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Sensitive and Selective Methods for the Determination of Olanzapine in Pharmaceuticals Using N-bromosuccinimide and Two Dyes

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ABSTRACT: Three indirect methods for the quality control of olanzapine (OLP) in commercial formulations based on titrimetric and spectrophotometric techniques are described using N-bromosuccinimide (NBS) and two dyes, namely amaranth and janus green B as reagents. In titrimetry, a measured excess of NBS is added to an acidified solution of OLP and the unreacted NBS is determined iodometrically. Spectrophotometry involves the addition of a known excess of NBS to OLP in acid medium followed by estimation of residual NBS by reacting with a fixed amount of either amaranth and measuring the absorbance at 520 nm (method A) or janus green B and measuring the absorbance at 620 nm (method B). Titrimetric procedure is applicable over the range 0.5-5.0 mg OLP, and the reaction stoichiometry is found to be 1: 6 (OLP: NBS). The spectrophotometric methods are applicable over the ranges 0.1-0.9 µg/ml (method A) and 0.1-1.2 µg/ml (method B). The proposed methods have been applied successfully for the determination of OLP in pure form and in its dosage forms and the results were compared with those of a literature method by applying the Student's t-test and F-test. The validity of the methods was established by recovery studies.
Keywords: olanzapine, titrimetry, spectrophotometry, pharmaceuticals.

INTRODUCTION

Olanzapine (OLP), chemically known as 2-methyl-4-(4-methyl-1-piperazinyl)-10H-thieno [2, 3-b] [1, 5] benzodiazepine (Fig. 1), is an atypical antipsychotic agent, also known as second-generation antipsychotic (SGA)[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]. HPLC has also been used for the assay of OLP in pharmaceutical formulations when present either alone[13,14] or in combination with fluoxetine[15,16]. Various other techniques including HPTLC[16], non aqueous titrimetry and UV-spectrophotometry[17], derivative spectrometry[13], capillary zone electrophoresis[13] and linear voltammetry[13] have also been reported for the assay of OLP in pharmaceuticals.

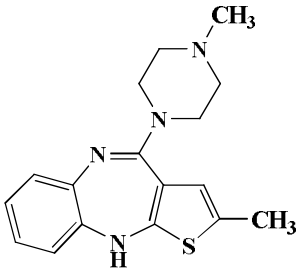

Fig.1: Chemical structure of olanzapine

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 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 one or the other disadvantage such as poor sensitivity, complicated experimental setup and meticulous control of experimental variables (Table 1).

The present investigation aims to develop more sensitive and cost-effective methods for the determination of OLP in pure form and in dosage forms using titrimetric and spectrophotometric techniques. The methods employ N-bromosuccinimide which acts as an oxidizing as well as brominating agent and two dyes amaranth and janus green B as auxiliary reagents. 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.

Table 1: Performance characteristic of the existing spectrophotometric methods and the proposed methods

Sl. No.	Reagent/s used	Methodology	λ_{max} (nm)	Linear range (µg/ml) and ε ($l\ mol^{-1}cm^{-1}$)	LOQ (µg/ml)	Reaction time	Remarks	Ref.
1.	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	25
	Potassium hexacyano ferrate (III)	Radical cation measured	540	0.5-250	-		FIA assembly required	
	Cerium (IV) sulphate	-do-	540	0.05-300	-		-do-	
2.	NBS	Radical cation measured	532	10-120 ($\varepsilon = 4.19\times10^3$)	6.99		Uses 1:1 mixture of H_2SO_4 and H_3PO_4 as the reaction medium, colour stable for only 30 S	26
	NBS-Celestine blue	Unbleached dye colour measured	538	0.5-6.0 ($\varepsilon = 6.41\times10^4$)	0.30	10 min	-	
	Cerium(IV)-Celestine blue	-do-	538	0.6-3.0 ($\varepsilon = 1.48\times10^5$)	0.37	35 min	-	
3.	KIO_3	Initial rate of formation of radical cation measured	537	up to 4.0	-	Within 30s	Scrupulous control of experimental variables and special equipment for kinetic measurement required	27
	KIO_3	Maximum absorbance measured	537	Up to 7.0	-			

4.	NBS- Amaranth	Unbleached dye colour measured	520	0.1 -0.9 $(\varepsilon = 1.54\times10^5)$	0.15	15 min	Highly sensitive and selective, no heating or extraction required.	Present methods
	NBS- Janus Green B	-do-	620	0.1-1.2 $(\varepsilon = 1.29\times10^5)$	0.28	15 min	Colour product stable for several days	

EXPERIMENTAL

Apparatus

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

Materials and Reagents

Pharmaceutical grade olanzapine (OLP) which is reported to be 99.85 % pure was received from Cipla Ltd., India. All pharmaceutical preparations were obtained from commercial sources in the local market.

All the reagents used were of analytical-reagent grade and distilled water was used throughout the investigation. N-bromosuccinimide (NBS): An approximately 0.01 M solution was prepared by dissolving about 1.8 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[21] and kept in an amber coloured bottle and stored in a refrigerator; and used in titrimetry. It was diluted appropriately to get 70 and 80 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), 10% Potassium iodide (Merck, Mumbai, India), 0.02 M Sodium thiosulphate (Sisco-chem Industries, Mumbai, India, assay 98 %), 0.1 M and 5 M Hydrochloric acid (Merck, Mumbai, India; sp. gr. 1.18) and 1% Starch indicator GR (LOBA Chemie, Mumbai, India), 200 μg/ml Amaranth (S.d fine-chem. Ltd., Mumbai, India) for method A and 60 μg/ml Janus Green B for method B (LOBA Chemie, Mumbai, India) were prepared in water.

Preparation of stock solution

A stock standard solution equivalent to 0.5 mg/ml of OLP was prepared by dissolving accurately weighed 50 mg of pure drug in 0.1 M H_2SO_4 and diluted to the mark in a 100 ml calibrated flask. This solution was used in titrimetric work. Another stock solution equivalent to 150 μg/ml of OLP was prepared by dissolving accurately weighed 15 mg of pure drug in 0.1 M HCl and diluting to the mark in a 100 ml calibrated flask. The second stock solution (150 μg/ml OLP) was diluted appropriately with 0.1 M HCl to get a working concentration of 4 μg/ml OLP for use in spectrophotometric methods. The standard solutions were kept in an amber coloured bottle and stored in a refrigerator when not in use.

Assay Procedures

Titrimetry

A 10 ml aliquot of pure drug solution containing 0.5-5.0 mg of OLP was accurately measured and transferred into a 100 ml titration flask. The solution was acidified by adding 2.5 ml of 5 M sulphuric acid followed by the addition of 10 ml of 0.01 M NBS. The content was mixed well and the flask was kept aside for 15 min with occasional swirling. Then, 5 ml of 10 % potassium iodide was added to the flask and the liberated iodine was titrated with 0.02 M sodium thiosulphate to a starch end point. A blank titration was run under the same conditions. The drug content in the aliquot was calculated.

Spectrophotometry using amaranth (method A)

Different aliquots (0.25,0.5,1.0,----2.25 ml) of a standard 4 μg/ml OLP solution were transferred into a series of 10 ml calibrated flasks by means of a micro burette and the total volume was adjusted to 2.5 ml by adding adequate quantity of 0.1 M HCl. To each flask 2 ml of 5 M HCl and 1.0 ml of NBS solution (70 μg/ml) were added, the last being measured accurately. The flasks were stoppered, content mixed and allowed to stand for 15 min with occasional shaking. Finally, 1.0 ml of 200 μg/ml amaranth solution was added (accurately measured) and the volume was adjusted to the mark with water and mixed well. The absorbance of each solution was measured at 520 nm against a reagent blank after 5 min.

Spectrophotometry using janus green B (method B)

Varying aliquots (0.25,0.5,1.0,----3.0 ml) of a standard 4 μg/ml OLP solution were transferred into a series of 10 ml calibrated flasks by means of a micro burette and the total volume was brought to 3 ml by adding 0.1 M HCl . To each flask 2 ml of 5 M hydrochloric acid and 1.0 ml of NBS solution (80 μg ml^{-1}) were added by means of a micro burette. The content was mixed well and the flasks were kept aside for 15 min with intermittent shaking. Finally, 1.0 ml of 60 μg/ml janus green B solution was added to each flask, the volume was adjusted to the mark with water, mixed well and the absorbance was measured against a reagent blank at 620 nm after 5 min.

In either spectrophotometric method, a standard graph 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.

Procedure for the dosage forms

Twenty tablets each containing 10 or 20 mg of OLP were weighed accurately and ground into a fine powder. An amount of the powder equivalent to 50 mg of OLP was accurately weighed into a 100 ml volumetric flask, 60 ml 0.1 M H_2SO_4 was added and content shaken thoroughly for about 15 min. The volume was adjusted 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 rejected and a convenient aliquot of filtrate (containing 0.5 mg/ml OLP) was taken for assay by titrimetric procedure. An amount of the powder equivalent to 15 mg of OLP was accurately weighed into a 100 ml volumetric flask, 60 ml 0.1 M HCl was added and content shaken thoroughly for about 15 min. The volume was adjusted 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 rejected and a convenient aliquot of filtrate (containing 150 µg/ml OLP) was diluted with 0.1 M HCl to get 4 µg/ml of OLP for use in spectrophotometric methods. A suitable aliquot was then subjected to analysis following the procedures described earlier.

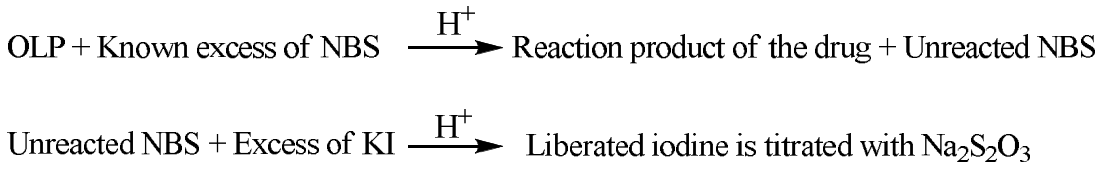

Fig.2: Reaction scheme for titrimetric method.

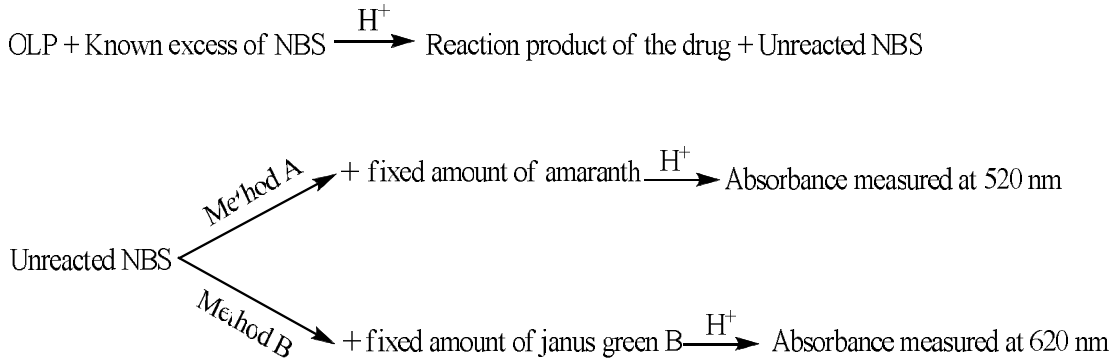

Fig.3: Reaction scheme for spectrophotometric methods.

RESULTS AND DISCUSSION

N-bromosuccinimide (NBS) has been used widely as a brominating and oxidizing agent for organic compounds[22]. There are many reported methods based on the oxidation of OLP by potassium hexacyanoferrate(III)[18], cerium(IV)sulphate[18,19], potassium iodate[20] which exclusively oxidize OLP resulting in the lower mole ratio and hence lesser sensitivity of the methods when compared to the proposed methods. In the proposed methods, NBS not only oxidizes OLP but also brominates it. This resulted in enhancing the mole ratio 1: 6 (OLP: NBS) and the sensitivity of the methods. The proposed methods are indirect and are based on the oxidation and bromination reaction between OLP and NBS, and determination of residual NBS after allowing the reaction between OLP and a measured amount of NBS to be complete. The amount of NBS reacted corresponds to the OLP content in all the methods.

Method Development

Titrimetry

In the proposed titrimetric procedure, the quantitative nature of reaction between OLP and NBS

was checked by treating (0.5 to 5.0 mg) of OLP with a measured excess of NBS in acid medium and determining the residual NBS iodometrically (Fig. 2). In the range studied (0.5 to 5.0 mg), the reaction stoichiometry was found to be 1:6 (OLP: NBS). Sulphuric acid was found to be an ideal medium for the assay and the reaction stoichiometry was found to be unaffected in the presence of 1 to 5 ml of 5 M H_2SO_4 in a total volume of 25 ml, and 2.5 ml was chosen as the optimum volume. The reaction was found to be complete in 15 min and contact time upto 60 min had no effect on the stoichiometry or the results. A 10 ml volume of 0.01 M NBS was found adequate for quantitative oxidation of OLP in the range investigated.

Spectrophotometry

Many dyes are irreversibly destroyed to colorless species by oxidizing agents in acid medium[23]. The proposed spectrophotometric methods are based on the reaction between OLP and measured excess of NBS and subsequent determination of the latter by reacting it with a fixed amount of either amaranth or janus green B dye, and measuring the absorbance at 520 or 620 nm (Fig. 3). These methods make use of the bleaching action of NBS on the dyes, the decolorization being caused by the oxidative destruction of the dyes. 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 concentrations of NBS, a concomitant increase in the concentration of dye is obtained. Consequently, a proportional increase in the absorbance at the respective λ_{max} is observed with increasing concentration of OLP.

Preliminary experiments were performed to fix the upper concentrations of the dyes that could be determined spectrophotometrically in acid medium, and these were found to be 20 and 6 µg/ml for amaranth and janus green B, respectively. A NBS concentration of 7 µg/ml was found to irreversibly destroy the red colour of 20 µg/ml amaranth, whereas 8 µg/ml NBS was required to destroy the blue colour of 6 µg/ml janus green B, in HCl medium. Hence, different concentrations of OLP were reacted with 1.0 ml of 70 µg/ml NBS in method A, and 1.0 ml of 80 µg/ml 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 two methods. However, since 1 M acid concentration was found optimum for the oxidation and bromination reaction in a reasonable time of 15 min in both methods, 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. The absorbance of either dyes solution even in the presence of reaction product was found to be stable for several days.

Method Validation

Analytical Parameters of Spectrophotometric Methods

A linear correlation was found between absorbance at λ_{max} and concentration of OLP. Beer's law was obeyed over the concentration ranges given in Table 2, and 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) obtained by the method of least squares. The limits of detection (LOD) and quantification (LOQ) calculated according to the current ICH guidelines[24] are presented in Table 2. The other sensitivity parameters such as molar absorptivity and Sandell sensitivity are also contained in Table 2.

Accuracy

The accuracy of an analytical method expresses the closeness between the reference value and the found value[24]. Accuracy was evaluated as percentage relative error between the measured concentrations and taken concentrations for OLP (Bias %). The results obtained are compiled in Table 3 and show that the accuracy is good for all methods.

Precision

The precision of the methods was calculated in terms of intermediate precision (intra-day and inter-day)[25]. Three different concentrations 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 all methods (Table 3).

Selectivity

To determine the selectivity of the proposed methods, the analytical placebo was prepared and subjected to analysis by the proposed methods. It was confirmed that the change in the titrant value and absorbance with respect to the water blank was caused only by the analyte. To identify the interference by common tablet excipients, a synthetic mixture with the composition: OLP (50 mg), talc (80 mg), starch (160 mg), calcium gluconate (80 mg), lactose (80 mg), sodium alginate (40 mg) and magnesium stearate (40 mg), was prepared and subjected to analysis by the proposed methods after solution preparation using the procedure described for tablets. The percent recoveries of OLP were 102.3 ± 1.14 (n = 5), 98.62 ±1.28 and

97.89 ± 2.06 (n = 5) by titrimetry and spectrophotometry (A and B), respectively, suggesting no interference by the excipients in the assay of OLP under the described optimum conditions.

Application to formulations

The proposed methods were applied to the determination of OLP in two representative tablets oliza-10 and oliza-20. 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 by the proposed methods and literature method[24] for the same batch of material is presented in Table 4. The literature method consisted measurement of the absorbance of the methanolic extract of the tablets at 226 nm. The results agree well with the claim and also are in agreement with the results obtained by the literature 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 methods and the literature method at the 95 % confidence level with respect to accuracy and precision (Table 4).

Recovery experiment was performed via standard addition technique to ascertain the accuracy and validity of the proposed methods. To a fixed and known amount / concentration 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. Each experiment was repeated three times and the percent recovery of the added standard was calculated. Results of this study presented in Table 5 indicate that the commonly excipients present in the formulations did not interfere in the assay.

In conclusion, three useful simple, rapid, and cost-effective methods for the determination of OLP have been developed and validated. The proposed methods are more sensitive than many existing methods, and are free from such experimental variables as heating or extraction step (Table 1). The stability of the colour system is an advantage over the earlier methods. The methods rely on the use of simple and cheap chemicals and techniques but provide a sensitivity comparable to that achieved by sophisticated and expensive technique like HPLC. Thus, they can be used as alternatives for rapid and routine determination of bulk sample and tablets.

Table 2: Analytical and regression parameters of spectrophotometric methods

Parameter	Method A (n=6)	Method B (n= 7)
λ_{max}, nm	520	620
Beer's law limits, µg/ml	0.1 – 0.9	0.1 – 1.2
Molar absorptivity, l/mol/cm	1.54×10^{5}	1.29×10^{5}
Sandell sensitivity*, µg/cm^2	0.0020	0.0024
Limit of detection, µg/ml	0.05	0.09
Limit of quantification, µg/ml	0.15	0.28
Regression equation, Y**		
Intercept, (a)	- 0.0015	0.0004
Slope, (b)	0.5010	0.4152
Correlation coefficient, (r)	0.9995	0.9980
Standard deviation of intercept (S_a)	0.0044	0.0084
Variance (S_a^2)	1.94×10^{-5}	7.06×10^{-5}
$\pm tS_a/\sqrt{n}$	4.62×10^{-3}	7.77×10^{-3}
Standard deviation of slope (S_b)	0.00758	0.01167
$\pm tS_b/\sqrt{n}$	7.96×10^{-3}	0.0108

*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, $\pm tS_a/\sqrt{n}$ = confidence limit for intercept, $\pm tS_b/\sqrt{n}$ = confidence limit for slope.

Table 3: Intra-day and inter-day precision and accuracy studies

Method*	OLP taken	Intra-day (n=7)			Inter-day (n=5)		
		OLP found[a]	Precision[b]	Accuracy[c]	OLP found[a]	Precision[b]	Accuracy[c]
Titrimetry	1.0	1.01	1.42	1.00	1.02	1.72	2.00
	2.0	2.04	0.96	2.00	2.05	1.28	2.50
	3.0	3.05	0.54	1.67	3.08	1.34	2.67
Spectrophotometric (method A)	0.3	0.29	2.40	3.33	0.31	3.64	3.33
	0.5	0.51	2.45	2.00	0.51	3.85	2.00
	0.7	0.71	2.24	1.43	0.71	3.37	1.43
Spectrophotometric (method B)	0.3	0.31	2.21	3.33	0.31	2.96	3.33
	0.6	0.61	2.90	1.67	0.62	3.14	3.33
	0.9	0.92	1.14	2.22	0.92	2.88	2.22

* OLP taken / found in titrimetric method is in mg and the same in spectrophotometric methods are in µg/ml,

a. Mean value of five determinations, b. Relative standard deviation (%), c. Bias %: (found-taken/taken) x 100.

Table 4: Results of assay of tablets by the proposed methods and statistical evaluation

Tablet brand name	Nominal amount, mg	Found (% of nominal amount ± SD)*			
		Literature method	Proposed methods		
			Titrimetric method	Spectrophoto metric (method A)	Spectrophoto metric (method B)
Oliza**	10	96.58 ± 0.85	98.04 ± 1.36 **t** = 2.09 **F** = 2.56	97.33 ± 2.09 **t** = 0.81 **F** = 6.05	96.06 ± 2.12 **t** = 0.55 **F** = 6.22
	20	101.3 ± 0.98	100.8 ± 1.52 **t** = 0.63 **F** = 2.41	102.5 ± 2.46 **t** = 1.10 **F** = 6.30	100.6 ± 2.24 **t** = 0.69 **F** = 5.22

*Mean value of five determinations.

**Marketed by: 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 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	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	1.96	1.0	3.04	108.0 ± 1.73	0.39	0.2	0.60	105.0 ± 2.14	0.38	0.2	0.59	105.0 ± 1.67
	1.96	2.0	4.21	112.5 ± 1.24	0.39	0.4	0.82	107.5 ± 2.26	0.38	0.4	0.79	102.5 ± 1.88
	1.96	3.0	5.12	105.3 ± 2.01	0.39	0.6	1.01	103.3 ± 1.95	0.38	0.6	1.00	103.3 ± 2.06

*Mean value of three determinations.

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