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# Quantitative Determination of Olanzapine in Tablets with Visible Spectrophotometry using Cerium(IV)sulphate and Based on Redox and Complexation Reactions

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**Abstract**

Three sensitive spectrophotometric methods were developed and validated for the quantification of olanzapine (OLP) in bulk drug and in tablets. The method involved treating OLP with a known excess of cerium(IV) in acid medium followed by the determination of unreacted oxidant by three reaction schemes in which cerium(IV) was reduced by an excess of iron(II), and the resulting iron(III) was complexed with thiocyanate, tiron or ferrocyanide and absorbance measured at 480 nm (method A), 640 nm (method B) or 700 nm (method C). Absorbance decreased linearly with concentration over the ranges 0.2-2.0 µg $mL^{-1}$ (method A), 1.0-9.0 µg $mL^{-1}$ (method B) and 0.3-3.0 µg $mL^{-1}$ (method C). The calculated molar absorptivity values were 10.94 $x10^4$, 1.67 $x10^4$ and 4.52 $x10^4$ L $mol^{-1}$ $cm^{-1}$ for method A, method B and method C, respectively; and the corresponding limits of quantification (LOQ) were 0.02, 0.11 and 0.03 µg $mL^{-1}$. Precision results, expressed by the intra-day and inter-day relative standard deviation values, were satisfactory, better than 3 %, and accuracy expressed as relative error varied from 0.75 to 2.5 %. The methods were successfully applied to the determination of OLP in tablets with mean percentage recoveries of 95.84 to 102.5% .The method validation results showed that the sensitivity and selectivity of the methods were adequate for drug monitoring in industrial quality control laboratories.

***Keywords:***

Olanzapine; Assay; Spectrophotometry; Cerium (IV); Pharmaceuticals

## 1. Introduction

Olanzapine (OLP) is chemically known as 2-Methyl-10-(4-methyl-piperazin-1-yl)-4H-3-thia-4,9-diaza-benzo[f]azulene (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 plasma, serum, urine, breast milk and rat brain [2-12]. HPLC has also been used for the assay of OLP in pharmaceutical formulations also 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, There are only three reports on the use of visible spectrophotometry in the assay of OLP. Jasinska and Nalewajko [18] have developed one indirect and two direct

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flow-injection spectrophotometric methods using hexacyanoferrate(III) and cerium(IV)sulphate as reagents. Recently [19], 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 conjuction with Celestine Blue. Mohamed [20], very recently, 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 poor sensitivity, colour instability and meticulous control of experimental variables (Table 1). One of the present authors [21], has recently studied the forced degradation of OLP. The aim of the present study is to develop a simple and sensitive spectrophotometric methods for the determination of OLP in pure form as well as in tablet form using cerium(IV)sulphate, which in recent years has widely been used for pharmaceutical analysis in the authors' laboratory [22-25]. The methods rely on the usage of cerium(IV)sulphate as the oxidimetric reagent, and thiocyanate, tiron or ferrocyanide as the colour forming complexing agents. The proposed methods have been demonstrated to be superior to the reported methods with respect to speed, simplicity, sensitivity, cost effectiveness and eco-friendliness.

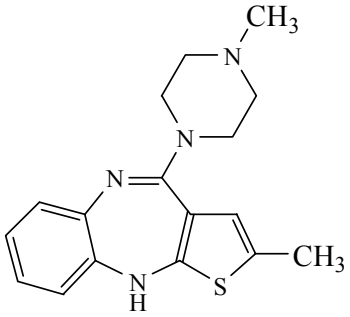



**Fig.1** Structure of olanzapine

**Table 1**. Performance characteristics of the proposed and reported methods.

| Reagent/s used | Methodology | $\lambda_{max}$ (nm) | Linear range (µg $mL^{-1}$) | LOQ (µg $mL^{-1}$) | Remarks | Ref. |
|---|---|---|---|---|---|---|
| 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 | 18 |
| Hexacyano ferrate (III) | Radical cation measured | 540 | 0.5-250 | | | |
| Cerium (IV) sulphate | Radical cation measured | 540 | 0.05-300 | | | |
| $KIO_3$ | Initial rate of formation of radical cation measured | 537 | up to 4.0 | | Scrupulous control of experimental variables and special equipment for kinetic measurement required | 19 |
| $KIO_3$ | Maximum absorbance measured | 537 | up to 7.0 | | | |
| NBS | Radical cation measured | 532 | 10-120 ($\varepsilon = 4.2 \times 10^4$) | 7.0 | Uses 1:1 miture of H2SO4 and H3PO4 as the reaction medium, colour stable for only 30 sec. | 20 |

**Table 1**. (continued)

| Reagent/s used | Methodology | $\lambda_{max}$ (nm) | Linear range (µg $mL^{-1}$) | LOQ (µg $mL^{-1}$) | Remarks | Ref. |
|---|---|---|---|---|---|---|
| NBS | Radical cation measured | 532 | 10-120 ($\varepsilon = 4.2x10^4$) | 7.0 | Uses 1:1 miture of $H_2SO_4$ and $H_3PO_4$ as the reaction medium, colour stable for only 30 sec. | 20 |
| NBS-Celestine blue | Unbleached dye colour measured | 538 | 0.5-6.0 ($\varepsilon = 6.41x10^4$) | 0.30 | High acidic conditions required | 20 |
| Cerium(IV)-Celestine blue | Unbleached dye colour measured | 538 | 0.6-3.0 ($\varepsilon = 1.48 \times 10^5$) | 0.37 | | |
| Ce(IV)-iron(II)-Thiocyanate | Fe(III)-$SCN^-$ complex measured | 480 | 0.2-2.0 ($\varepsilon$=10.94x$10^4$) | 0.02 | Mild experimental conditions, highly sensitive | Present work |
| Ce(IV)-iron(II)-Tiron | Fe(III)-tiron complex measured | 640 | 0.5-9.0 ($\varepsilon$=1.67x $10^4$) | 0.11 | Low acid conditions, very sensitive | Present work |
| Ce(IV)-iron(II)-ferrocyanide | Fe(III)-ferrocyanide complex measured. | 700 | 0.2-3.0 ($\varepsilon = 4.52 \times 10^4$) | 0.03 | Low acid conditions, very sensitive | Present work |

ε Molar absorptivity in L $mol^{-1}cm^{-1}$

## 2. Experimental

### 2.1. Apparatus

A Systronics model 106 digital spectrophotometer provided with 1-cm matched quartz cell was used for all absorbance measurements.

### 2.2. Materials

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

#### 2.2.1. Cerium(IV)sulphate solution (400, 1400 and 360 µg $mL^{-1}$)

A stock standard solution of cerium(IV)sulphate (0.05 mol $L^{-1}$) was first prepared by dissolving accurately weighed 5.5 g of $Ce(SO_4)_2.2H_2O$ (Merk, Mumbai, India) in 0.5 mol $L^{-1}$ $H_2SO_4$ with the aid of heat, filtering through glass wool and diluting to 250 mL in a calibrated flask with the same acid. The stock solution after standardization [26], was diluted stepwise to get the working concentrations of 400, 1400 and 360 µg $mL^{-1}$ cerium(IV)sulphate for method A, method B and method C, respectively, with 0.5 mol $L^{-1}$ $H_2SO_4$.

#### 2.2.2. Ferrous ammonium sulphate :FAS (430, 1400 and 360 µg $mL^{-1}$)

A stock solution equivalent to 0.01 mol $L^{-1}$ FAS was prepared by dissolving about 400 mg of the salt (S.D. Fine Chem, Mumbai, India) in 50 mL of water containing 1 mL of dilute $H_2SO_4$, diluted to 100 mL with water, and standardised [26] using pure potassium dichromate.

The stock solution was then diluted appropriately with water to get 430, 1400 and 360 µg $mL^{-1}$ for method A, method B and method C respectively.

### 2.2.3. Ammonium thiocyanate (3 mol $L^{-1}$)

Prepared by dissolving 29.154 g of the chemical (S.D. Fine Chem, Mumbai, India) in 100 mL of water.

### 2.2.4. Tiron (1%)

About 1.0 g of tiron (Loba chemie, Mumbai, India) was dissolved in 100 mL of water.

### 2.2.5. Ferrocyanide (0.2 %)

About 200 mg of potassium ferrocyanide (S.D. Fine Chem, Mumbai, India) in 100 mL water.

### 2.2.6. Sodium acetate trihydrate (2 mol $L^{-1}$)

Prepared by dissolving 27.22 g of the chemical (S.D. Fine Chem, Mumbai, India) in 100 mL of water.

### 2.2.7. Buffer of pH 1.09

Prepared by mixing 50 mL of 1 M sodium acetate and 70 mL of 1 M HCl and diluting to 250 mL with water.

### 2.2.8. Sodium lauryl sulphate (1 %)

Preapared by dissolving 1 g of sodium lauryl sulphate (S.D. Fine Chem, Mumbai, India) in 100 mL of water.

### 2.2.9. Sulphuric acid

Concentrated sulphuric acid (S.D. Fine Chem, Mumbai, India, sp. gr. 1.84) was diluted appropriately with water to get 5 and 0.1 mol $L^{-1}$ acid solutions.

### 2.2.10. Standard drug solution

Pharmaceutical grade OLP certified to be 99.85 % pure was procured from Cipla India Ltd, Mumbai, India, and used as received. A 500 µg $mL^{-1}$ standard solution of OLP was prepared by dissolving accurately weighed 50 mg of pure drug in 0.1 mol $L^{-1}$ $H_2SO_4$ and diluted to 100 mL with the same acid. The stock solution was diluted stepwise with 0.1 M $H_2SO_4$ to get working concentrations of 5, 20 and 10 µg $mL^{-1}$ OLP for use in method A, method B and method C, respectively.

### 2.2.11. Tablets

Commercially available tablets: Oleanz-20 (Sun Pharmaceuticals Industries Ltd, Mumbai, India), Olenz-5 (Cipla India Pvt Ltd, Mumbai, India) and Olimelt-2.5 (Ranbaxy Laboratories Ltd (Solus)-were used for the study.

## 3. Procedures

### 3.1. Method A

Different aliquots (0.0, 0.4, 0.8, 1.2, 1.6, 2.0 2.4, 2.8, 3.2, 3.6, 4.0 mL) of standard (5 μg $mL^{-1}$) OLP solution were accurately measured and transferred into a series of 10 mL standard flasks by means of micro burette and the volume was adjusted to 4.0 mL by adding 0.1 mol $L^{-1}$ $H_2SO_4$. To each flask was added 1 mL of each of 5 mol $L^{-1}$ $H_2SO_4$ and 400 μg $mL^{-1}$ cerium(IV)sulphate. The content was mixed well and flasks were let stand for 10 min. with occasional shaking. Then, 1 mL of 430 μg $mL^{-1}$ FAS was added to each flask (micro burette), and again flasks were let stand for 2 min. followed by the addition of 1 mL of 3 mol $L^{-1}$ thiocyanate. The volume was diluted to the mark with water, mixed well and absorbance was measured at 480 nm against water blank within 10 min.

### 3.2. Method B

Varying aliquots (0.0,0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 mL) of standard OLP solution (20 μg $mL^{-1}$) were accurately measured into a series of 10 mL calibrated flasks by means of micro burette and the total volume was adjusted to 4.5 mL with 0.1 mol $L^{-1}$ $H_2SO_4$. To each flask, 1 mL of 1400 μg $mL^{-1}$ cerium(IV)sulphate was added by means of micro burette, the content was mixed and allowed to stand for 10 min with occasional shaking. To each flask was then added 1 mL 1400 μg $mL^{-1}$ FAS, and after 5 min, 1 mL each of 2 mol $L^{-1}$ sodium acetate and 1 % tiron were added and diluted to the mark with buffer solution of pH 1.09. After mixing well, the absorbance was measured at 640 nm against a water blank.

### 3.3. Method C

To a series of 10 mL calibrated flasks were added different aliquots (0.0, 0.3, 0.6, 0.9, 1.2, 1.5, 1.8, 2.1, 2.4, 2.7, 3.0 mL) of standard OLP solution (10 μg $mL^{-1}$) by means of a micro burette, and the total volume was adjusted to 3 mL by adding 0.1 mol $L^{-1}$ $H_2SO_4$. To each flask was added 1 mL of 360 μg $mL^{-1}$ cerium(IV)sulphate using a micro burette. The flasks were stoppered, content was mixed well and kept aside for 15 min with occasional shaking. Then 1 mL of 360 μg $mL^{-1}$ FAS was added to each flask (micro burette), and again the flasks were let stand for 5 min followed by the addition of 1 mL each of 1 % sodium lauryl sulphate and 0.2 % ferrocyanide, and after 15 min the volume was made upto mark with water, mixed well and the absorbance was measured at 700 nm against a water blank within 20 min.

In each of the methods, a standard graph was prepared by plotting the decreasing absorbance values versus concentration of OLP. The concentration of unknown was read from the standard graph or from the respective regression equation derived using the Beer's law data.

### 3.4 Procedure for tablet

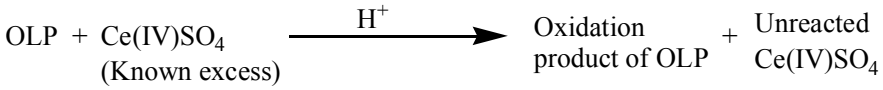
Twenty tablets were weighed and ground into a fine powder. An amount of powder equivalent to 10 mg of OLP was accurately weighed into a 100 mL calibrated flask, 25 mL of 0.1 mol $L^{-1}$ $H_2SO_4$ were added, and the flask was shaken for 20 min; and finally made upto the mark with the same 0.1 mol $L^{-1}$ $H_2SO_4$. The content was kept aside for 5 min, and filtered using Whatmann No 42 filter paper. First 10 mL portion of the filtrate was discarded and a suitable aliquot of the subsequent portion (100 μg $mL^{-1}$ OLP) was diluted appropriately to get 5, 20 and 10 μg $mL^{-1}$ OLP for analysis by method A, method B and method C, respectively.

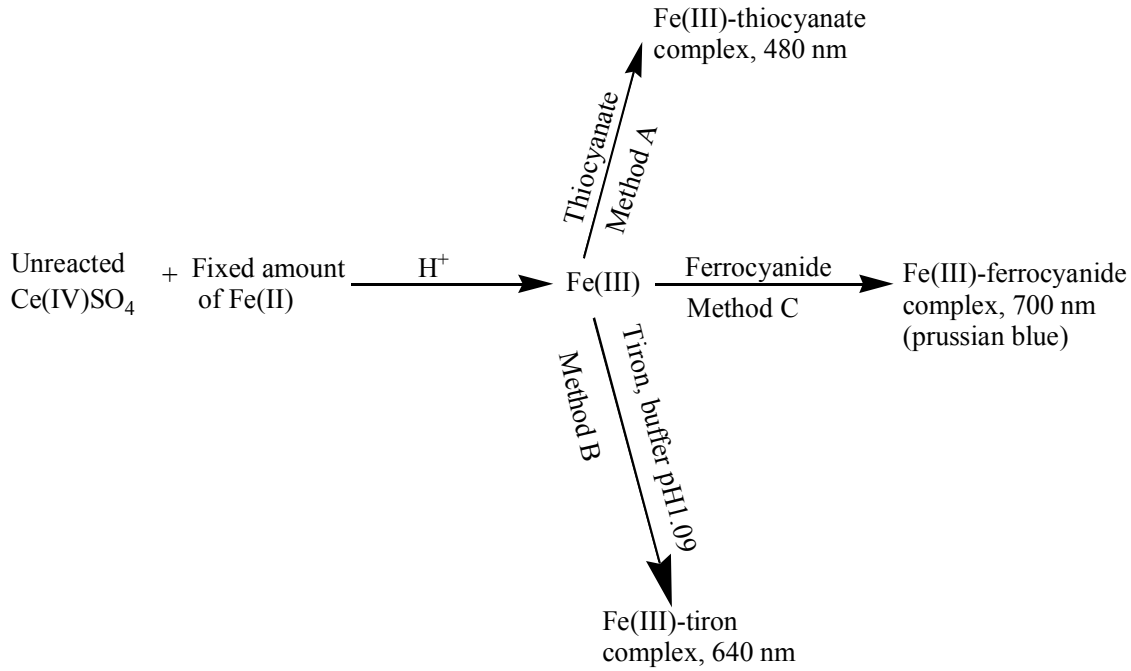
## 4. Results and Discussion

Jasinska and Nalewajko [18] have earlier used cerium(IV) as a reagent for the direct determination of OLP, but the flow injection spectrophotometric determination is found to be critically dependent on $H_2SO_4$ concentration besides being least sensitive with a linear dynamic range of 0.05-300 μg $mL^{-1}$.The method of Anna Krebs *et.al* [19] which also uses cerium(IV) in the presence of Celestine Blue though reported to be sensitive requires a contact time of 35 min for the reaction to proceed to completion.

Some popular methods for cerium(IV) are based on the oxidizing properties of the oxidant in which a known amount of iron(II) is added to a solution containing cerium(IV), which oxidizes an stoichiometric amount of iron(II) to iron(III); and iron(III) is determined with thiocyanate [27], tiron [28, 29] or hexacyanoferrate(II) [30]. The above observations prompted the autors to develop sensitive methods for OLP using cerium(IV) as the oxidimetric reagent, and the procedures were demonstrated to be more robust than reported previously [18, 19].

The proposed methods are indirect and are based on the determination of unreacted cerium(IV) after the reaction between OLP and the oxidant was ensured to be complete; and rely on three different well known reactions which are shown below.




The methods involve oxidation of OLP by a known excess of $Ce(IV)SO_4$ in sulphuric acid medium, reducing the unreacted oxidant by iron(II) and subsequent determination of iron(III) by thiocyante [27], tiron [28, 29] or ferrocyanide [30].

When a fixed concentration of cerium(IV)sulphate is made to react with increasing concentrations of OLP in $H_2SO_4$ medium, there occurs concomitant fall in the concentration of $Ce(IV)SO_4$. When the unreacted oxidant is reduced by a fixed concentration of iron(II), a stoichiometric but decreasing concentration of iron(III) resulted. This is observed as a

proportional decrease in the absorbance of iron(III)-thiocyanate, iron(III)-tiron or iron(III)-ferrocyanide complex on increasing the concentration of OLP which formed the basis for the determination of drug.

### 4.1. Method development

Preliminary experiments were performed to determine cerium(IV) *via* iron(III) and thiocyanate, tiron or hexacyanoferrate(II) as reagents.Under the described experimental conditions, the upper Beer's law limits were fixed at 40, 140 and 36 μg $mL^{-1}$ cerium(IV)sulphate by method A, method B and method C, respectively. Hence different concentrations of OLP were reacted with 1 mL of 400, 1400 or 360 μg $mL^{-1}$ cerium(IV)sulphate in acid medium in method A, method B or method C before determining the residual cerium(IV) *via* the reaction scheme illustrated earlier. This facilitates the optimization of the linear dynamic ranges over which methods could be applied for the assay of OLP.

In all the three methods, $H_2SO_4$ was found ideally suited for the oxidation of OLP by cerium(IV) and the latter's (residual) reduction by iron(II). Since a higher acid concentration of acid is required for iron(III)-thiocyanate reaction [31], 1 mL of 5 mol $L^{-1}$ $H_2SO_4$ in a total volume of 6 mL was used for the oxidation step and the same acid quantity was maintained for the reduction as well as complexation steps in method A. However, the formation of iron(III)-tiron complex (1:1) is pH dependant [28, 29] and hence, in method B, acid present in cerium(IV) solution was found adequate for the first two steps of the assay procedure. In order to raise the pH to the optimum value [29]; 1 mL of 2 mol $L^{-1}$ sodium acetate was added besides using buffer of pH 1.09 as the diluent. Even in method C, $H_2SO_4$ contained in cerium(IV)sulphate was sufficient for all the three steps of assay. However, addition of 1 mL of 1 % sodium lauryl sulphate prior to the addition of hexacyanoferrate(II) was found essential to arrest the flocculation of Prussian blue and to get consistent absorbance readings. The oxidation of OLP was complete in 5-10 min, but the reduction of residual cerium(IV) by iron(II) and subsequent formation of complexes with thiocyanate or tiron was instantaneous whereas the Prussian blue formation was slow taking 15 min after adding hexacyanoferrate(II).

For each system, two blanks were prepared. The first blank which contained all the reactants except OLP exhibited maximum absorbance (equal to the slope of the calibration graph). A second blank without OLP and cerium(IV) was prepared to determine the contribution of other reactants to the absorbance of the system. Since the second blank had negligible absorbance, all measurements were made against a water blank in each method.

### 4.2. Method Validation

#### 4.2.1. Analytical data

A linear correlation was found between absorbance at $\lambda_{max}$ and concentration of OLP in the ranges given in Table 2. Beer's law is obeyed in the inverse manner and the graphs are described by the regression equation:

$$Y = a + bX$$

(Where Y = absorbance of 1-cm layer of solution; a = intercept; b = slope and X = concentration in μg $mL^{-1}$). Regression analysis of the Beer's law data using the method of least squares was made to evaluate the slope (b), intercept (a) and correlation coefficient (r) for each system and the values are presented in Table 2. The optical characteristics such as Beer's law limits, molar absorptivity and sandell sensitivity values of all the three methods

are also given in Table 2. The limits of detection (LOD) and quantitation (LOQ) calculated according to ICH guidelines [32] are also presented in Table 2 and reveal the very high sensitivity of the methods.

**Table 2**. Quantitative and regression parameters of the proposed methods.

| Parameter | Method A | Method B | Method C |
|---|---|---|---|
| $\lambda_{max}$, nm | 480 | 640 | 700 |
| Color stability, min. | 10 | >24 h | 20 |
| Linear range, μg $mL^{-1}$ | 0.2-2.0 | 0.5-9.0 | 0.2-3.0 |
| Molar absorptivity, L $mol^{-1}$ $cm^{-1}$ | 10.940 x $10^4$ | 1.67 x $10^4$ | 4.52 x $10^4$ |
| Sandell sensitivity, μg $cm^{-2}$ | 0.0029 | 0.0187 | 0.0069 |
| Limit of detection, μg $mL^{-1}$ | 0.01 | 0.04 | 0.01 |
| Limit of quantification, μg $mL^{-1}$ | 0.02 | 0.11 | 0.03 |
| Regression equation, Y* | | | |
| Intercept (a) | 0.68 | 0.5292 | 0.7658 |
| Slope (b) | -0.3333 | -0.0565 | -0.2047 |
| Correlation coefficient (r) | -0.9940 | -0.9957 | -0.9956 |
| Standard deviation of b ($S_b$) | 0.0616 | 0.0047 | 0.1259 |
| Standard deviation of a (Sa) | 0.0998 | 0.0364 | 0.3160 |

*Y=a+bX, Where Y is the absorbance and X is concentration in μg $mL^{-1}$.

### 4.2.2 Specificity

The specificity of an analytical method may be defined as the ability to unequivocally determine the analyte in the presence of additional components such as impurities, degradation products and matrix [32-34]. The specificity in the present case was evaluated by preparing the analytical placebo and it was confirmed that the change in absorbance with respect to the reagent blank was caused only by the analyte. A solution of the analytical placebo (containing all the tablet excipients except OLP) was prepared according to the sample preparation procedure and subjected to analysis using the procedures described earlier. The absorbance measured was nearly the same as that of the reagent blank. To identify the interference by these excipients, a synthetic mixture of inactive ingredients (placebo) including OLP with the following composition: OLP (20 mg), talc (30 mg) starch (50 mg), lactose (10 mg), gum acacia (10 mg), calcium dihydrogen orthophosphate (20 mg), sodium alginate (15 mg) and magnesium stearate (30 mg), was prepared. The entire mixture was transferred into a 100 mL calibrated flask, 60 mL 0.1 mol $L^{-1}$ $H_2SO_4$ added and the content shaken for 20 min; volume diluted to the mark with the same acid, mixed well and filtered. The filtrate after suitable dilution was analysed by proposed methods. The difference between the measured absorbance of the above extract and that of a standard OLP solution of the same concentration was less than 3% indicating the absence of interference by the excipients. In addition, the slopes of the standard calibration curves were compared with the slopes of calibration curves prepared with the solutions of the synthetic mixture prepared above. It was found that there was no significant difference between the slopes which indicated that excipients did not interfere in OLP determination.

### 4.2.3 Precision

The precision of the methods was evaluated in terms of intermediate precision (intra-day and inter-day) [32-34]. Three different concentrations of OLP within the Beer's law limits in each method were analysed in seven replicates during the same day (intra-day precision) and five consecutive drugs (inter-day precision). For inter-day precision, each day analysis was performed in triplicate and pooled-standard deviation was calculated. The RSD values of intra-day and inter-day studies for OLP showed that the precision of the methods was good (Table 3).

**Table 3**. Results of accuracy and precision studies.

| Method | OLP Taken, µg mL$^{-1}$ | Intra-day accuracy and precision | | | Inter-day accuracy and precision | | |
|---|---|---|---|---|---|---|---|
| | | OLP found*, µg mL$^{-1}$ | RE % | RSD % | OLP found*, µg mL$^{-1}$ | RE % | RSD % |
| A | 0.5 | 0.51 | 1.5 | 2.24 | 0.49 | 1.0 | 2.64 |
| | 1.0 | 1.02 | 1.7 | 1.56 | 1.02 | 1.5 | 1.85 |
| | 1.5 | 1.47 | 2.0 | 1.28 | 1.48 | 1.6 | 2.06 |
| B | 2.0 | 1.99 | 0.75 | 1.85 | 1.98 | 1.2 | 2.14 |
| | 4.0 | 4.04 | 1.0 | 1.23 | 4.06 | 1.6 | 1.72 |
| | 6.0 | 5.91 | 1.5 | 0.95 | 6.09 | 1.5 | 1.16 |
| C | 1.0 | 1.02 | 2.25 | 1.02 | 1.02 | 2.3 | 2.58 |
| | 2.0 | 1.97 | 1.5 | 1.95 | 1.95 | 2.5 | 1.42 |
| | 3.0 | 3.03 | 1.0 | 3.06 | 3.06 | 2.15 | 1.72 |

*Mean value of seven determination
RE. relative error; RSD. Relative standard deviation.

### 4.2.4 Accuracy

The accuracy of an analytical method expresses the closeness between the reference value and found value [32-34]. Accuracy was evaluated as percentage relative error (RE) between the measured mean concentrations and taken concentrations for OLP. The results obtained are shown in Table 3, from which it is clear that accuracy is satisfactory for the active ingredient.

With respect to accuracy, method B with an intra-day relative error of ≤ 1.5 % is found to be more accurate than the method A and method C which have an upper RE (%) of 2.0 and 2.25 respectively. Similarly, method B (RSD ≤ 1.85 %) is more precise than the remaining two methods with RSD values of 2.24-3.06 %. Almost similar trend is noticed with respect to inter-day accuracy and precision.

### 4.2.5. Robustness and ruggedness

For the evaluation of the method robustness, an important experimental variable, reaction time was slightly varied deliberately. The analysis was performed at ±2 min of the optimum reaction time by taking three different concentrations of OLP and found to remain unaffected as shown by the RSD values in the range of 0.78 to 2.12 %. Method ruggedness was expressed as the RSD of the same procedure applied by four different analysts as well as using three different instruments. The inter-analysts RSD were within 0.92 % whereas the inter-instruments RSD for the same OLP concentrations ranged from 1.32 to 3.46 % suggesting that the developed method was rugged. The results are shown in Table 4.

### 4.3. Application to analysis of tablets

The proposed methods were successfully applied to the determination of OLP in three representative tablets and the results are summarized Table 5. For the brands/ doses examined, the methods gave results which were in agreement with the declared content. The results obtained were compared with those of the reference method [17]. The reference method consisted of measurement of the absorbance of the methanolic extract of the tablets at 226 nm. As can be seen from the Table 5, the results agree well with the claim and also are in agreement with the results obtained by the reference method. When the results were statistically compared with those of the reference method by applying the Student's t-test for accuracy and F-test for precision, 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 methods and the reference method with respect to accuracy and precision.

**Table 4**. Results of method robustness and ruggedness (all values in %RSD) studies.

| Method | Nominal concentration µg $mL^{-1}$ | Reaction times (n=3) | Different analysts, (n=4) | Different instruments (n=3) |
|---|---|---|---|---|
| A | 0.5 | 1.65 | 0.45 | 2.24 |
| | 1.0 | 0.78 | 0.36 | 1.63 |
| | 1.5 | 1.24 | 0.86 | 3.46 |
| B | 2.0 | 2.12 | 0.72 | 1.87 |
| | 4.0 | 1.24 | 0.92 | 2.45 |
| | 6.0 | 1.05 | 0.46 | 1.32 |
| C | 1.0 | 0.65 | 0.52 | 1.56 |
| | 2.0 | 0.86 | 0.85 | 2.64 |
| | 3.0 | 1.34 | 0.36 | 1.78 |

**Table 5**. Results of determination of olanzapine in formulations and statistical comparison with the literature method.

| Tablet brand name** | Nominal amount mg | % Found* ±SD | | | |
|---|---|---|---|---|---|
| | | Literature method | Method A | Method B | Method C |
| Oleanz-20[a] | 20 | 98.64±0.74 | 97.76±1.26<br>t=1.39<br>F=2.89 | 99.12±0.96<br>t=0.89<br>F=1.68 | 98.04±1.38<br>t=0.89<br>F=3.50 |
| Olenz-5[b] | 5.0 | 101.3±0.62 | 102.1±1.46<br>t=1.22<br>F=5.53 | 100.8±1.58<br>t=0.72<br>F=6.5 | 102.5±0.92<br>t=2.46<br>F=2.20 |
| Olimelt-2.5[c] | 2.5 | 96.56±0.84 | 97.31±1.64<br>t=0.96<br>F=3.81 | 95.84±1.72<br>t=0.89<br>F=4.20 | 97.14±1.26<br>t=0.87<br>F=2.25 |

*Mean value of five determinations.
**Marketed by: [a]Sun Pharmaceuticals Industries Ltd, Mumbai, India. [b]Cipla India Pvt Ltd (Mumbai), [c]Ranbaxy Laboratories Ltd (Solus).

### 4.4. Recovery study

To further assess the accuracy of the methods, 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 test was done by spiking the pre-analysed tablet powder with pure OLP at three different levels (50, 100 and 150% of the content present in the tablet powder (taken) and the total was found by the proposed methods (Table 6). Each test was repeated three times. The recovery percentage values ranged between 96.58 and 106.2 with relative standard deviation in the range 1.58 to 3.26 %. Closeness of the results to 100 % showed the fairly good accuracy of the methods.

**Table 6**. Results of recovery experiments by standard addition method

| Method Type | Tablets studied | OLP in tablet (µg/mL) | Pure drug added (µg/mL) | Total found (µg/mL) | Pure drug recovered* Percent±SD |
|---|---|---|---|---|---|
| Method A | Oleanz-20 | 0.49 | 0.25 | 0.71 | 96.58±2.65 |
| | | 0.49 | 0.50 | 0.98 | 99.32±2.31 |
| | | 0.49 | 1.00 | 1.49 | 100.3±1.98 |
| | Olenz-5 | 0.51 | 0.25 | 0.79 | 104.3±1.76 |
| | | 0.51 | 0.50 | 1.04 | 102.5±2.14 |
| | | 0.51 | 1.00 | 1.56 | 103.6±2.52 |
| Method B | Oleanz-20 | 2.97 | 1.5 | 4.41 | 98.56±1.64 |
| | | 2.97 | 3.0 | 6.05 | 101.3±2.25 |
| | | 2.97 | 4.5 | 7.43 | 99.46±1.65 |
| | Olenz-5 | 3.02 | 1.5 | 4.68 | 103.5±2.62 |
| | | 3.02 | 3.0 | 6.07 | 100.8±1.58 |
| | | 3.02 | 4.5 | 7.99 | 106.2±1.89 |
| Method C | Oleanz-20 | 0.98 | 0.5 | 1.45 | 97.85±1.64 |
| | | 0.98 | 1.0 | 1.98 | 100.3±2.76 |
| | | 0.98 | 1.5 | 2.52 | 101.7±3.16 |
| | Olenz-5 | 1.03 | 0.5 | 1.54 | 100.6±2.72 |
| | | 1.03 | 1.0 | 2.09 | 103.1±3.26 |
| | | 1.03 | 1.5 | 2.59 | 102.5±2.46 |

*Mean value of three determination

## 5. Conclusions

Three spectrophotometric methods for the determination of olanzapine in bulk drug and in tablets were developed and validated for accuracy, precision, linearity, robustness and ruggedness. The methods employ mild acid conditions than those previously reported, and rely on well-characterised redox and complex formation reactions. Besides, the methods have the advantages of simplicity without involving heating or extraction step and high sensitivity. Infact, method A with an ε value of $10.94 \times 10^4$ L $mol^{-1}cm^{-1}$ is the most sensitive spectrophotometric method ever reported for olanzapine. These advantages coupled with the

use of inexpensive and eco-friendly chemicals substantiate the usefulness of the methods for routine use.

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