Utility of N-bromosuccinimide as an Environmental-Friendly Reagent for Sensitive Determination of Olanzapine in Pharmaceuticals

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ABSTRACT

One titrimetric and two spectrophotometric methods are described for the determination of olanzapine (OLP) in bulk drug and dosage forms. The methods use N-bromosuccinimide (NBS) and two-dyes, namely quinoline yellow and metanil yellow, as reagents. In titrimetry, an acidified solution of OLP was titrated directly with NBS using methyl orange as indicator. Spectrophotometry involves the addition of a known excess of NBS to OLP in acid medium followed by determination of unreacted NBS by reacting with a fixed amount of either quinoline yellow and measuring the absorbance at 410 nm (method A) or metanil yellow and measuring the absorbance at 530 nm (method B). Titrimetric method is applicable over a range of 1-10 mg of OLP, and the reaction stoichiometry is found to be 1:6 (OLP: NBS). In spectrophotometry, Beer's law was obeyed in the concentration ranges of 0.1–1.2 and 0.1–1.5 μg ml^{-1} OLP for method A and method B, respectively. The molar absorptivity, Sandell sensitivity, limits of detection and quantification are also reported for both the spectrophotometric methods. The proposed methods were applied successfully to the determination of OLP in tablets. The reliability and accuracy of the methods were further ascertained by recovery studies. A major advantage of the use of NBS is that the by-product succinimide can be easily recovered and recycled to NBS.

Keywords: Olanzapine, Titrimetry, Spectrophotometry, N-bromosuccinimide, Pharmaceuticals.

INTRODUCTION

Olanzapine (OLP), chemically known as 2-methyl-4-(4-methyl-1-piperazinyl)-10H-thieno[2, 3-b] [1, 5] benzodiazepine (Figure 1), is an atypical antipsychotic drug used in the treatment of schizophrenia and other psychotic syndromes.[1] 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. Various techniques have been developed for the determination of OLP in pharmaceuticals which include high performance liquid chromatography (HPLC)[2-10], high performance thin layer chromatography (HPTLC)[2,7,11-13], capillary zone electrophoresis[9], voltammetry[3,9], spectrofluorimetry[14], UV-spectrophotometry[3,9,15-17], flow injection visible spectrophotometry[18], visible spectrophotometry[19-31] and non-aqueous titrimetry.[17,32] Visible spectrophotometry, because of its simplicity and cost-effectiveness, sensitivity and fair accuracy and precision, is still being used for quality control in laboratories of developing and under-developed nations which can ill-afford other expensive and sophisticated techniques.

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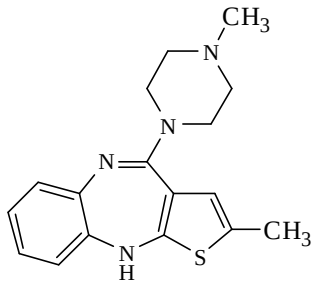


Figure 1: Structure of olanzapine.

Fourteen reports on the use of visible spectrophotometry were found in the literature for the determination of OLP in pharmaceuticals[18,19-31] Jasinska and Nalewajko[18] have reported indirect batch and direct flow injection visible spectrophotometric methods using potassium hexacyanoferrate(III) and cerium(IV) sulphate as oxidimetric reagents for the determination of OLP. Two more visible spectrophotometric methods based on the oxidation of OLP followed by complexation between OLP and o-phenanthroline or potassium ferricyanide in presence of ferric chloride to form a coloured product were developed by Siva Prasad et al.[19] Rajendraprasad and Basavaiah[20] have reported two methods based on the oxidation of OLP with potassium permanganate in either acidic or alkaline medium followed by the determination of unreacted permanganate or bluish-green colour of manganate. Rajendraprasad et al.[21] have reported three methods based on the oxidation of OLP with a known excess of cerium(IV) sulphate in acidic medium followed by the determination of unreacted oxidant by its reduction using an excess of iron(II), and the resulting iron(III) was complexed with thiocyanate, tiron or ferrocyanide. A method based on the oxidation of OLP with a known excess of iodine monochloride (ICl) in acidic medium followed by iodinating of thymol blue by unreacted ICl was reported by Revanasiddappa and Veena.[22] Few reports[22-24] described different methods for the determination of OLP based on the oxidation of OLP by a known excess of cerium(IV) sulphate in acidic medium followed by the determination of unreacted oxidant by reacting with leuco crystal violet[22], N-phenylanthranilic acid or sulphanilic acid[23], ferroin or iron(II)-2,2'-bipyridyl[24] and measuring the absorbance at the appropriate wavelength. Krebs and coworkers[25] described three methods for the assay of OLP by direct and indirect spectrophotometry using N-bromosuccinimide and cerium(IV) sulphate as oxidimetric reagents. Mohamed[26] has reported kinetic and maximum-absorbance spectrophotometric methods for the determination of OLP in its dosage forms. Basavaiah et al.[27,28] have reported two methods based on the oxidation of OLP with an excess of potassium iodate in acidic medium followed by the determination of the liberated iodine either by reacting with a fixed amount of Nile blue[27] or by extraction of the resulting iodine into chloroform.[28] The extraction of yellow ion-pair complex between OLP and bromocresol green with dichloromethane and breaking of that complex in alkaline medium was reported by Basavaiah et al.[29] Abdulaziz et al.[30] have reported three methods based on the oxidation of OLP with an excess of sodium periodate in buffer medium followed by the determination of unreacted oxidant by iodometric titration and spectrophotometry by measuring the absorbance of either liberated iodine (I_3^-) directly or starch-iodine chromogen. Basavaiah et al.[31] described two extraction-free procedures based on the formation of yellow coloured ion-pair complexes between OLP and two dyes, namely, bromocresol purple and bromothymol blue. However, the reported methods suffered from one or the other disadvantage such as poor sensitivity or selectivity, less stability of the measured species, use of organic solvents, heating or extraction step, meticulous control of experimental variables and complicated experimental setup.

The present investigation aims at developing more sensitive and cost-effective method for the determination of OLP in pure form and in dosage forms using titrimetric and spectrophotometric techniques. The methods employ N-bromosuccinimide as eco-friendly reagent which acts as an oxidizing as well as brominating agent, and two dyes, quinoline yellow and metanil yellow as auxiliary reagents. The proposed methods have the advantages of speed and simplicity. Besides being accurate and precise

they can be adopted by the pharmaceutical laboratories for industrial quality control.

EXPERIMENTAL SECTION

Instrument

A Systronics model 106 digital spectrophotometer (Systronics, Ahmedabad, Gujarat, India) equipped with 1 cm matched quartz cells was used for all absorbance measurements.

Reagents and Materials

All the reagents used were of analytical reagent grade and distilled water was used throughout the investigation.

N-bromosuccinimide (NBS): An approximately 0.02 M solution was prepared by dissolving about 3.6 g of NBS (SRL Research Chemicals, Mumbai, India) in water with the aid of heat and diluted to one litre with water. The solution was standardized iodometrically[33] and kept in an amber coloured bottle, stored in a refrigerator and used in titrimetry. It was appropriately diluted to get 70 and 100 μg mL^{-1} NBS for use in spectrophotometric method A and method B, respectively.

Solutions of 0.1 M and 5 M sulphuric acid (Merck, Mumbai, India; sp. gr. 1.84), 0.1% methyl orange indicator (S.D. Fine Chem. Ltd., Mumbai, India), 0.1 M and 5 M hydrochloric acid (Merck, Mumbai, India; sp. gr. 1.18), 140 μg mL^{-1} quinoline yellow (LOBA Chemie, Mumbai, India, 70% dye content) for method A and 50 μg mL^{-1} metanil yellow (S.D. Fine Chem., Mumbai, India, 70 % dye content) for method B were prepared in water.

Standard OLP solution: Pharmaceutical grade OLP which is reported to be 99.8% pure was received from Cipla Ltd., India. A stock standard solution equivalent to (1 mg mL^{-1}) of OLP was prepared by dissolving accurately weighed 250 mg of the pure drug in 0.1 M H_2SO_4, diluted to the mark in a 250 mL volumetric flask with the same solvent and used in the titrimetric work. Another stock solution equivalent to 100 μg mL^{-1} of OLP was prepared by dissolving accurately weighed 10 mg of the pure drug in 0.1 M HCl and diluting to the mark in a 100 mL volumetric flask. The second stock solution (100 μg mL^{-1} OLP) was diluted appropriately with 0.1 M HCl to get working concentrations of 4 and 5 μg mL^{-1} OLP for use in spectrophotometric method A and method B, respectively. The standard solutions were kept in amber coloured bottles and stored in a refrigerator when not in use.

Procedures

Titrimetry

A 1.0-10.0 mL aliquot of standard OLP (1.0 mg mL^{-1}) solution containing 1-10 mg of OLP was measured accurately, transferred into a 100 mL Erlenmeyer flask, and the total volume was made to 10 mL with 0.1 M H_2SO_4. The solution was acidified by adding 5 mL of 5 M sulphuric acid. Two drops of methyl orange indicator were added, and the flask was kept on a magnetic stirrer. The titration was performed by drop wise addition of standard 0.02 M NBS solution with vigorous and continuous stirring during the titration to a colourless end point. The amount of the drug present in the measured aliquot was calculated from:

$$Amount(mg) = \frac{V \times Mol.wt \times R}{n}$$

where V = volume of NBS solution consumed, mL; Mol.wt = relative molecular mass of drug (g_mol^{-1}); R = concentration of NBS, mol L^{-1} and n = number of moles of NBS reacting with each mole of drug.

Spectrophotometry using quinoline yellow (method A)

Different aliquots (0.25, 0.5, 1.0, …, 3.0 mL) of a standard OLP (4 μg mL^{-1}) solution were transferred into a series of 10 mL volumetric flasks using a micro burette, and the total volume was adjusted to 3 mL by adding adequate quantity of 0.1 M HCl. To each flask, 2 mL of 5 M HCl and 1.00 mL of NBS solution (70 μg mL^{-1}) were added. The flasks were stoppered, content mixed and let to stand for 15 min with occational shaking. Finally, 1.0 mL of 140 μg mL^{-1} quinoline yellow solution was added, and the mixture was diluted to the mark with water and mixed well. The absorbance of each solution was measured at 410 nm against a reagent blank after 5 min.

Spectrophotometry using metanil yellow (method B)

Varying aliquots (0.2, 0.5, 1.0, …, 3.0 mL) of a standard OLP (5 μg mL^{-1}) solution were transferred into a series of 10 mL calibrated flasks as described above and the total volume was brought to 3 mL by adding 0.1 M HCl . To each flask, 2 mL of 5 M HCl was added and followed by 1.0 mL of NBS solution (100 μg mL^{-1}). The content was mixed well, and the flasks were kept aside for 25 min with intermittent shaking. Finally, 1.0 mL of 50 μg mL^{-1} metanil yellow solution was added to each flask, the volume was diluted to the mark with water and mixed well, and the absorbance was measured against a reagent blank at 530 nm after 5 min.

In either spectrophotometric methods, a standard curve was prepared by plotting the absorbance *versus* the concentration of OLP. The concentration of the unknown was read from the calibration graph or computed from the regression equation derived using Beer's law data.

Assay Procedure for Tablets

Twenty tablets each containing 10 or 20 mg of OLP were accurately weighed and ground into fine powder. An amount of the powder equivalent to 100 mg of OLP was accurately weighed into a 100 mL volumetric flask. Sixty mL of 0.1 M H_2SO_4 was added, and the solution was 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. First 10 mL portion of the filtrate was discarded, and a convenient aliquot of filtrate (containing ca. 1 mg mL^{-1} OLP) was taken for assay by titrimetric procedure. An amount of the powder equivalent to 10 mg of OLP was accurately weighed into a 100 mL volumetric flask, 60 mL 0.1 M HCl was added and the mixture was shaken for about 15 min. The volume was diluted to the mark with 0.1 M HCl, mixed well and filtered using Whatman No.42 filter paper. First 10 mL portion of the filtrate was discarded, and a convenient aliquot of filtrate (containing ca. 100 μg mL^{-1} OLP) was diluted with 0.1 M HCl to get 4 and 5 μg mL^{-1} of OLP for use in spectrophotometric methods A and B, respectively. A suitable aliquot was then subjected to analysis by the procedures described earlier.

RESULTS AND DISCUSSION

Recently, N-bromosuccinimide (NBS) has gained much attention as oxidation and bromination agent in organic reactions.[34] Many reactions using NBS have found extensive applications in the determination of a variety of organic compounds including those of pharmaceutical interest.[35,36] The use of molecular bromine as oxidizing and brominating agent has several drawbacks as it is harmful, hazardous and there are difficulties in handling and maintaining the stoichiometric ratio during the reaction.[37] From the green chemistry point of view, the replacement of such harmful reagents with non-toxic, inexpensive, commercially available, non-polluting and more selective reagents is an important goal. Among various brominating reagents, NBS is environmentally friendly, easy to work with and extensively used for allylic, benzylic, and aromatic nuclear bromination under mild conditions.[38] NBS can be considered a convenient source of molecular bromine, or it can also act as a source of hypobromous acid which is the actual oxidizing agent.

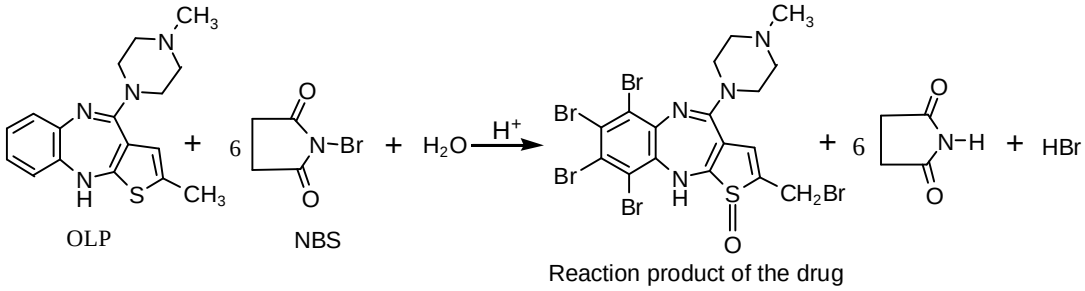
All the present proposed methods are based on the oxidation and bromination reactions involving OLP and NBS in acidic medium. In the proposed methods, NBS not only oxidizes OLP but also brominates it and thus resulting in an enhanced mole-ratio of 1:6 (OLP: NBS). The titrimetric method is direct whereas the spectrophotometric methods are indirect and based on the determination of the residual NBS by reacting with a fixed amount of either quinoline yellow or metanil yellow. The spectrophotometric methods make use of the bleaching action of NBS on either dye. The amount of NBS reacted corresponds to the drug content in all the methods.

Method Development

Titrimetry

The proposed titrimetric procedure is based on the oxidation and bromination reaction between OLP and NBS in the presence of methyl orange as indicator. The

reaction stoichiometry is calculated to be 1:6 (OLP: NBS) in the 1-10 mg range investigated, and outside these limits deviant results were obtained. Several factors like nature of the acid and its concentration were optimized. Sulphuric acid was found to be an ideal medium for the titration. Reproducible and stoichiometric results were obtained when 3-8 mL of 5 M sulphuric acid in a total volume of 15-20 mL was used; hence 5 mL of 5 M H_2SO_4 in 15 mL was maintained in the assay procedure. As the reaction between OLP and NBS was slow, the solution was kept on a magnetic stirrer, and the titration was carried out by drop wise addition of standard NBS solution and stirring vigorously and continuously during the titration, to a colourless end point. Even though we did not isolate the reaction product, we have proposed the reaction mechanism between OLP and NBS based on the literature knowledge as well as the reactivity of the functional groups present in OLP, and based on the experimentally found reaction stoichiometry (Figure 2). At the end point, NBS will react with methyl orange, and the latter becomes colourless due to its bromination. The bromination of methyl orange with NBS was reported by Pande and Gopal[39], and they have isolated the product and identified its structure by infrared and NMR spectra. They have reported that o- and p-positions of the nucleus A are activated by the $-N(CH_3)_2$ group, and two atoms of bromine will substitute at the free ortho positions. Also, the nucleus B bears the electron- attracting $-SO_3H$ group which increases the electron density at positions meta to the $-SO_3H$ group and facilitates the substitution at these two positions. Finally the $-SO_3H$ group is replaced by bromine (Figure 2).



At the end point:

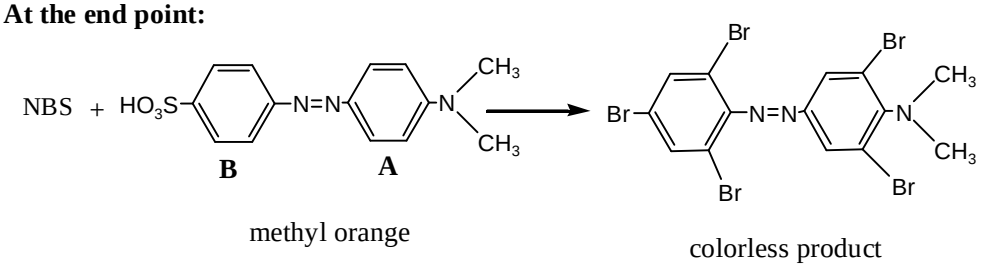


Figure 2: A tentative reaction scheme for titrimetric method.

Spectrophotometry

Many dyes are irreversibly destroyed to colourless species by oxidizing agents in acidic medium[40], and this observation has been exploited for the indirect spectrophotometric determination of some bioactive compounds.[41,42] In the proposed spectrophotometric

methods, the ability of NBS to affect oxidation and bromination of OLP and irreversibly destroy quinoline yellow, and metanil yellow dyes to colourless products in acidic medium has been capitalized. Both methods are based on the oxidation and bromination of the drug by measured excess of NBS and subsequent determination of the latter by reacting with quinoline yellow or metanil yellow and measuring the absorbance at 410 or 530 nm (Figure 3). In either method, the absorbance increased linearly with increasing concentration of the drug. OLP when added in increasing concentrations to a fixed concentration of NBS consumes the latter, and there will be a concomitant decrease in the concentration of NBS. When a fixed concentration of either dye is added to decreasing concentration of NBS, a concomitant increase in the concentration of dye is obtained. This is observed as a proportional increase in the absorbance at the respective wavelengths of maximum absorption with increasing concentration of OLP.

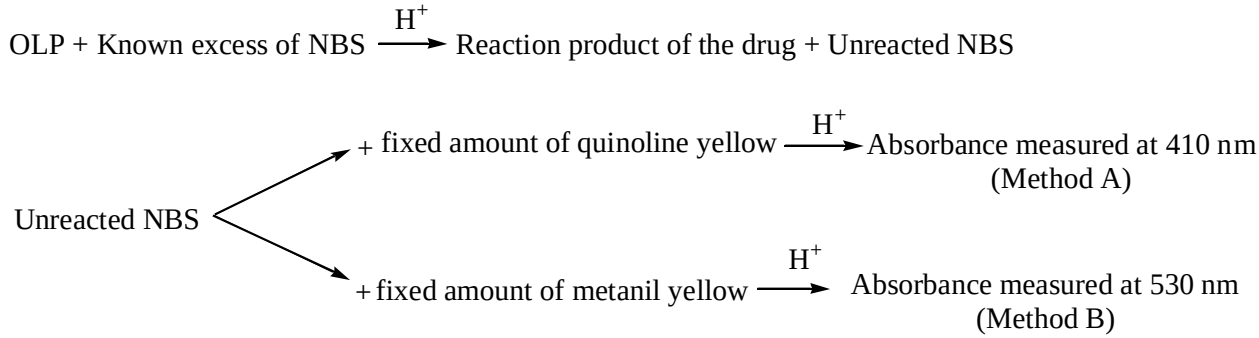


Figure 3: A tentative reaction scheme for spectrophotometric methods.

Preliminary experiments were performed to fix the upper limits of the dyes that could be measured spectrophotometrically, and these were found to be 14 and 5 µg mL^{-1} for quinoline yellow and metanil yellow, respectively. A 7 µg mL^{-1} NBS was found to destroy irreversibly the yellow colour of 14 µg mL^{-1} quinoline yellow, whereas 10 µg mL^{-1} NBS was required to destroy 5 µg mL^{-1} metanil yellow to a colourless product in HCl medium. Hence, different concentrations of OLP were reacted with 1.0 mL of 70 µg mL^{-1} NBS in method A, and with 1.0 mL of 100 µg mL^{-1} NBS in method B, followed by the determination of residual NBS as described under the respective procedures. Hydrochloric acid was found to be a convenient medium for the two steps involved in both spectrophotometric methods. However, since 1.0 mol L^{-1} acid concentration was found optimum for the oxidation and bromination reactions in a reasonable time of 15 and 25 min, in methods A and B, respectively. The same concentration was maintained for the determination of the unreacted NBS with the dyes, and even this reaction time is not critical. Any delay up to 30 min had no effect on the absorbance. A contact time of 5 min is necessary for the complete destruction of the dyes by the residual NBS. Though the colour of acid form of either dye was indefinitely stable in hydrochloric acid medium, it was found to be stable up to 30 min in the presence of the reaction product of the drug. Hence, the absorbance of the dye solution should be measured within 30 min after its addition.

Method Validation

Analytical parameters of spectrophotometric methods:

A linear correlation was found between absorbance at λ_{max}, and concentration ranges of drug were given in Table 1 for both methods. Correlation coefficients, intercepts and slopes for the calibration data were also presented in Table 1. The calibration graphs showed negligible intercept as described by the regression equation. $Y = a + bX$ (where Y is the absorbance, a is the intercept, b the slope and X the concentration in µg

mL^{-1}) obtained by the method of least squares. The apparent molar absorptivity and the Sandell's sensitivity values together with the limits of detection and quantification were also summarized in Table 1 and indicate the high sensitivity of the methods.

Table 1: Analytical and regression parameters of spectrophotometric methods.

Parameter	Method A (n =7)	Method B (n =7)
λ_{max}, nm	410	530
Beer's law limits, µg mL^{-1}	0.1 – 1.2	0.1 – 1.5
Molar absorptivity, L mol^{-1} cm^{-1}	1.64×10^{5}	1.29×10^{5}
Sandell sensitivity*, µg cm^{-2}	0.0019	0.0024
Limit of detection, µg mL^{-1}	0.02	0.02
Limit of quantification, µg mL^{-1}	0.07	0.05
Regression equation, Y**		
Intercept, (a)	- 0.0108	0.0003
Slope, (b)	0.5557	0.4149
Correlation coefficient, (r)	0.9997	0.9961
Standard deviation of intercept (S_a)	0.0042	0.0148
Variance (S_a^2)	1.76×10^{-5}	2.19×10^{-4}
$\pm tS_a/\sqrt{n}$	3.88×10^{-3}	0.0137
Standard deviation of slope (S_b)	0.00582	0.0164
$\pm tS_b/\sqrt{n}$	5.38×10^{-3}	0.0152

*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 concentration in µg mL^{-1}, $\pm tS_a/\sqrt{n}$ = confidence limit for intercept, $\pm tS_b/\sqrt{n}$ = confidence limit for slope.

Accuracy and precision

To evaluate the accuracy and precision of the methods, pure drug solutions at three different levels (within the working limits) were analysed, each determination being repeated seven times. The intra-day relative error (%) and relative standard deviation (%) were less than 2.5 and indicate the high accuracy and precision for the methods (Table 2). For a better picture of reproducibility on a day-to-day basis, a series of experiments were performed in which standard drug solution at three different levels was determined each day for five days with all solutions being prepared a freshly each day. The day-to-day (inter-day) relative standard deviation values were in the range of 1.24-3.44% and represent the best appraisal of the methods in routine use. These results were also given in Table 2.

Table 2: Intra-day and inter-day precision and accuracy studies.

Method[a]	OLP taken	Intra-day (n=7)			Inter-day (n=5)		
		OLP found[b]	Precision[c]	Accuracy[d]	OLP found[b]	Precision[c]	Accuracy[d]
	2.5	2.54	1.13	1.60	2.57	1.24	2.80
Titrimetry	5.0	4.89	0.93	2.20	5.08	1.52	1.60
	7.5	7.42	0.82	1.07	7.61	1.72	1.47
	0.3	0.31	2.27	2.00	0.31	1.58	2.00
Spectrophotometric	0.6	0.59	2.48	1.67	0.62	2.74	2.50
method A	0.9	0.88	2.41	2.22	0.93	3.12	2.89
	0.75	0.76	1.55	1.33	0.77	2.36	2.67
Spectrophotometric	1.00	0.98	1.34	2.00	1.02	1.82	2.00
method B	1.25	1.26	1.78	0.80	1.27	3.44	1.60

a OLP taken / found in titrimetric method is in mg and the same in spectrophotometric methods are in μg mL^{-1},
b. Mean ± standard error, c. Relative standard deviation (%), d. Bias %: [(found-taken)/taken] × 100.

Selectivity

The proposed methods were tested for selectivity by placebo blank and synthetic mixture analyses. Placebo blanks containing talc, starch, lactose, calcium carbonate, calcium dihydrogen orthophosphate, methyl cellulose, sodium alginate and magnesium stearate were extracted with 0.1 M H_2SO_4 in titrimetric method and with 0.1 M HCl in spectrophotometric methods and the solutions were made as described above under "assay procedure for tablets". A convenient aliquot of the solutions was subjected to analysis by titrimetry and spectrophotometry (method A and method B) according to the recommended procedures. In all the cases, 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 prepared above, different amounts of OLP were added, homogenized, and the solutions of the synthetic mixture were prepared as described above for tablets. The filtrates were collected and a portion of 5.0 mL of the resulting solution (1.0 mg mL^{-1}) was assayed (n=5) by titrimetry which yielded a % recovery of 97.96 ± 1.62. The synthetic mixture solution (100 μg mL^{-1} in OLP) was appropriately diluted with 0.1 M HCl to get 4 and 5 μg mL^{-1} solutions, and 1.5 mL was analysed by spectrophotometric method A and method B, separately, and the corresponding % recoveries of OLP were 102.9 ± 1.31 and 101.2 ± 2.07. These results demonstrate the accuracy as well as the precision of the proposed methods. These results complemented the findings of the placebo blank analysis with respect to the selectivity.

Application to formulations

The proposed methods were applied to the determination of OLP in two representative tablets oliza-10 and oliza-20 purchased from local stores and containing other inactive ingredients. The results in Table 3 show 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 of OLP determination by the proposed methods and reference method[17] is presented in Table 3. The reference method is based on the measurements of

the absorbance of the methanolic extract of the tablets at 226 nm. The results agreed 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 2.78 and 6.39, respectively, for four degrees of freedom. Hence, no significant difference exists between the proposed and reference method with respect to accuracy and precision.

Table 3: Results of assay of tablets by the proposed methods and statistical evaluation.

Tablet brand name	Nominal amount, mg	Found (% of nominal amount ± SD)[a]			
		Reference method	Proposed methods		
			Titrimetric method	Spectrophotometric (method A)	Spectrophotometric (method B)
Oliza[b]	10	96.58 ± 0.85	97.46 ± 1.26 **t** = 1.32 **F** = 2.20	96.14 ± 1.82 **t** = 0.52 **F** = 4.58	95.72 ± 1.52 **t** = 1.15 **F** = 3.20
	20	101.3 ± 0.98	102.6 ± 1.54 **t** = 1.63 **F** = 2.47	100.8 ± 2.14 **t** = 0.51 **F** = 4.77	101.7 ± 1.38 **t** = 0.54 **F** = 1.98

Tabulated t-value at the 95% confidence level is 2.78; Tabulated F-value at the 95% confidence level is 6.39

[a]Mean value of five determinations

[b]Marketed by: Intas Pharmaceuticals, Dehradun, India;

Recovery study

To study the reliability and reproducibility of the proposed methods, a standard addition technique was followed. A fixed amount of drug from preparations was taken and pure (standard) drug at three different concentrations was added. The total concentration was found by the proposed methods. The determination with each concentration was repeated three times and the percent recovery of the added standard was calculated from:

$$\%\,\mathrm{Recovery} = \frac{[C_F - C_T]}{C_P} \times 100$$

where C_F is the total concentration of the analyte found; C_T, concentration of the analyte present in the formulation; C_P, concentration of analyte (pure drug) added to formulation. Results of this study presented in Table 4 reveal that the accuracy of methods was unaffected by the various excipients present in the formulations.

Table 4: Results of recovery experiments by standard addition method.

Formulation studied	Titrimetric method				Spectrophotometric (method A)				Spectrophotometric (method B)			
	OLP in tablet, mg	Pure OLP added, mg	Total found, mg	Pure OLP recovered*, Percent ±SD	OLP in tablet, µg mL^{-1}	Pure OLP added, µg mL^{-1}	Total found, µg mL^{-1}	Pure OLP recovered*, Percent ±SD	OLP in tablet, µg mL^{-1}	Pure OLP added, µg mL^{-1}	Total found µg mL^{-1}	Pure OLP recovered*, Percent ± SD
Oliza, 10 mg	3.86	2	5.81	97.50 ± 1	0.385	0.2	0.59	102.5 ± 2.45	0.383	0.25	0.64	102.8 ± 1.62
	3.86	4	7.87	100.2 ± 1	0.385	0.4	0.79	101.2 ± 1.92	0.383	0.50	0.87	97.40 ± 1.2
	3.86	6	10.02	102.7 ± 1	0.385	0.6	1.01	104.2 ± 2.24	0.383	1.00	1.41	102.7 ± 1.56

*Mean value of three determinations

CONCLUSION

Three useful micro methods for the determination of OLP have been developed and validated based on the current ICH guidelines.[43] The assay results demonstrate that it is possible to use NBS as an environmentally friendly reagent for the direct titrimetric and indirect spectrophotometric determination of OLP in authentic samples. The titrimetric method is applicable over a micro scale (1-10 mg), and as small as a concentration of 0.1 µg mL^{-1} drug can be determined by spectrophotometric methods with a fair degree of accuracy and precision. The proposed methods have the advantages of simplicity, cost-effectiveness and easily accessible technique in under-developed and developing countries. The proposed spectrophotometric methods rely on the use of simple and cheap chemicals. Inexpensive techniques provide a sensitivity comparable to that achieved by sophisticated and expensive technique like HPLC. These advantages coupled with good accuracy and precision make the proposed methods highly suitable for routine use in laboratories as a part of industrial quality control.

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فائدة ن-بروموسكسينيمايد ككاشف صديق البيئة لتحديد تراكيز عقار الأولانزبين بدقة في المستحضرات الصيدلانية

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ملخص

تم وصف طريقتين لقياس تراكيز عقار الأولانزبين olanzapineبالجملة و في أشكال صيدلانية. كانت إحدى هاتين الطريقتين باستعمال أسلوب المعايرة والأخرى طيفية. تعتمد الطرق الموصوفة على استخدام مركب ن-بروموسكسينيمايد (N-Bromosuccinimide) واثنين من الأصباغ هما : الكينولين الأصفر و الميتانيل الأصفر ككواشف. في طريقة القياس بالمعايرة تم معايرة محلول حامضي من عقار الأولانزبين بشكل مباشر بمحلول ن-بروموسكسينيمايد و باستعمال صبغة الميثيل البرتقالي ككاشف. طريقة القياس الطيفي تتضمن إضافة كمية زائدة و محددة من ن-بروموسكسينيمايد الى محلول الأولانزبين الحامضي و من ثم قياس تركيز ن-بروموسكسيمايد غير المتفاعل طيفياً بواسطة إضافة إما الكونولين الأصفر و قراءة الأمتصاص الطيفي على 410 nm (الطريقة أ) أو إضافة صبغة الميتانيل الأصفر و قراءة الأمتصاص الطيفي على530nm (الطريقة ب). لقد وجد أنه يمكن تطبيق طريقة المعايرة على تراكيز من الأولانزبين تتراوح بين 1-10مغ ووجد أن نسبة التفاعل هي 1: 6 (الأولانزبين: ن-بروموسكسينيمايد). في طريقتي التحليل الطيفي وجد أنتتم إطاعة قانون بير ضمن تراكيز تتراوح بين 0.1-1.2 و 0.1-1.5 مايكروغرام أولانزبين للطريقتين أ و ب على التوالي. تم أيضاً تحديد الأمتصاصية المولارية ، حساسية ساندیل ، حدود الكشف و القياس لكل من الطريقتين الطيفيتين.تم تطبيق الطريقتين لقياس تركيز الأولانزبين في أقراص صيدلانية. كما تم تأكيد دقة وإمكانية الإشتغال لطرق التحليل بواسطة دراسات أسترجاع.من أهم مميزات أستعمال ن-بروموسكسينيمايد هو أن ناتج التفاعل ، و هي مادة السكسينيمايد ، يمكن أسترجاعها و إعادة تدويرها الى ن-بروموسكسينيمايد.

الكلمات الدالة: عقار الأولانزبين ، القياس بالمعايرة ، القياس الطيفي ، ن-بروموسكسينيمايد، والمستحضرات الصيدلانية.

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