New Extractive Spectrophotometric Methods for the Determination of Olanzapine in Pharmaceutical Formulations Using Bromocresol Green

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Abstract

Two simple, rapid, accurate, and sensitive spectrophotometric methods have been described for the assay of olanzapine (OLP) in bulk and in pharmaceutical formulations. The first method (method A) is based on the formation of yellow ion-pair complex between the OLP and bromocresol green (BCG) at pH 5.50 ± 0.10. The ion-pair complex formed was extracted with dichloromethane and the absorbance was measured at 410 nm. The second method (method B) is based on the breaking of the yellow OLP-BCG ion-pair complex in alkaline medium after its extraction into dichloromethane followed by the measurement of the blue colour of the dye at 620 nm. Beer's law is obeyed in the concentration ranges 0.25-12.50 µg/mL for method A and 0.2-5.0 µg/mL for method B. The molar absorptivity, Sandell sensitivity, detection and quantification limits are also calculated. The methods were validated for intra-day and inter-day accuracy and precision; selectivity and robustness and ruggedness. The proposed methods were applied successfully to the determination of OLP in their pharmaceutical formulations and the results were in good agreement with those obtained by the official method. The accuracy and reliability of the proposed methods were further ascertained by recovery studies via standard addition technique.

Keywords: Olanzapine; Bromocresol green; Ion-pair complex; Spectrophotometry.

Introduction

Olanzapine (OLP) is an antipsychotic agent, chemically known as 2-methyl-4-(4-methyl-1-piperazinyl)-10H-thieno[2,3-b][1,5] benzodiazepine[1]. It is the most commonly prescribed second-generation neurloteptic for the treatment of psychiatric patients suffering from schizophrenia[2]. Since its introduction in a therapy of psychiatric disorders in 1996, the need for reliable, sensitive and selective methods for its analysis in bulk samples and pharmaceutical preparations is obvious. In literature several methods have been already reported for the determination of OLP in body fluids and in pharmaceutical preparations, including: high performance liquid chromatography (HPLC) with UV[3–14], electrochemical[15–21] or coulometric detection[22-23]. Olanzapine was also determined by capillary zone electrophoresis[7], derivative spectrometry[7, 11], voltammetry[7, 11], liquid chromatography (LC)–tandem mass spectrometry (MS)[24–27], LC–atmospheric pressure ionization MS[28], gas chromatography–MS[29], HPTLC[30], non aqueous titrimetry and UV-spectrophotometry[31].

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To the best of our knowledge, there are six reports on use of visible spectrophotometry in the determination of OLP in pharmaceutical preparations, but all these methods are based on the oxidation of OLP using different oxidizing reagents such as N-bromosuccinimide[2], Ce(IV)sulphate[2,32-34], potassium hexacyanoferrate(III)[32], potassium iodate[35], and potassium permanganate[36]. The reported spectrophotometric methods[2, 32-34, 36] are based on the reaction of OLP with known excess of the oxidizing reagent followed by the determination of the unreacted oxidizing agent. However, the reported methods suffer from one or the other disadvantage such as poor sensitivity or selectivity, less stability of the measured species, meticulous control of experimental variables and complicated experimental setup (Table 1).

Extractive spectrophotometric procedures are popular for their sensitivity in the assay of some drugs[37-43]; therefore, ion-pair extractive spectrophotometry has received considerable attention for the quantitative determination of many pharmaceutical compounds. This technique depends on the reaction of a drug that has a basic cationic nitrogen and an anionic dye at a suitable pH, where a highly colored ion-pair complex is formed. However, no reports have appeared dealing with the extractive spectrophotometric method for the determination of OLP in drug forms so far. Therefore, this paper proposes two accurate and sensitive spectrophotometric methods for the assay of OLP. The first method (method A) is based on the formation of OLP-BCG ion-pair complex at pH 5.50 ± 0.10 which was extracted into dichloromethane for measurement at 410 nm whereas in the second method (method B), the drug-dye ion-pair was broken in alcoholic KOH medium and the resulting blue coloured dianion dye was measured at 620 nm. The proposed methods have been demonstrated to be superior to the reported methods with respect to simplicity, sensitivity, cost effectiveness and eco-friendliness, and can be adopted by the pharmaceutical laboratories for industrial quality control.

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	Color stability	Reaction time	Remarks	Ref. No.
1.	a) NBS	Radical cation measured	532	10-120 $(\varepsilon = 4.19\times10^{3})$	30 sec	0.5 min	Uses 1:1 mixture of H_2SO_4 and H_3PO_4 as the reaction medium, colour stable for only 30 S	2
	b) NBS-Celestine blue	Unbleached dye colour measured	538	0.5-6.0 $(\varepsilon = 6.41\times10^{4})$	-	10 min	Non selective reagent used. High acidic conditions required and NBS unstable in solution.	
	c) Cerium(IV)-Celestine blue	-do-	538	0.6-3.0 $(\varepsilon = 1.48\times10^{5})$	-	35 min		
2.	a) Potassium hexacyano ferrate (III)	Unreacted oxidant measured	425	2.5-40.0 $(\varepsilon = 2.59\times10^{3})$	-	60 min	Reaction requires 1:1 mixture of H_2SO_4 and H_3PO_4. Colour of the oxidation product is unstable.	32
	b) Potassium hexacyano ferrate (III)	Radical cation measured	540	0.5-250				
	c) Cerium (IV) sulphate	-do-	540	0.05-300			FIA assembly required in the methods b & c.	
3.	a) Cerium-NPA	Colored product formed between oxidant and NPA/ SAA measured	440	0.3-1.8 $(\varepsilon = 1.50\times10^{5})$	30 min	15 min	Non-selective and strong oxidizing agents used. Less stable color product.	33
	b) Cerium (IV)-SAA		545	5.0-75.0 $(\varepsilon = 1.30\times10^{4})$	30 min	20 min		
4.	a) Ce(IV)- iron(II)-Thiocyanate	Fe(III)-thiocyanate complex measured	480	0.2-2.0 $(\varepsilon = 10.94\times10^{4})$	10 min	12 min	Non-selective and strong oxidizing agents used. Less stable color product in the methods a & c.	34
	b) Ce(IV)- iron(II)-Tiron	Fe(III)-tiron complex measured	640	0.5-9.0 $(\varepsilon = 1.67\times10^{4})$	>24 hrs	15 min		
	c) Ce(IV)- iron(II)-ferrocyanide	Fe(III)-ferrocyanide complex measured.	700	0.2-3.0 $(\varepsilon = 4.52\times10^{4})$	20 min	20 min		
5.	a) KIO_3	Initial rate of formation of radical cation measured	537	up to 4.0	30 sec	Within 30s	Scrupulous control of experimental variables and special equipment for kinetic measurement required.	35
	b) KIO_3	Maximum absorbance measured	537	Up to 7.0				
6.	a) $KMnO_4$ acid medium	Unreacted KMnO4 measured	550	2.0-20.0 $(\varepsilon = 1.34\times10^{4})$	45 min	15 min	Non-selective and strong oxidizing agents used. Less stable color product.	36
	b) $KMnO_4$ alkaline medium	Bluish-green manganate measured.	610	1.0-10.0 $(\varepsilon = 2.54\times10^{4})$	40 min	15 min	Extraction was required to overcome the interferences.	
7.	a) BCG - NaOAc	Absorbance of the ion-pair complex measured	410	0.25-12.5 $(\varepsilon = 2.41\times10^{4})$	>20 hrs	5 min	Highly selective and sensitive, no heating required. Highly stable measured species and use stable reagents.	Present methods
	b) BCG - NaOAc - KOH	Absorbance of the blue colour of dianionic form of the dye measured	620	0.2-5.0 $(\varepsilon = 6.11\times10^{4})$	>24 hrs	5 min		

Materials and Methods

Apparatus

A Systronics model 106 digital spectrophotometer provided with 1.0 cm matched quartz cells was used for all absorbance measurements.

Materials

Pharmaceutical grade OLP which is certified to be 99.85 % pure was received as a gift from Cipla Ltd., India, and used as received. All pharmaceutical preparations were obtained from commercial sources in the local market.

Reagents

All reagents used were of analytical grade, organic solvents were of HPLC grade and distilled water was used throughout the investigation. A stock standard solution of 200 µg/mL OLP was prepared in 0.1 M H_2SO_4 (Merck, Mumbai, India, sp. gr. 1.84) and was further diluted with the same acid to get a working solution of 25.0 µg/mL OLP. The working solutions of 1.0 M CH_3COONa (Merck, Mumbai, India) in water, 1.0 % (w/v) KOH (New India Chemical Enterpris, Kochi, India) in ethanol and 0.4 % (w/v) bromocresol green (Qualigens fine chemicals, Mumbai, India) in water after dissolving it in a few milliliters of ethanol were also prepared.

Method A

Different aliquots (0.1,0.5,1.0,2.0,---5.0 mL) of a standard 25.0 µg/mL OLP solution were accurately transferred into a series of 125-mL separating funnels and the total volume was adjusted to 5.0 mL by adding adequate quantity of 0.1 M H_2SO_4 to each funnel. To each funnel 13 mL of water and 5 mL of 1 M sodium acetate were added, followed by 2 mL dye (0.4 % w/v), and the content was mixed well and kept for 5 min. Dichloromethane (10 mL) was added to each of the separating funnels with a micro burette, the contents were shaken well for 1.0 min and left at room temperature for a minute. The two phases were allowed to separate and the dichloromethane layer was passed through anhydrous sodium sulphate. The absorbance of the yellow OLP-BCG ion-pair complex was measured at 410 nm against corresponding reagent blank.

Method B

Aliquots (0.1, 0.5, 1.0, ---2.5 mL) of OLP-BCG ion-pair complex (10 µg/mL in OLP; prepared in method A) were transferred into a series of 5-mL standard flasks and the total volume was adjusted to 2.5 mL by adding dichloromethane. To each flask, 1 mL of 1.0 % w/v alcoholic KOH was added, the content was mixed well and kept aside for 5 min. Finally, the volume was made up to the mark with ethanol and the absorbance of the blue coloured species was measured at 620 nm against corresponding reagent blank.

Procedure for the Dosage Forms

The contents of ten tablets each containing 5 or 10 or 20 mg of OLP were weighed, ground into a fine powder and mixed well. An amount of the powder equivalent to 10.0 mg of OLP was accurately weighed and transferred to a 100-mL

volumetric flask, 60 mL of 0.1 M H_2SO_4 was added and content shaken thoroughly for about 15 min. The volume was diluted to the mark with 0.1 M H_2SO_4, mixed well and filtered using Whatman No.42 filter paper in to a 100-mL volumetric flask. First 10 mL portion of the filtrate was rejected and a suitable aliquot of filtrate (containing 100 µg/mL OLP) was diluted with 0.1 M H_2SO_4 to get a working concentration of 25.0 µg/mL and used for the assay by method A. The ion-pair complex of these tablets OLP-BCG (10.0 µg/mL; in OLP) was prepared for assay by applying the procedure described in method B.

Results and Discussion

Absorption Spectra

OLP forms an ion-pair complex with BCG. Extraction of the yellow ion-pair complex from the aqueous reaction medium with dichloromethane was investigated. The ion-pair formed was found to be quantitatively extracted into dichloromethane and its absorption spectrum (Figure 1) displays an absorption peak at 410 nm (method A). Neither OLP nor BCG alone exhibits any significant absorption at 410 nm under the same conditions. In method B, this OLP-BCG ion-pair complex was treated with alcoholic base to yield a chromogen, the dianionic form of the dye, which exhibits bathochromic shift to maximum absorbance 620 nm (Figure 1).

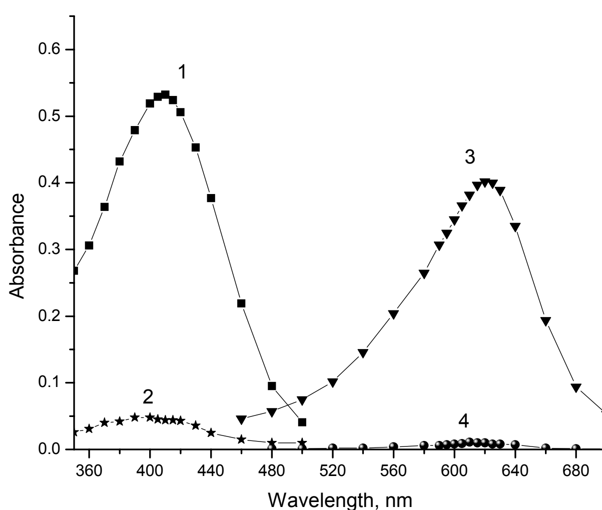

Figure 1: Absorption spectra 1: ion-pair complex of OLP–BCG (7.5 µg/mL OLP) in method A; 2: reagent blank in method A; 3: dianionic form of the dye (2.0 µg/mL, in OLP) in method B; 4: reagent blank in method B.

Reaction Mechanism

OLP forms ion-pair complex with BCG, since the drug contains tertiary amino group which is protonated in acid medium. 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[41]. BCG is an example of sulphonphthalein type of dye and the colour of such dye is due to the opening of lactoid ring and subsequent formation of quinoid group[38]. 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-pair complex with BCG which is quantitatively extracted into dichloromethane. The possible reaction mechanism for method A is given in figure 2. The BCG color reagent occurs in two acid–base forms (pKa = 4.66) in weakly acidic aqueous solutions with the absorption maximum at 430 nm ($BCGH^-$ form) and 615 nm (BCG^{2-})[44]. When this triarylmethane dye complexes with OLP forms ion-pair at pH 5.50 ± 0.10 and develops a yellow color solution that exhibits absorbance maximum at 410 nm and when in basic medium change to blue color solution with maximum absorbance at 620 nm, which are soluble in dichloromethane. In aqueous solution[45], bromocresole green ionizes to give the monoanionic form ($BCGH^-$ yellow), that further deprotonates at higher pH (method B) to give the dianionic form (BCG^{2-} blue), which is stabilized by resonance shown in figure 2.

Optimization of Reaction Conditions

The optimization of the methods was carefully studied to achieve complete reaction formation, quantitative extraction of the ion-pair complex and highest sensitivity. Reaction conditions of the ion-pair complex were found by studying with preliminary experiments such as pH, type of organic solvent, volumes of the dye, and shaking time for the extraction of ion-pair complex. In method B, alcoholic KOH concentration required for complete breaking of the complex was optimized.

Selecting of the Extracting Solvent.

A number of organic solvents such as chloroform, dichloromethane, 1, 2-dichloroethane and benzene were examined for extraction of the ion-pair complex in order to provide an applicable extraction procedure. Although dichloromethane is not an environmental friendly solvent, it was preferred for its efficient and quantitative extraction of ion-pair complex and the greater stability of the extracted ion-pair (>20 h), its high sensitivity, very low absorbance of the reagent blank, maximum absorbance of the measured species and shortest time to reach the equilibrium between both phases.

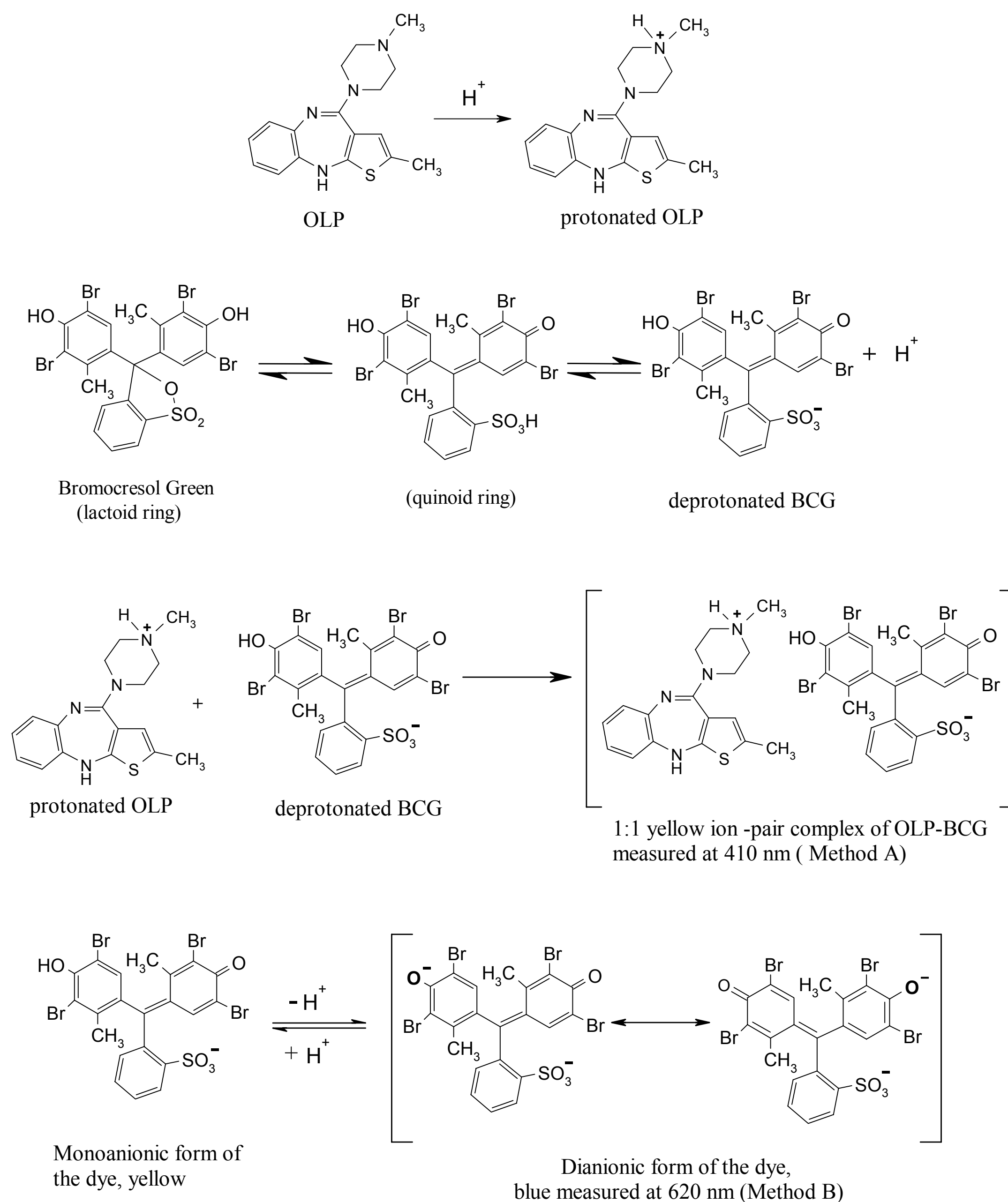

Figure 2: The possible reaction mechanisms.

Effect of pH on the Ion-pair Formation

The effect of pH of the aqueous phase was studied by extracting the coloured complex at (pH= 4.80-5.80). It was noticed that the maximum colour intensity, minimum absorbance of the reagent blank and constant absorbance were observed at pH 5.50 ± 0.10. The results are shown in figure 3. At pH values greater than 5.80 ± 0.10, the decrease in absorbance of the ion-pair complex was observed and at pH values below than 4.80 ± 0.10 the increase in absorbance of the reagent blank was observed. Hence the pH 5.50 ± 0.10 was fixed in all subsequent work.

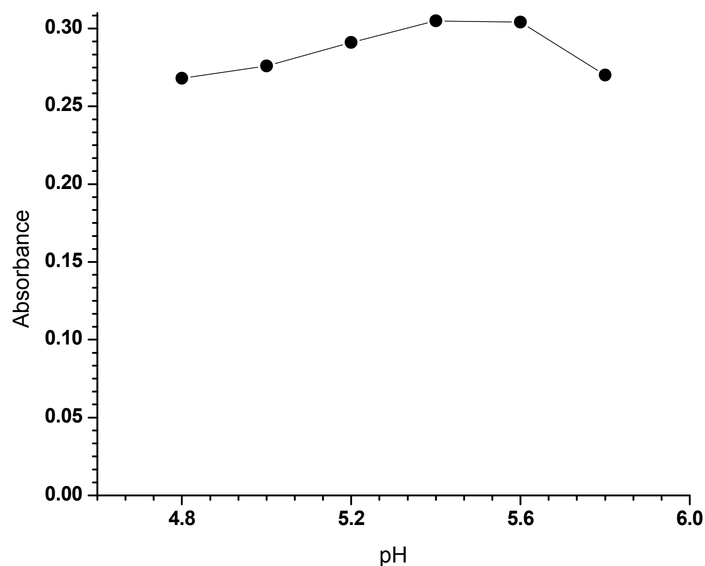

Figure 3: Effect of pH on the OLP–BCG ion-pair complex (6.0 µg/mL OLP).

Effects of Reagents Concentration

The effect of the dye concentration was studied in method A by measuring the absorbance of solutions containing a fixed concentration of OLP (10.0 µg/mL) and varied amounts of the BCG. It is apparent from figure 4 that the maximum absorbance was found with 1.0 ml of 0.4 % BCG, beyond which absorbance was constant. Thus, 2 ml of 0.4 % BCG was used for ion-pair formation throughout the investigation. Also, the effect of volume of aqueous phase was studied by extracting different volumes of aqueous phase (including drug, BCG and sodium acetate) such as 15, 20, 25, 30 and 35 mL with 10 mL of dichloromethane (Figure 5). The use of 25 mL of aqueous phase was found to be sufficient to achieve maximum absorbance of measured species and minimum absorbance of reagent blank and hence an aqueous phase of 25 mL was fixed throughout. Different volumes of sodium acetate were added to the acidic solution of OLP to bring the pH to 5.50 ± 0.10. This pH was achieved when the volume of 1 M sodium acetate was 5.0 mL in a total volume of aqueous phase 25 mL. For method B, the effect of alcoholic potassium hydroxide concentration required to break the ion-pair complex and formation of the dianionic form of the dye was studied by measuring the absorbance of solutions containing a fixed concentration of ion-pair complex (5.0 µg/mL; in OLP) and different volumes of alcoholic KOH (Figure 6). It was found that 1 mL of 1.0 % (w/v) alcoholic KOH was sufficient to yield a maximum absorbance at 620 nm, although larger volumes of base had no pronounced effect on the absorbance of the measured species.

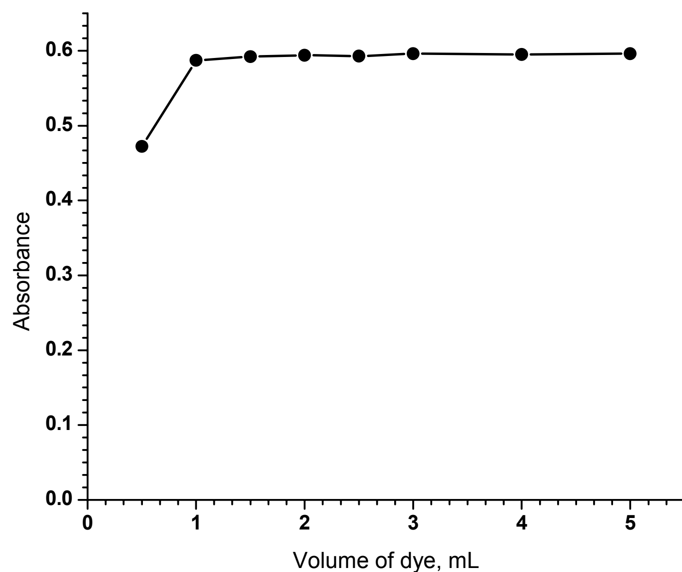

Figure 4: Effect of the volume of 0.4 % dye (10.0 µg/mL OLP).

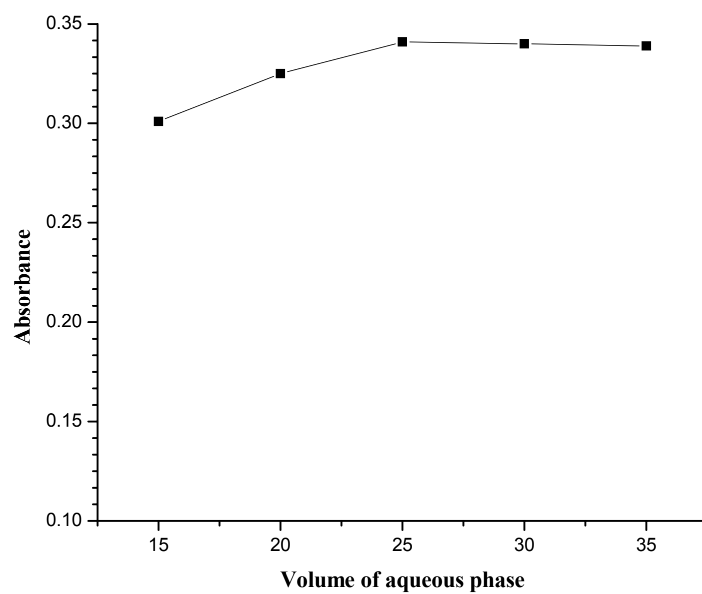

Figure 5: Effect of the volume of aqueous phase (7.5 μg/ml OLP).

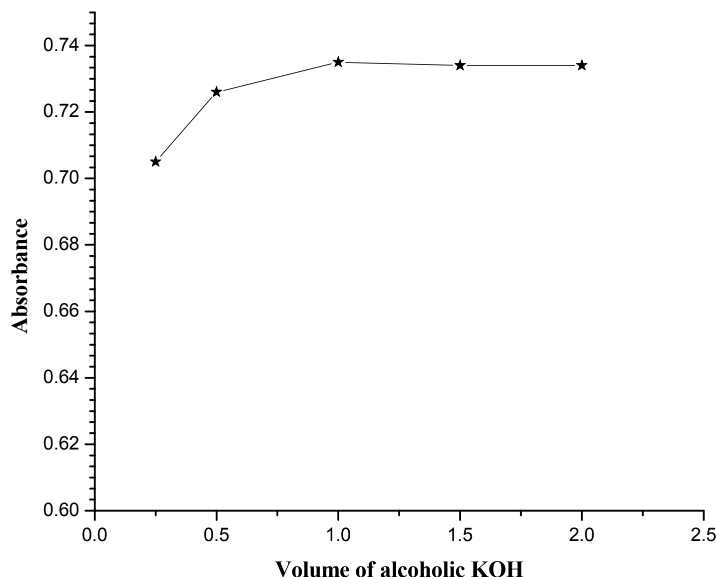

Figure 6: Effect of alcoholic KOH (5.0 μg/ml OLP).

Effects of Time and Sequence of Addition

The effect of contact time between OLP and BCG in the presence of sodium acetate was studied in the time range 0-30 min before extraction and it was found that 5 min is sufficient to achieve maximum absorbance at 410 nm. Shaking times of 0.5–3 min produced a constant absorbance in method A, and hence a shaking time of 1 min was used throughout. In method B, the effect of the time required to break the complex was studied after the addition of alcoholic KOH to the complex and it was found that 5 min was sufficient for that breaking. There was no appreciable change in the absorbance or colour of the measured species if the order of addition of the reactants was varied.

Composition of the Ion-pair Complex

The composition of the ion-pair complex formed in method A between OLP and BCG was established by applying Job's method of continuous variations. In this method, 2.4×10^{-4} M solutions of OLP and BCG were used and mixed in varying volume ratios in such a way that the total volume of each mixture was the same. The absorbance of each solution was measured and plotted against the mole fraction of the drug (Figure 7). The plot reached a maximum value at a mole fraction of 0.5 which indicated that a 1:1 (OLP: BCG) ion-pair complex is formed through the electrostatic attraction between positive protonated OLP and BCG anion. The conditional stability constant (K_f) of the ion-pair complex was calculated[46] from the data of continuous variations method and found to be 4.18×10^6.

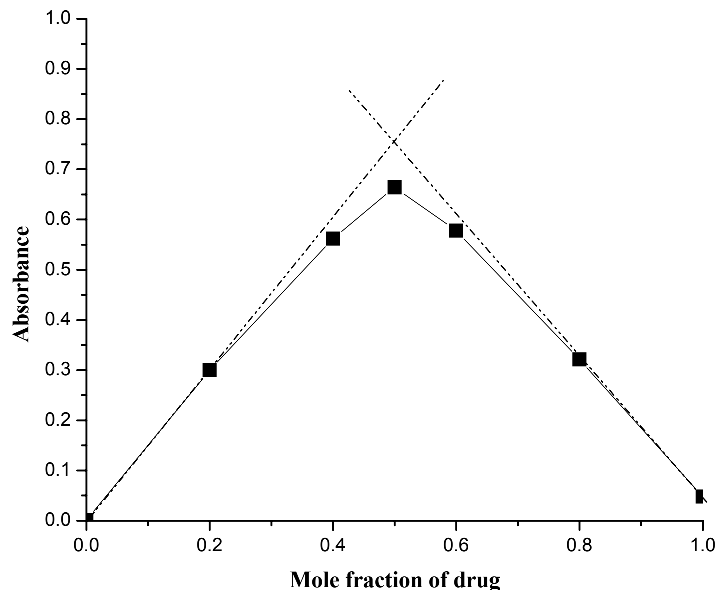

Figure 7: Job's Continuous-variations plot for method A: OLP + BCG.

Stability of the Measured Species

The stability of the ion-pair complex formed in method A between OLP and BCG was evaluated. The formation of the ion-pair was rapid and the yellow color extract was stable for at least 20 hours without any change in color intensity at room temperature. Also, the absorbance of the blue colour of dianionic form of the dye in method B at 620 nm was stable for more than 24 hours.

Method Validation

Linearity

At described experimental conditions for OLP determination, the absorbance–concentration plots were found to be linear over the concentration ranges stated in table 2. The statistical parameters were given in the regression equation calculated from the calibration graphs, along with the standard deviations of the slope (S_b) and the intercept (S_a). The linearity of calibration graphs was proved by the high values of the correlation coefficient (r) and the small values of the y-intercepts of the regression equations. The apparent molar absorptivity, Sandell sensitivity of the methods A and B were also calculated and recorded in table 2.

Table 2: Analytical and regression parameters.

Parameter	Method A	Method B
λ_{max}, nm	410	620
Beer's law limits, µg/mL	0.25-12.5	0.2-5.0
Molar absorptivity, L /mol/cm	2.41×10^{4}	6.11×10^{4}
Sandell sensitivity*, µg/cm^{2}	0.0130	0.0051
Limit of detection, µg/mL	0.28	0.03
Limit of quantification, µg/mL	0.86	0.08
Regression equation, Y**		
Intercept, (a)	0.0055	0.0033
Slope, (b)	0.0685	0.1920
Correlation coefficient, (r)	0.9986	0.9999
Standard deviation of intercept (S_a)	0.01127	0.00283
Variance (S_a^2)	1.27×10^{-4}	8.01×10^{-6}
$\pm tS_a/\sqrt{n}$	0.0104	2.97×10^{-3}
Standard deviation of slope (S_b)	0.00160	0.00093
$\pm tS_b/\sqrt{n}$	1.48×10^{-3}	9.76×10^{-4}

* 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 1.0 cm^2 and l = 1.0 cm. $Y^{**} = a + bX$, where Y is the absorbance and X concentration in µg/mL, $\pm tS_a/\sqrt{n}$ = confidence limit for intercept, $\pm tS_b/\sqrt{n}$ = confidence limit for slope.

Sensitivity

The detection limits (DL) for the proposed methods were calculated using the following equation[47]:

$$DL = \frac{3.3\times\sigma}{S}$$

where σ is the standard deviation of replicate determination values under the same conditions as for the sample analysis in the absence of the analyte and S is the sensitivity, namely the slope of the calibration graph. In accordance with the formula, the detection limits were found to be 0.28 and 0.03 µg/mL for method A and method B, respectively.

The quantitation limit QL, defined as[47]:

$$QL = \frac{10\times\sigma}{S}$$

According to this equation, the quantitation limits were found to be 0.86 and 0.08 µg/mL for method A and method B, respectively.

Accuracy and Precision

In order to evaluate the precision of the proposed methods, solutions containing three different concentrations of the OLP were prepared and analyzed in five replicates. The analytical results obtained from this investigation are summarized in table 3. The low values of the relative standard deviation (% R.S.D) and percentage relative error (% R.E) also indicate the high precision and the good accuracy of the proposed methods. The assay procedure was repeated seven times, and percentage relative standard deviation (% R.S.D) values were obtained within the same day to evaluate repeatability (intra-day precision) and over five different days to evaluate intermediate precision (inter-day precision).

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

Method*	OLP taken μg/mL	Intra-day			Inter-day		
		OLP found[a] μg/mL	Precision[b]	Accuracy[c]	OLP found[a] μg/mL	Precision[b]	Accuracy[c]
Method A	2.50	2.54	1.26	1.60	2.55	2.34	2.00
	6.25	6.36	1.42	1.76	6.39	1.72	2.24
	10.0	10.2	1.37	2.00	10.1	2.66	1.00
Method B	2.0	1.98	0.97	1.00	2.02	1.04	1.00
	3.0	2.99	0.90	0.33	3.02	0.85	0.67
	4.0	3.97	0.86	0.75	4.05	1.11	1.25

a. Mean value of n determinations, b. Relative standard deviation (%), c. Relative Error (%).

Selectivity

In order to evaluate the selectivity of the proposed methods for the analysis of pharmaceutical formulations, the effect of the presence of common excipients, such as talc, starch, lactose, glucose, sodium alginate, calcium gluconate and magnesium stearate was tested for possible interference in the assay by placebo blank and synthetic mixture analyses and no significant interference was observed from these excipients.

Robustness and Ruggedness

For the evaluation of the method robustness, some parameters were interchanged; volume of H_2O/ alcoholic KOH and contact time and the effect of the changes were studied on the absorbance of the colored systems. Method ruggedness was expressed as % R.S.D of the same procedure applied by three analysts and also by a single analyst performing analysis on three different instruments. The results showed no statistical differences between different analysts and instruments suggesting that the proposed methods were robust and rugged. The results are presented in table 4.

Table 4: Method robustness and ruggedness expressed as intermediate precision (% RSD).

Method	OLP taken, μg/mL	Robustness		Ruggedness	
		Parameters interchanged		Inter-analysts (%RSD), (n = 3)	Inter-instruments (%RSD), (n = 3)
		Volume of H_2O/Ethanolic KOH*	Reaction/Breaking time**		
A	5.0	1.60	1.28	0.63	2.58
B	3.0	0.86	0.52	0.72	1.85

*In method A, the volumes of H_2O were 10, 13 and 16 mL, and in method B the volumes of ethanolic KOH added were 0.7, 1.0 and 1.3 mL. **In method A, the reaction times were 3, 5 and 7 min and in method B breaking times were 3, 5 and 7 min.

Applications

The proposed methods have been successfully applied to the determination of OLP in three representative tablets oleanz-5, oliaz-10 and oliza-20. The results obtained and shown in table 5 were compared to those obtained by the official method[31] by means of t- and F-tests at 95 % confidence level. The official method consisted measurement of the absorbance of the methanolic extract of the tablets at 226 nm. In all cases, the average results obtained by the proposed methods and official method were statistically identical, as the difference between the average values had no significance at 95 % confidence level with respect to accuracy and precision.

Table 5: Comparison of assay results of proposed and official methods.

Tablet brand Name**	Nominal amount, mg	Found (% of nominal amount ± SD)*		
		Official method	Proposed methods	
			Method A	Method B
Oleanz [1]	5	98.62 ± 0.96	99.46 ± 1.38 **t =** 1.13 **F**= 2.07	98.04 ± 0.78 **t =** 1.05 **F**= 1.52
Oliza [2]	10	102.5 ± 0.78	102.8 ± 1.51 **t** = 0.41 **F**= 3.75	101.3 ± 0.76 **t =** 2.46 **F**= 1.05
	20	97.66 ± 0.82	98.42 ± 1.32 **t =** 1.12 **F**= 2.59	96.82 ± 1.12 **t =** 1.37 **F**= 1.87

* 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.

Recovery Study

To ascertain the 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 OLP 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 6 indicate that the above mentioned excipients did not interfere in the assay.

Table 6: Results of recovery study by standard-addition method.

Formulation studied	Method A				Method B			
	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
Oleanz, 5 mg	3.98	2.0	5.95	98.05 ± 1.58	1.96	1.0	2.91	95.00 ± 1.14
	3.98	4.0	8.03	101.2 ± 1.92	1.96	2.0	3.91	97.50 ± 1.62
	3.98	6.0	10.1	102.0 ± 2.14	1.96	3.0	4.97	100.3 ± 1.34
Oliza, 10 mg	5.14	2.5	7.78	105.6 ± 2.31	2.03	1.0	2.99	96.00 ± 0.80
	5.14	5.0	10.7	111.2 ± 1.87	2.03	2.0	3.90	93.50± 1.28
	5.14	7.5	13.3	108.8 ± 2.60	2.03	3.0	4.81	92.70± 1.23

*Mean value of three determinations.

Conclusion

A significant advantage of the extractive spectrophotometric methods is that it can be applied for the determination of individual compounds in a multi component mixture. The proposed methods make use of simple reagent, which an ordinary analytical laboratory can afford and the procedures do not involve any critical reaction conditions or tedious sample preparation. The proposed methods are highly reliable owing to the stability of the ion-pair complex and dianionic form of the dye, which are ultimately measured. Moreover, the methods are accurate, reproducible, adequately sensitive and free from interference caused by the excipients expected to be present in tablets. The methods are unaffected by slight variations in experimental conditions such as pH and reagent concentration. The wide applicability of the new procedures for routine quality control is well established by the assay of OLP in pure form, as well as in pharmaceutical preparations.

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