. The yellow colored iodinated dye was measured at 400 nm (Fig. 2) and related to OLP concentration. The possible reaction scheme is given in Fig. 3.

The various experimental parameters affecting the formation of the yellow colored product were optimized and used throughout the experiment.

Optimization of variables

The optimum conditions of the proposed method responsible for the oxidation of OLP and the formation of the yellow product were studied and maintained throughout the experiment.

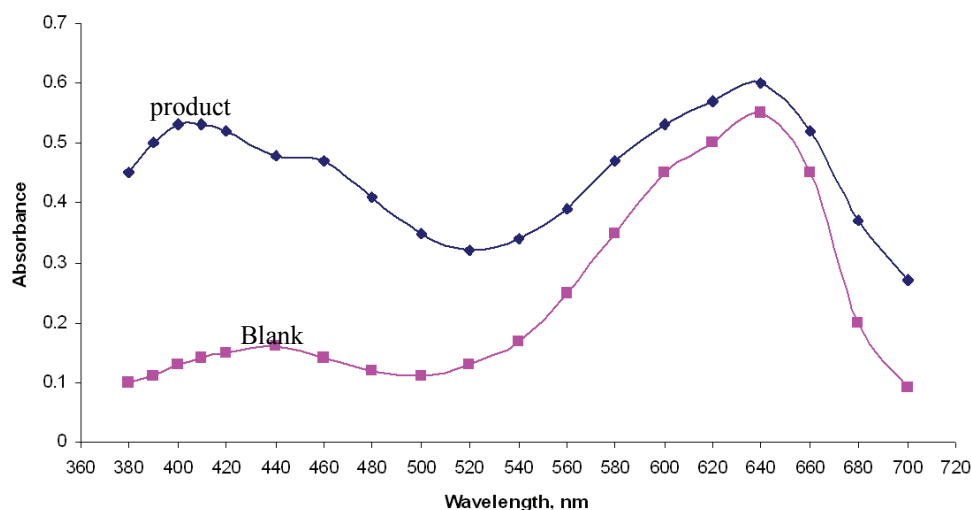
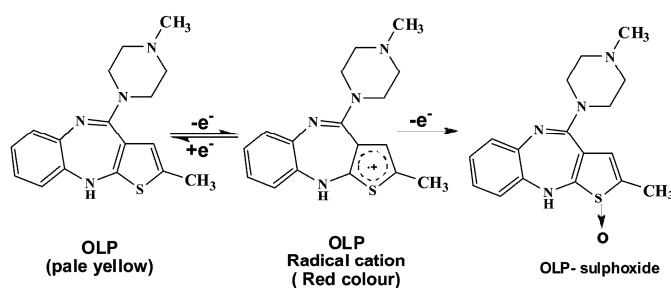


Fig. 2. Absorption spectra of both the product and blank.



Possible mechanism for the oxidation of olanzapine by IO_3^- ion

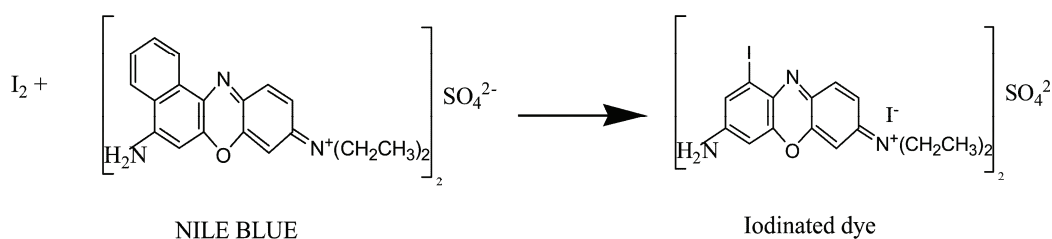


Fig. 3. Tentative reaction scheme.

Effect of acid

Sulphuric acid was found ideally suited for the oxidation of OLP, as well as the conversion of blue Nile blue to the yellow product. The absorbance value was unaffected when 0.8–1.2 M H₂SO₄ was maintained. Higher concentrations yielded less sensitivity. Hence, 2 ml of 2 M H₂SO₄ was used for both steps of the reaction.

Effect of time

The effect of time on the oxidation of OLP by iodate and the formation of yellow color product were studied. The reaction between OLP and iodate was complete in 15 min as indicated by the discharge of purple color of the radical cation [20] and the reaction between liberated I₂ and Nile blue is found to be instantaneous, and constant absorbance readings were obtained when the reaction time was varied from 0–30 min. The developed yellow color was stable for 30 min.

Effect of iodate concentration

To study the effect of iodate concentration, the change in the absorbance due to varying concentrations of iodate on fixed concentrations of OLP and Nile blue was measured against the respective reagent blank. The absorbance values were found to be constant in the range of 1–3 ml of 5% KIO₃ in a total volume of 10 ml; after which further increase in the concentration of iodate showed a decreasing trend in absorbance (Fig. 4). Therefore, 1 ml of 5% KIO₃ in a total volume of 10 ml was recommended for the determination procedure.

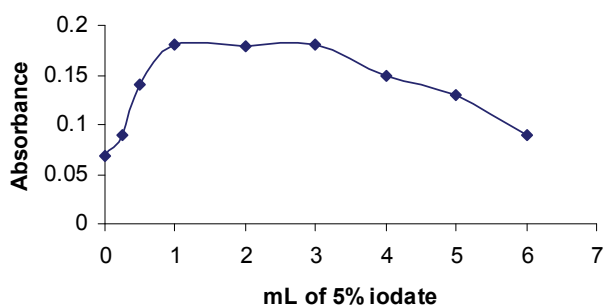


Fig. 4. Effect of iodate concentration (90 $\mu\text{g mL}^{-1}$ OLP).

Effect of Nile blue concentration

The effect of the Nile blue concentration on the formation of yellow the color product was investigated in the range of (0.25–1.25 ml) of 0.01% Nile blue. The maximum absorbance value was obtained with 0.5 ml 0.01% Nile blue and no change in the absorbance value up to 1.25 ml (Fig. 5). Thus, the 0.5 ml of 0.01%

Nile blue was found to be the most suitable concentration for the determination.

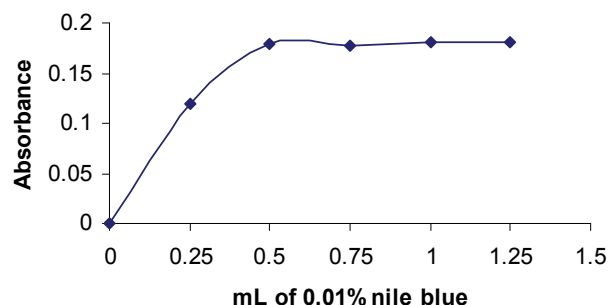


Fig. 5. Effect of Nile blue concentration (90 $\mu\text{g mL}^{-1}$ OLP).

Method validation

Linearity and sensitivity

The relationship between the absorbance and OLP concentration was studied under the optimum conditions established. A linear calibration graph was obtained in the range 15–120 $\mu\text{g mL}^{-1}$ (Table 2). The regression analysis of the plot using the method of least squares was made to evaluate the intercept (a), slope (b), regression coefficient (r) and standard deviations of the slope and intercept. The high value of the regression coefficient (close to unity) of the regression equation and the negligible value of the intercept (0.005) corroborate the linearity of the calibration plot. The moderately high sensitivity of the method was indicated by the fairly high value of molar absorptivity and low values of Sandell sensitivity, LOD and LOQ . These data are summarized in Table 2.

Table 2. Analytical and regression parameters

Parameter	Value
λ_{nm} , nm	400
Beer's law limits, $\mu\text{g mL}^{-1}$	15–120
Molar absorptivity, $\text{L mol}^{-1}\text{cm}^{-1}$	0.66×10^3
Sandell sensitivity, $\mu\text{g cm}^{-2}$	0.475
Limit of detection (LOD), $\mu\text{g mL}^{-1}$	3.93
Limit of quantification (LOQ), $\mu\text{g mL}^{-1}$	11.90
Regression equation, y^*	
Intercept (a)	0.005
Slope (b)	0.002
Correlation coefficient (r)	0.997
Standard deviation of the intercept, S_a	0.02098
Standard deviation of the slope, S_b	0.00022

* $y = a + bx$, where y is the absorbance and x is the concentration in $\mu\text{g mL}^{-1}$

The validation of the regression line and the correlation coefficient was further ascertained by back calculating the “theoretical” concentrations from the

regression line and comparing them with the original concentration values. The calculated percent residuals (*RE*, %) were in the range of ± 2 to ± 5 %. The results are shown in Table 3 and demonstrate that the method provides good accuracy and linearity in the given range of concentrations.

Table 3. The results of validation of the regression line

OLP taken, $\mu\text{g ml}^{-1}$	OLP found, $\mu\text{g ml}^{-1}$	<i>RE</i> , %
60	58.3	2.83
90	87.5	2.78
120	122.5	2.08

Selectivity

The recommended procedure was applied to the analysis of placebo blank and to determine OLP in a synthetic mixture. The absorbance of the placebo blank extract was nearly the same as that of the reagent blank. The results (Table 4) showed that the Student's *t* and *F*-values at 95% confidence level were less than the theoretical values, which confirmed that there is no significant difference between the placebo blank absorbance and reagent blank absorbance. In order to study the ability of the method to determine the analyte in the presence of matrix, the method was applied to the synthetic mixture. It was confirmed that the change in signal measured (absorbance) was caused only by the analyte. The results of the study are presented in Table 5 indicating that the common tablet excipients did not interfere in the assay. In addition, the slope of the calibration plot obtained from the synthetic mixture solution and pure OLP solution were not significantly different. From the calibration plot obtained from the synthetic mixture solution, the value of the parameters like slope (0.0019), intercept (0.005), standard deviation of slope (0.021) and standard deviation of intercept (0.00024) are almost equal to those obtained from the pure drug.

Assay of precision and accuracy

The precision of the method was calculated in terms of the intermediate precision (intra-day and inter-day). The *RSD* values of intra-day and inter-day studies showed that the precision was good (Table 6). The accuracy of an analytical method expresses the

closeness between the reference value and found value. Accuracy was evaluated as a percentage relative error between the measured concentrations and taken concentrations for OLP (bias %). The results obtained are compiled in Table 6 and show that the accuracy is good.

Table 4. Statistical comparison for the absorbance values of placebo blank and reagent blank

Parameter	Placebo blank	Reagent blank
Mean ^a	0.069	0.067
<i>RE</i> , %	2.98	-
<i>SD</i> ^a	0.003	0.0048
<i>RSD</i> ^a	0.043	0.072
Variance ^a	0.000009	0.000023
95% <i>CL</i> ^b	0.069 \pm 0.0037	0.067 \pm 0.0059
<i>F</i> -value ^c	2.56	-
Student's <i>t</i> -value ^c	0.79	-

^aMean of five independent analytes; ^bconfidence limit; ^ctabulated *t* and *F*-values at 95% confidence level for 4 degrees of freedom are 2.77 and 6.39, respectively

Table 5. Recovery of the drug from synthetic mixture

OLP in synthetic mixture taken, $\mu\text{g mL}^{-1}$	OLP recovered ^a \pm <i>SD</i>	<i>RE</i> , %
60	98.01 \pm 1.87	2.12
90	102.6 \pm 1.62	1.81
120	97.21 \pm 1.56	1.43

^aMean value of five determinations

Robustness and ruggedness

In the robustness study, the intermediate precision expressed as *RSD* was calculated and found to be less than 2.8%. The method ruggedness was expressed as inter-instruments and inter-analysts precision. *RSD* values in both the instances were less than 3% indicating the acceptable ruggedness.

Application to analysis of tablets

The proposed method was successfully applied to the determination of OLP in three brands of tablets and the results are summarized Table 7. The results obtained were statistically compared with those of the reference method [17] by applying the Student's *t*-test for accuracy and *F*-test for precision. The reference method involved the titration of the acetous solution of

Table 6. Intra-day and inter-day accuracy and precision

OLP taken, $\mu\text{g ml}^{-1}$	Intra-day accuracy and precision (<i>n</i> = 7)			Inter-day accuracy and precision (<i>n</i> = 5)		
	OLP found, $\mu\text{g ml}^{-1}$	<i>RE</i> , %	<i>RSD</i> , %	OLP found, $\mu\text{g ml}^{-1}$	<i>RE</i> , %	<i>RSD</i> , %
30	30.56	1.87	1.72	30.7	2.33	2.52
60	60.85	1.42	1.64	61.28	2.14	2.46
90	90.77	0.86	1.28	91.84	2.04	2.26

Table 7. Results of assay in tablets and statistical comparisons with the reference method

Tablet brand name	Label claim, mg/tablet	Found ^a (% of label claim \pm SD)	
		Reference method	Proposed method
Oleazn ^b	2.5	101.4 \pm 0.78	102.1 \pm 1.76 t = 0.87 F = 5.09
Oltal ^c	5.0	96.38 \pm 1.11	97.04 \pm 1.85 t = 0.71 F = 2.77
Oliza ^d	20.0	103.3 \pm 0.83	102.8 \pm 1.57 t = 0.66 F = 3.58

^aMean value of five determinations. Tabulated t -value at the 95% confidence level is 2.77; tabulated F -value at the 95% confidence level is 6.39; ^bSun Pharmaceuticals Industries Ltd., Mumbai, India; ^cTalent Pharma, Ahmedabad, India; ^dIntas Pharmaceutical Ltd., Ahmedabad, India

the sample with perchloric acid in acetic acid medium. As it can be seen from the Table 6, the calculated t -value and F -value at 95% confidence level did not exceed the tabulated values of 2.77 and 6.39 respectively, for four degrees of freedom. The tests indicate that there is no difference between the proposed method and the reference method with respect to accuracy and precision.

Recovery study

To further assess the accuracy and reliability of the method, recovery experiments were performed by applying the standard-addition technique. The recovery was assessed by determining the agreement between the measured standard concentration and added known concentration to the sample [32-34]. The recovery values ranged between 97.64 and 103.5% with a relative standard deviation <3%. The results of this study indicated that the recovery was good and that the co-formulated substances did not interfere in the determination.

CONCLUSIONS

A simple, rapid and cost effective spectrophotometric method was developed and validated for the determination of olanzapine. Statistical analysis for the results of assay reveals that the method is repeatable and selective for the analysis of the active ingredient and in tablet formulations without interference from excipients. Though the method is less sensitive, it presents some advantages over the existing spectrophotometric methods. The method employs milder acidic conditions unlike the methods of Jasinska and Nalewajko [19] and Krebs *et al.* [20]. The method is faster than the previously reported method [19] which requires a contact time of 60 min. The present method is based on the measurement of a stable colored species unlike the method of Krebs

et al. [20] where the purple colored radical cation is reported to be stable for 30 s only. The kinetic methods using KIO_3 as the reagent [21] are prone to give inaccurate and imprecise results even under very slightly altered conditions of temperature, acid concentrations and ionic strength whereas the present method has been demonstrated to be both robust and rugged. The method employs a less expensive and simple instrument compared to the flow injection [19], HPLC [13,14], voltammetric [17,18] and capillary zone electrophoresis [13] techniques. The method has been demonstrated to be more sensitive relative to the reported HPLC [14] method besides enjoying better accuracy and precision compared to the reported spectrophotometric and voltammetric methods [13]. The method was found to be quite useful to the determination of olanzapine in the concentration range in which the response is linear.

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