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Simple and Sensitive Spectrophotometric Determination of Olanzapine in Pharmaceutical Formulations Using Two Sulphonphthalein Acid Dyes

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ABSTRACT

Two simple, sensitive and extraction-free spectrophotometric methods have been developed for the determination of olanzapine (OLP) both in pure form and in pharmaceutical formulations. The methods are based on the formation of yellow coloured ion-pair complexes between OLP and two sulphonphthalein acid dyes, bromocresol purple (BCP) and bromothymol blue (BTB) with absorption maximum at 405 nm and 410 nm for BCP and BTB, respectively. The stoichiometry of the complex in either case was found to be 1: 1 and the conditional stability constant (K_F) of the complexes has also been calculated. Reaction conditions were optimized to obtain the maximum colour intensity. Beer's law was obeyed in the concentration ranges of 1.0 - 10.0 and 1.0 - 8.0 μg/mL with BCP and BTB, respectively. Various analytical parameters have been evaluated and the results have been validated by statistical data. The proposed methods have been applied successfully to the analysis of OLP in pure form and in its dosage forms and no interference was observed from common excipients present in pharmaceutical formulations.

Key words: olanzapine, ion-pair complexes, bromocresol purple, bromothymol blue

INTRODUCTION

Olanzapine (OLP), chemically known as 2-methyl-4-(4-methyl-1-piperazinyl)-10H-thieno [2, 3-b] [1, 5] benzodiazepine, 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, human breast milk and rat brain[2-12]. High performance liquid chromatography (HPLC) has also been used for the assay of OLP in pharmaceutical formulations when present either alone[13-15] or in combination with fluoxetine[16,17]. Various other techniques including HPTLC[17], non aqueous titrimetry and UV-spectrophotometry[18], derivative spectrometry[13,15], capillary zone electrophoresis[13], cyclic voltammetry[15], differential pulse voltammetry[15], osteryoung square wave voltammetry[15] 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[19] have developed one indirect and two direct flow-injection spectrophotometric methods using hexacyanoferrate (III) and cerium (IV) sulphate as reagents. Recently, 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, very recently, has reported two kinetic spectrophotometric methods for the determination of OLP in its dosage forms and spiked serum samples[21]. However, the reported methods suffered from one or the other disadvantage such as poor sensitivity, complicated experimental setup and meticulous control of experimental variables (Table 1).

The well-established spectrophotometric method employed ion-pair extraction. In this case, an ion-pair is formed between basic compounds and an anionic dye such as bromophenol blue, bromocresol purple (BCP), methyl orange, etc. At a specific pH, the ion-pair is extracted into an organic solvent, which is immiscible with water, and the concentration of the resulting ion pair in the organic phase is determined spectrophotometrically[22-24]. The

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Table 1. Comparison of the existing visible spectrophotometric methods and the proposed methods

Sl. No.	Reagent/s used	Methodology	λ_{max} (nm)	Linear range (μg/mL) and ε (L/mol/cm)	LOQ (μg/mL)	Reaction time	Remarks	Ref. No.
1.	a) Potassium hexacyano ferrate (III)	Unreacted oxidant measured	425	2.5 - 40.0 ($\varepsilon = 2.59 \times 10^3$)	NA*	60 min	Reaction requires 1:1 mixture of H_2SO_4 and H_3PO_4. Colour of the oxidation product is unstable FIA assembly required	19
	b) Potassium hexacyano ferrate (III)	Radical cation measured	540	0.5 - 250				
	c) Cerium (IV) sulphate	-do-	540	0.05 - 300			-do-	
2.	a) NBS	Radical cation measured	532	10 - 120 ($\varepsilon = 4.19 \times 10^3$)	6.99		Uses 1:1 mixture of H_2SO_4 and H_3PO_4 as the reaction medium, colour stable for only 30s	20
	b) NBS-Celestine blue	Unbleached dye colour measured	538	0.5 - 6.0 ($\varepsilon = 6.41 \times 10^4$)	0.30	10 min	High acidic conditions required and NBS unstable in solution.	
	c) Cerium(IV)-Celestine blue	-do-	538	0.6 - 3.0 ($\varepsilon = 1.48 \times 10^5$)	0.37	35 min		
3.	a) KIO_3	Initial rate of formation of radical cation measured	537	up to 4.0	NA	Within 30s	Scrupulous control of experimental variables and special equipment for kinetic measurement required	21
	b) KIO_3	Maximum absorbance measured	537	Up to 7.0				
4.	a) BCP in dichloromethane	Absorbance of the ion-pair complex measured	405	1.0 - 10.0 ($\varepsilon = 2.80 \times 10^4$)	0.46	5 min	Simple, fast, sensitive, extraction-free, no pH-adjustment, only one reagent required	Present methods
	b) BTB in dichloromethane	-do-	410	1.0 - 8.0 ($\varepsilon = 3.33 \times 10^4$)	0.96	5 min		

*NA: Not available.

ion-pair extraction technique has some difficulties and inaccuracies due to incomplete extraction or the formation of emulsions between the hydrocarbon solvent and the basic compound-containing solution. In response to the problems resulting from extraction of the ion-pair, few articles were published for the analysis of pharmaceutical compounds through ion pair formation without extraction[25-27].

This paper describes, for the first time, the application of acidic dyes to the spectrophotometric determination of OLP. The formed ion-pair complexes between the OLP and two sulphonphthalein acid dyes, namely, BCP and bromothymol blue (BTB) require no extraction step and are measured directly in dichloromethane. The proposed methods have been demonstrated to be superior to the reported methods with respect to speed, simplicity, sensitivity, cost effectiveness and eco-friendliness, and can be adopted by the pharmaceutical laboratories for industrial quality control.

MATERIALS AND METHODS

I. *Apparatus*

A Systronics model 106 digital spectrophotometer

(Ahmedabad, Gujarat, India) equipped with 1 cm matched quartz cells was used for all absorbance measurements.

II. *Reagents and Materials*

All reagents used were of analytical reagent grade and HPLC grade organic solvents were used throughout the investigation.

(I) *0.1% Bromocresol Purple (BCP)*

One hundred milligrams of the chemical (Rolex Laboratory Reagent Ltd., India) was dissolved in dichloromethane (Merck, Mumbai, India, Sp. gr. 1.32) and made up to 100 mL with dichloromethane.

(II) *0.1% Bromothymol Blue (BTB)*

One hundred milligrams of the chemical (Qualigens Fine Chemicals, Mumbai, India) was dissolved in dichloromethane and made up to 100 mL with dichloromethane.

(III) *Standard Drug Solution*

Pharmaceutical grade OLP which is reported to be 99.85% pure was received from Cipla Ltd., India. A stock standard OLP solution (100 µg/mL) was prepared by dissolving 10.0 mg of pure OLP in dichloromethane and diluting to the mark in a 100 mL calibrated flask with dichloromethane. The working standard solution (20 µg/mL) was then prepared by suitable dilution of the stock solution with dichloromethane.

III. *Methods*

(I) *Spectrophotometry Using Bromocresol Purple (BCP)*

Different aliquots (0.25, 0.5, 1.0, 1.5, 2.0 and 2.5 mL) of a standard OLP (20 µg/mL) solution were transferred into a series of 5 mL calibrated flasks using a micro burette and to each flask was added 1 mL of 0.1% BCP solution. The mixture was diluted to the volume with dichloromethane and mixed well. The absorbance of each solution was measured at 405 nm against a reagent blank after 5 min.

(II) *Spectrophotometry Using Bromothymol Blue (BTB)*

Different aliquots (0.25, 0.5, 1.0, 1.5 and 2.0 mL) of a standard OLP (20 µg/mL) solution were transferred into a series of 5 mL calibrated flasks, as described above. Each flask was added 2 mL of 0.1% BTB solution and diluted to the volume with dichloromethane and mixed well. The absorbance of each solution was measured at 410 nm against a reagent blank after 5 min.

IV. *Assay Procedure for Tablets*

Ten tablets were weighed accurately and ground into fine powder. An amount of the powder equivalent to 20 mg of OLP was weighed into a 100 mL calibrated flask containing about 60 mL of dichloromethane. The solution was shaken thoroughly for about 15-20 min, diluted to the mark with dichloromethane, and filtered using a Whatman No. 42 filter paper. First 10 mL portion of filtrate was discarded and subsequent portions were subjected to analysis by the procedure described above after dilution to 20 µg/mL with dichloromethane.

RESULTS AND DISCUSSION

I. *Absorption spectra*

The absorption spectra of the ion-pair complexes, formed between OLP and each of BCP and BTB, were recorded at 360 - 480 nm against the blank solution and are shown in Figure 1. The yellow ion-pair complexes showed maximum absorbance at 405 and 410 nm for OLP-BCP and OLP-BTB, respectively. The measurements were thus made at these wavelengths for bulk and tablet samples.

II. *Reaction Mechanism*

Olanzapine forms ion-pair complexes with dyes such as BCP and BTB, since it contains tertiary amino group which is protonated. In the ring of 1H-[1, 4] diazepine, protonation is very difficult due to resonance and steric effects. Therefore, the only site in OLP vulnerable for protonation is the nitrogen bonded to electron donating methyl group in the piperazine ring[23]. BCP and BTB are dyes of sulphonphthalein type. The colour of such dyes is

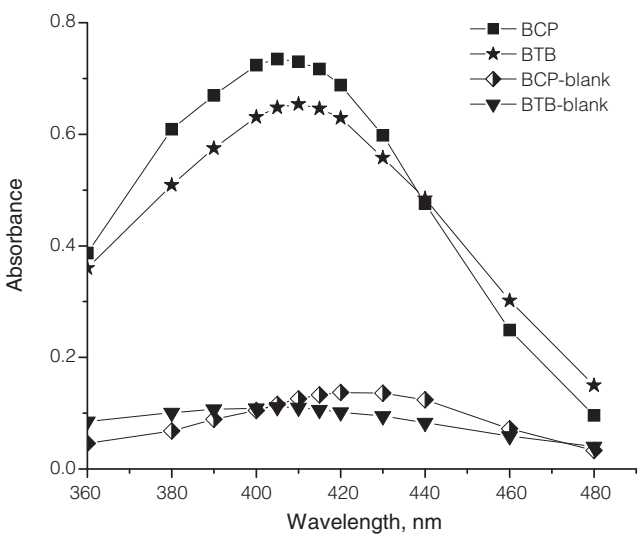

Figure 1. Absorption spectra of ion-pair complexes of OLP (6 µg/mL) with BCP and OLP (6 µg/mL) with BTB against reagent blank.

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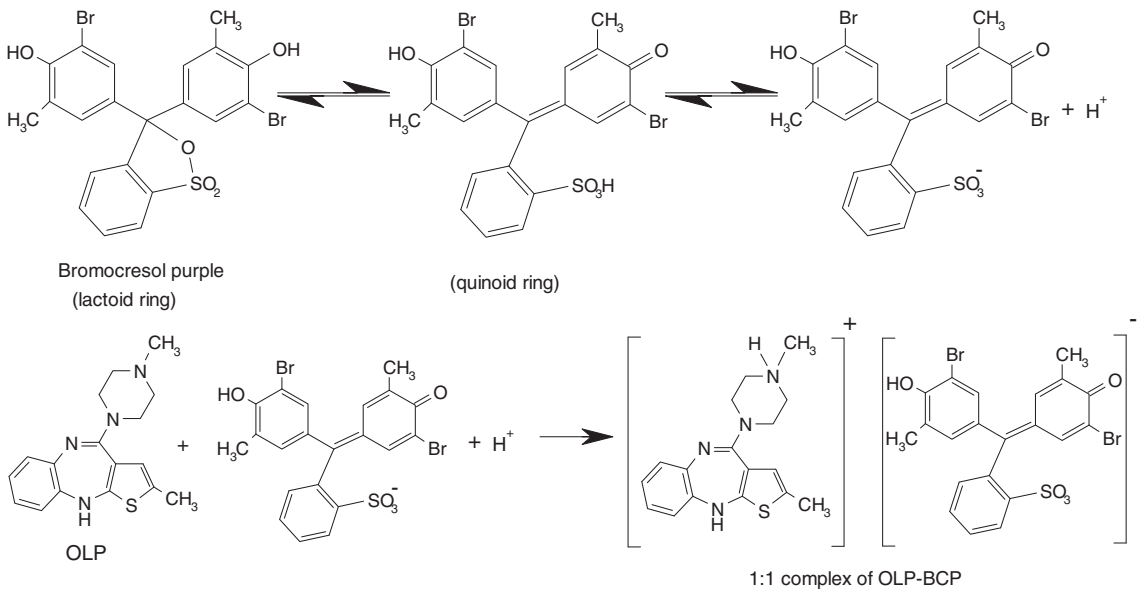
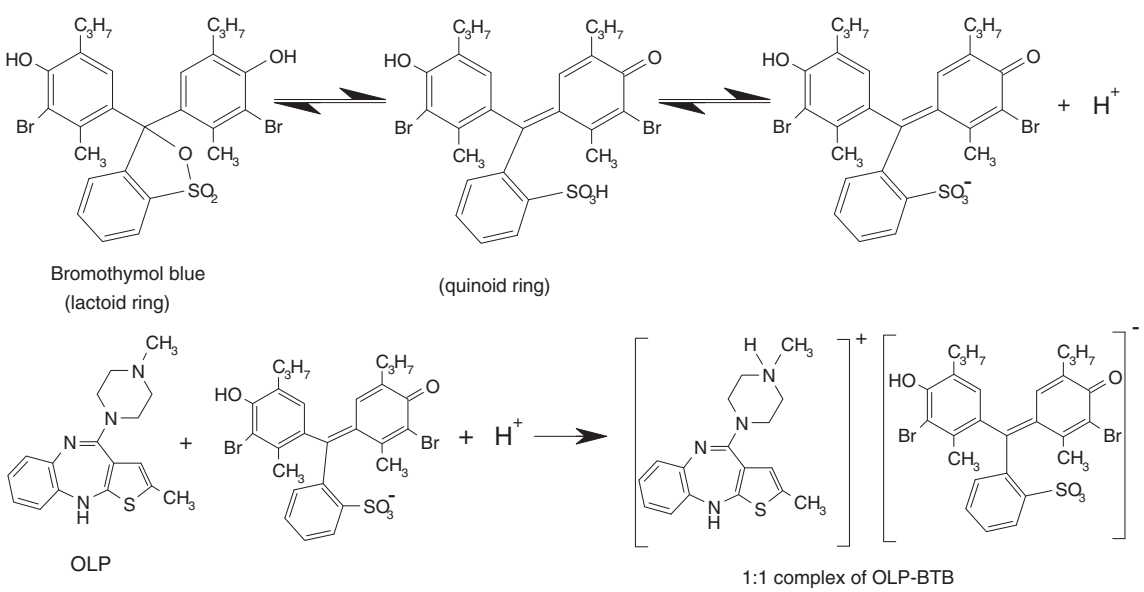
due to the opening of lactoid ring and subsequent formation of quinoid group[24]. It is supposed that the two tautomers are present in equilibrium but due to strong acidic nature of the sulphonic acid group, the quinoid body must predominate. Finally the protonated OLP forms ion-pairs with the dyes. The possible reaction mechanisms are proposed and illustrated in Schemes 1 and 2.

III. *Optimization of Reaction Conditions*

The effect of the dye-concentration on the intensity of the colour developed at selected wavelengths was studied by measuring the absorbances of solutions containing a fixed concentration of OLP (8 μg/mL) and different amounts of the respective dye of 0.1% BCP solution (0.25 - 2.5 mL) and of 0.1% BTB (0.5 - 3.0 mL) solution. Maximum color intensity of the complex was achieved with 1 mL of BCP and 2 mL of BTB solution and excess dyes did not affect the absorbance of the complex. The addition of the dye solution resulted in an immediate full color development at room temperature and the formed ion pairs were stable for at least 1.5 h and 1 h with BCP and BTB, respectively. The reaction was found complete and quantitative when the reaction mixture was allowed to stand for 5 min and any delay in the absorbance measurements of the yellow ion pair complexes up to 1 hour had no



Scheme 1. The possible reaction mechanism of formation of OLP-BCP ion-pair complex.



Scheme 2. The possible reaction mechanism of formation of OLP-BTB ion-pair complex.

effect on the reaction stoichiometry which is determined to be 1: 1 (OLP: dye) for the ranges studied.

IV. *Composition of the Ion-pair Complexes*

The composition of the ion-pair complex formed between OLP and BCP/BTB was established by applying Job's method of continuous variations. In this method, solutions of 6.4×10^{-5} M standard OLP and 6.4×10^{-5} M dye (BCP/BTB) were mixed in varying volume ratios in such a way that the total volume of each mixture was kept the same. The absorbance of each solution was plotted against the mole fraction of the drug, $\frac{V_{OLP}}{V_{OLP} + V_{dye}}$ (Figure 2). The plot reached a maximum value at a mole fraction of 0.5 which indicated the formation of 1: 1 (OLP: dye) complexes. The conditional stability constants (K_F) of the ion-pair complexes were calculated[28] from the data of continuous variations method and found to be 9.45×10^5 and 2.05×10^6 for OLP-BCP and OLP-BTB complexes, respectively.

V. *Method Validation*

(I) *Analytical Parameters*

Under optimum experimental conditions for OLP determination, the standard calibration curves for OLP with BCP and BTB were constructed by plotting absorbance versus concentration. The regression parameters calculated from the calibration graphs data, along with the standard deviations of the slope (S_b) and the intercept (S_a) are presented in Table 2. Beer's law was obeyed over the concentration ranges given in Table 2, and the linearity of calibration graphs was demonstrated by the high values of the correlation coefficient (r) and the small values of the y-intercepts of the regression equations. The apparent molar absorptivities of the resulting colored ion-pair complexes and Sandell sensitivities were

Table 2. Analytical and regression parameters

Parameter	BCP method	BTB method
λ_{max}, nm	405	410
Beer's law limits (μg/mL)	1.0-10.0	1.0-8.0
Concentration of the dye	0.02%	0.04%
Molar absorptivity (L/mol/cm)	2.80×10^4	3.33×10^4
Sandell sensitivity* (μg/cm^2)	0.0111	0.0094
Limit of detection (μg/mL)	0.15	0.32
Limit of quantification (μg/mL)	0.46	0.96
Regression equation, Y**		
Intercept (a)	-0.0072	-0.0133
Slope (b)	0.0923	0.1124
Correlation coefficient (r)	0.9998	0.9998
Standard deviation of intercept (S_a)	0.0060	0.0059
Variance (S_a^2)	3.61×10^{-5}	3.43×10^{-5}
$\pm tS_a / \sqrt{n}$	6.31×10^{-3}	7.28×10^{-3}
Standard deviation of slope (S_b)	0.00099	0.00119
$\pm tS_b / \sqrt{n}$	1.04×10^{-3}	1.48×10^{-3}

*Limit of determination as the weight in μg per mL of solution, which corresponds to an absorbance of A = 0.001 measured in a cuvette of cross-sectional area 1 cm^2 and l = 1 cm.

** $Y = a + bX$, where Y is the absorbance and X is the concentration in μg/mL; $\pm tS_a / \sqrt{n}$ = confidence limit for intercept, $\pm tS_b / \sqrt{n}$ = confidence limit for slope.

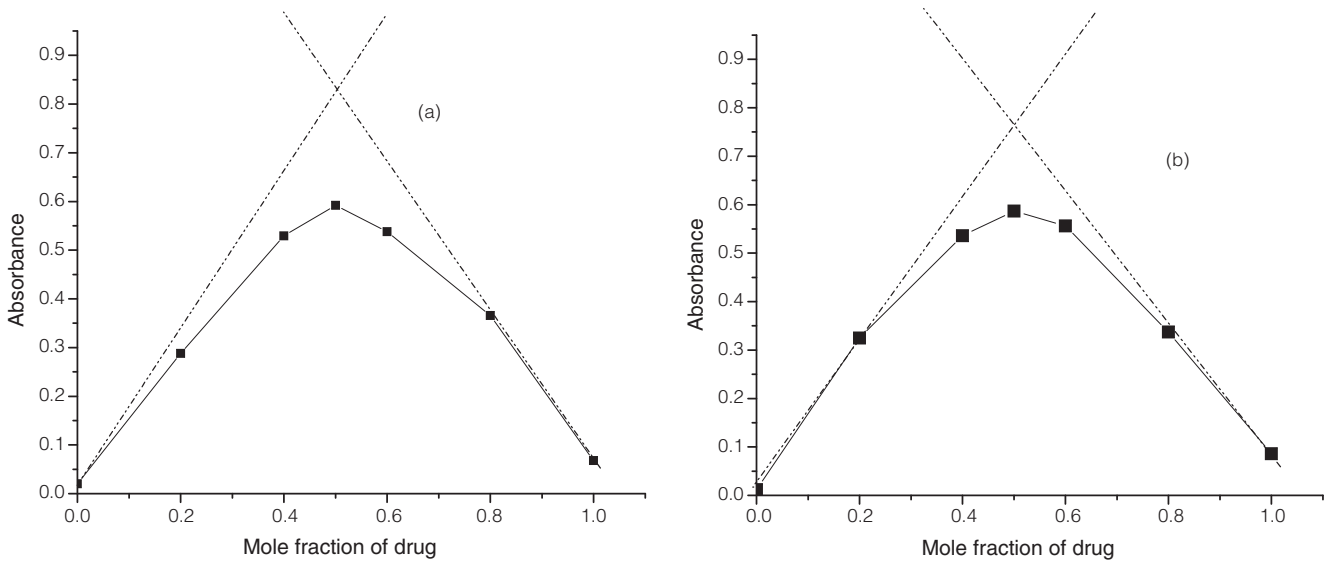


Figure 2. Job's Continuous - variations plots (a) OLP + BCP; (b) OLP + BTB.

also shown in Table 2. The detection and quantification limits[29] were calculated from the standard deviation of the absorbance measurements in a series of 6 and 5 blank solutions for BCP and BTB methods, respectively.

(II) *Accuracy and Precision*

The accuracy of an analytical method expresses the closeness between the reference value and the found value[29]. Accuracy was evaluated as percentage relative error between the measured concentrations and taken concentrations for OLP (Bias %). The results, compiled in Table 3, show that the accuracy is good for both methods. The precision of the methods was calculated in terms of intermediate precision (intra-day and inter-day) [30]. Three different concentration of OLP (within the working limits) were analysed in seven replicates during the same day (intra-day precision) and five consecutive days (inter-day precision). The RSD (%) values of intra-day and inter-day studies showed that the precision was good for both methods, too (Table 3).

(III) *Selectivity*

The proposed methods were tested for selectivity by placebo blank and synthetic mixture analyses. A placebo blank containing talc (50 mg), starch (40 mg), lactose (30 mg), calcium carbonate (20 mg), calcium dihydrogen orthophosphate (20 mg), methyl cellulose (30 mg), sodium alginate (25 mg) and magnesium stearate (20 mg) was extracted with dichloromethane in both methods and solutions were made as described above under "assay procedure for tablets". A convenient aliquot of solution was subjected to analysis by both methods according to the recommended procedures. In both methods, there was no interference from the inactive ingredients.

A separate test was performed by applying the proposed methods to the determination of OLP in a synthetic mixture. To the placebo blank of similar composition, 10 mg of OLP was added and homogenized. The solution of the synthetic mixture equivalent to 200 µg/mL of OLP was prepared as described above for tablets. The filtrate was collected in a 50 mL flask and

Table 3. Evaluation of intra-day and inter-day precision and accuracy

Method*	OLP taken (µg/mL)	Intra-day (n = 7)			Inter-day (n = 5)		
		OLP found[a] (µg/mL)	Precision[b]	Accuracy[c]	OLP found[a] (µg/mL)	Precision[b]	Accuracy[c]
BCP	3.0	3.06	1.97	2.00	3.07	2.65	2.33
	6.0	5.98	1.99	0.33	6.08	3.12	1.33
	9.0	8.82	1.35	2.00	9.28	2.76	3.11
BTB	2.0	2.04	1.86	2.00	2.04	2.56	2.00
	4.0	4.07	2.18	1.75	4.09	3.09	2.25
	6.0	6.13	2.24	2.17	6.19	2.42	3.17

[a]Mean value of n determinations.
[b]Relative standard deviation (%).
[c]Bias (%): (found – taken / taken) ×100.

Table 4. Comparison of assay results of proposed and reference methods

Tablet brand name**	Nominal amount (mg)	Found (% of nominal amount ± SD)*		
		Reference method	Proposed methods	
			BCP method	BTB method
Oleanz[1]	5	98.56 ± 1.14	99.12 ± 1.64 **t** = 0.64 **F** = 2.07	99.72 ± 1.85 **t** = 1.23 **F** = 2.63
Oliza[2]	10	100.3 ± 1.28	101.1 ± 1.74 **t** = 0.84 **F** = 1.85	99.36 ± 2.05 **t** = 0.89 **F** = 2.56

*Mean value of five determinations.
**Marketed by: [1]Sun pharmaceuticals Industries Ltd., Mumbai, India. [2] Intas Pharmaceuticals, Dehradun, India;
Tabulated t-value at the 95% confidence level is 2.78; Tabulated F-value at the 95% confidence level is 6.39.

Table 5. Results of recovery study by standard-addition method

Formulation studied	BCP method				BTB method			
	OLP in tablet (μg/mL)	Pure OLP added (μg/mL)	Total found, (μg/mL)	Pure OLP recovered* (Percent ± SD)	OLP in tablet (μg/mL)	Pure OLP added (μg/mL)	Total found (μg/mL)	Pure OLP recovered* (Percent ± SD)
Oliza, 10 mg	4.04	2.0	6.16	106.0 ± 0.03	1.99	1.0	3.03	104.0 ± 0.10
	4.04	4.0	8.32	107.0 ± 0.07	1.99	2.0	4.13	107.0 ± 0.14
	4.04	6.0	10.33	104.8 ± 0.17	1.99	3.0	4.85	95.33 ± 0.16

*Mean value of three determinations.

1.5 mL of the resulting solution was assayed (n = 5) by BCP method which yielded a% recovery of 103.4 ± 2.47 and 1.0 mL of the resulting solution was assayed (n = 5) by BTB method which yielded a % recovery of 96.44 ± 2.53. These results complemented the findings of the placebo blank analysis with respect to selectivity.

(IV) *Application to Formulations*

The proposed methods were applied to the determination of OLP in two representative tablets oleanz-5 and oliza-10. The results in Table 4 showed that the methods are successful for the determination of OLP and that the excipients in the dosage forms do not interfere. A statistical comparison of the results for determination of OLP from the same batch of material by the proposed and reference method[18] is shown in Table 4. The reference method consisted measurements of the absorbance of the methanolic extract of the tablets at 226 nm. The results agreed well with the label claim and also are in agreement with the results obtained by the reference method. Statistical analysis of the results using Student's t-test for accuracy and F-test for precision revealed no significant difference between the proposed and reference method at the 95 % confidence level with respect to accuracy and precision (Table 4).

(V) *Recovery Study*

To ascertain the accuracy and validity of the proposed methods, recovery experiment was performed *via* standard addition technique. To a fixed and known amount of OLP in tablet powder (pre-analysed), pure drug was added at three levels (50, 100 and 150% of the level present in the tablet) and the total was found by the proposed methods. Results of this study presented in Table 5 indicated that the commonly added excipients such as talc, starch, lactose, sodium alginate, magnesium stearate, calcium gluconate and calcium dihydrogen orthophosphate did not interfere in the assay.

CONCLUSIONS

Unlike GC and HPLC techniques, spectrophotometry is simple and inexpensive. The importance of the technique also lies in the chemical reactions upon which the procedures are based rather than upon the sophistication of the instrument. This aspect of spectrophotometric analysis is of major interest in analytical pharmacy since it offers distinct possibility in the assay of a particular component in complex dosage formulations. The proposed methods require only dyes as reagents which are cheaper and readily available; no pH-adjustment is required and the procedures do not involve any critical reaction conditions or tedious sample preparation. Moreover, both methods are simple, fast, accurate, extraction-free, adequately sensitive and free from interference by common additives and excipients. The present methods are superior to the reference method with respect to both sensitivity and selectivity. The calculated ε values of the proposed methods are 2.80×10^4 and 3.33×10^4 L/mol/cm whereas that of the reference method is 2.37×10^4. Further, in the reference method, the absorbance is measured at 226 nm where the interference from excipients particularly with unsaturation is very serious and the present methods are free from such interference. Moreover, the reference method uses methanol, a toxic solvent, as the medium whilst the present methods use a non-toxic solvent, one of the characteristic features of green analytical chemistry. The wide applicability of the new procedures for routine quality control is well established by the assay of OLP in pure form and in pharmaceutical preparations.

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REFERENCES

1. Shen, W. W. 1999. The metabolism of atypical antipsychotic drugs: an update. Ann. Clin. Psychiatry 11: 145-158.
2. Concetta, D., Gaetana, M., Vincenza, S. and Edoardo, S. 2006. Determination of olanzapine in human plasma by reversed-phase high-performance liquid chromatography with ultraviolet detection. Ther. Drug Monit. 28: 388-393.
3. Raggi, M. A., Casamenti, G., Mandrioli, R., Fanali, S., De Ronchi, D. and Volterra, V. 2000. Determination of the novel antipsychotic drug olanzapine in human plasma using HPLC with amperometric detection. Chromatographia 51: 562-566.
4. Raggi, M. A., Mandrioli, R., Sabbioni, C., Ghedini, N., Fanali, S. and Volterra, V. 2001. Determination of olanzapine and desmethylolanzapine in the plasma of schizophrenic patients by means of an improved HPLC method with amperometric detection. Chromatographia 54: 203-207.
5. Dusci, L. J., Hackett, L. P., Fellows, L. M. and Ilett, K. F. 2002. Determination of olanzapine in plasma by high-performance liquid chromatography using ultraviolet absorbance detection. J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 773: 191-197.
6. Saracino, M. A., Koukopoulos, A., Sani, G., Amore, M. and Raggi, M. A. 2007. Simultaneous high-performance liquid chromatographic determination of olanzapine and lamotrigine in plasma of bipolar patients. Ther. Drug Monit. 29: 773-780.
7. Raggi, M. A., Casamenti, G., Mandrioli, R. and Volterra, V. 2001. A sensitive high-performance liquid chromatographic method using electrochemical detection for the analysis of olanzapine and desmethylolanzapine in plasma of schizophrenic patients using a new solid-phase extraction procedure. J. Chromatogr. B Biomed. Sci. Appl. 750: 137-146.
8. Olesen, O. V. and Linnet, K. 1998. Determination of olanzapine in serum by high-performance liquid chromatography using ultraviolet detection considering the easy oxidability of the compound and the presence of other psychotropic drugs. J. Chromatogr. B Biomed. Sci. Appl. 714: 309-315.
9. Harald, W., Sebastian, H., Sabine, M., Werner, K., Godehard, K., Gerd, D. and Christoph, H. 2001. Simultaneous determination of olanzapine, clozapine and demethylated metabolites in serum by on-line column-switching high-performance liquid Chromatography. J. Chromatogr. B Biomed. Sci. Appl. 759: 63-71.
10. Olesen, O. V., Poulsen, B. and Linnet, K. 2001. Fully automated on-line determination of olanzapine in serum for routine therapeutic drug monitoring. Ther. Drug Monit. 23: 51-55.
11. Kasper, S. C., Mattiuz, E. L., Swanson, S. P., Chiu, J. A., Johnson, J. T. and Garner, C. O. 1999. Determination of olanzapine in human breast milk by high-performance liquid chromatrography with electrochemical detection. J. Chromatogr. B Biomed. Sci. Appl. 726: 203-209.
12. Saracino, M. A., Gandolfi, O., Dall'Olio, R. O., Albers, L., Kenndler, E. and Raggi, M. A. 2006. Determination of Olanzapine in rat brain using liquid chromatography with coulometric detection and a rapid solid-phase extraction procedure. J. Chromatogr. A 1122: 21-27.
13. Raggi, M. A., Casamenti, G., Mandrioli, R., Izzo, G. and Kenndler, E. 2000. Quantitation of olanzapine in tablets by HPLC, CZE, derivative spectrometry and linear voltammetry. J. Pharm. Biomed. Anal. 23: 973-981.
14. Xuejun, X. and Zhonghua, T. 2004. Determination of olanzapine and its tablets by HPLC. Zhongguo Yiyao Gongye Zazhi 35: 46-48.
15. Biryol, I. and Erk, N. 2003. Voltammetric, spectrophotometric, and high performance liquid chromatographic analysis of olanzapine. Anal. Lett. 36: 2497-2513.
16. Reddy, B. V., Suresh Reddy, K. V. N., Sreeramulu, J. and Kanumula, G. V. 2007. Simultaneous determination of olanzapine and fluoxetine by HPLC. Chromatographia 66: 111-114.
17. Shah, C. R., Shah, N. J., Suhagia, B. N. and Patel, N. M. 2007. Simultaneous assay of olanzapine and fluoxetine in tablets by column high-performance liquid chromatography and high-performance thin-layer chromatography. J. AOAC Int. 90: 1573-1578.
18. Firdous, S., Aman, T. and Nisa, A. 2005. Determination of olanzapine by UV spectrophotometry and non-aqueous titration. J. Chem. Soc. Pak. 27: 163-167.
19. Jasinska, A. and Nalewajko, E. 2004. Batch and flow-injection methods for the spectrophotometric determination of olanzapine. Anal. Chim. Acta 508: 165-170.
20. Krebs, A., Starczewska, B., Puzanowsha-Tarasiewicz, H. and Sledz, J. 2006. Spectrophotometric determination of olanzapine by its oxidation with N-bromosuccinimide and cerium (IV) sulfate. Anal. Sci. 22: 829-833.
21. Mohamed, A. A. 2008. Kinetic and maximum-absorbance spectrophotometric methods for the determination of olanzapine. Monatsh Chem. 139: 1005-1010.
22. Basavaiah, K. and Charan, V. S. 2004. Ion-pair complexometric determination of cyproheptadine hydrochloride using bromophenol blue. Sci. Asia 30: 163-170.
23. Harikrishna, K., Nagaralli, B. S. and Seetharamappa, J. 2008. Extractive spectrophotometric determination of sildenafil citrate (viagra) in pure and pharmaceutical formulations. J. Food Drug Anal. 16: 11-17.
24. Ashour, S., Chehna, M. F. and Bayram, R. 2006. Spectrophotometric determination of alfuzosin HCl in pharmaceutical formulations with some sulphonephthalein dyes. Int. J. Biomed. Sci. 2: 273-278.

25. Al-Ghannam, S.M. 2006. A simple spectrophotometric method for the determination of beta-blockers in dosage forms. J. Pharm. Biomed. Anal. 40: 151-156.
26. Abdine, H., Belal, F. and Zoman, N. 2002. Simple spectrophotometric determination of cinnarizine in its dosage forms. Il Farmaco 57: 267-271.
27. Manjunatha, D. H., Shaikh, S. M. T., Harikrishna, K., Sudhirkumar, R., Kandagal, P. B. and Seetharamappa, J. 2008. Simple and sensitive spectrophotometric methods for the determination of acebutolol hydrochloride in bulk sample and pharmaceutical preparations. Eclet. Quím. 33: 37-40.
28. Amin, A. S., Gouda, A. A. E., El-Sheikh, R. and Zahran, F. 2007. Spectrophotometric determination of gatifloxacin in pure form and in pharmaceutical formulation. Spectrochim. Acta A Mol. Biomol. Spectrosc. 67: 1306-1312.
29. International Conference on Hormonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, ICH Harmonised Tripartite Guideline. 2005. Validation of Analytical Procedures: Text and Methodology, Q2 (R 1), Current Step 4 Version, Parent Guideline dated 27 October 1994; (Complementary Guideline on Methodology dated 06 November 1996, incorporated in November 2005), London. http://www.ich.org/LOB/media/MEDIA417.pdf
30. Shabir, G. A. 2003. Validation of high-performance liquid chromatography methods for pharmaceutical analysis: Understanding the differences and similarities between validation requirements of the US Food and Drug Administration, the US Pharmacopeia and the International Conference on Harmonization. J. Chromatogr. A 987: 57-66.