The Spectrophotometric Determination of Olanzapine via Coupling with Diazotized p-Nitroaniline

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Abstract

A new spectrophotometric method has been developed for the assay of olanzapine (OLN.) in pure and dosage forms. The method is based on the diazocoupling of (OLN.) with diazotized p-nitroaniline in alkaline medium to form a stable brown colored water-soluble azo dye with a maximum absorption at 405 nm. The variables that affect the completion of reaction have been carefully optimized. Beer's law is obeyed over the concentration range of (0.5-45.0 $\mu g.mL^{-1}$) with a molar absorptivity of 1.5777×10^4 $L.mol^{-1}.cm^{-1}$. The limit of detection was 0.3148 $\mu g.mL^{-1}$ and Sandell's sensitivity value was 0.0198 $\mu g.cm^{-2}$. The proposed method has been applied successfully to the determination of olanzapine in tablet pharmaceutical preparations.

Keywords: Spectrophotometry, Olanzapine, Diazotization, p-nitroaniline, tablet dosage form.

التقدير الطيفي للاولانزابين باقترانه مع العامل المؤزوت بارا-نيتروانيلين

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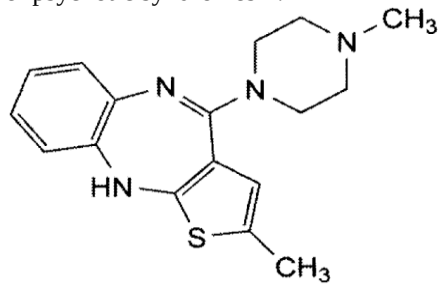
الخلاصة

تم تطوير طريقة طيفية جديدة لتقدير الاولانزابين بصورته النقية و في المستحضرات الصيدلانية. تعتمد الطريقة على اقتران الدايازو لعقار الاولانزابين مع كاشف البارا-نايتروانلين الدايازوتايزي في وسط قاعدي لتكوين صبغه مستقرة ذات لون بني ذائبه في الماء التي اعطت اعظم امتصاص عند الطول الموجي ٤٠٥ نانومتر. درست العديد من العوامل التي تؤثر في اكتمال التفاعل بدقة للحصول على الظروف الفضلى للتفاعل. لقد انطبق قانون بير ضمن مدى من التراكيز ما بين (٠,٥ – ٤٥,٠) مايكروغرام.مل$^{-1}$. حيث اعطت امتصاصيه مولارية تقدر ١.٥٧٧٧×١٠^{٤} لتر.مول$^{-1}$.سم$^{-1}$. لقد كان حد الكشف مساويا لـ ٠.٣١٤٨ مايكروغرام.مل$^{-1}$ اما حساسية ساندل فقد كانت تساوي ٠.٠١٩٨ مايكروغرام.سم$^{-٢}$. تم تطبيق الطريقة المقترحة بنجاح في تقدير الاولانزابين في مستحضرات الحبوب الصيدلانية.

الكلمات المفتاحية: التقدير الطيفي, اولانزابين, الازوته, بارا-نيتروانيلين, مستحضرات الحبوب الصيدلانية.

Introduction

Olanzapine (OLN.) ($C_{17}H_{20}N_4S$) is known chemically as 2-methyl-4-(4-methyl -1piperazinyl) 10H-thieno [2,3-b] [1,5] benzodiazepine (Scheme 1), has molar mass of 312.439 $g.mol^{-1}$ and it is a yellow crystalline solid substance with a melting point of 195ºC.[1,2] It is an atypical antipsychotic drug used in the treatment of schizophrenia and other psychotic syndromes[3].



Scheme (1):- The structural formula of olanzapine.[1]

The mode of action of olanzapines' of antipsychotic activity having efficacy in schizophrenia is unknown,[4] it has been proposed that drug achieve this seemingly highly selective approach to treatment is the antagonism of a specific serotonin receptor, 5-HT_{2A} .This receptor can be found on the axon terminal of neurons that produce dopamine. When serotonin activates those receptors, the release of dopamine decreases. By building into the typical antipsychotics a mechanism to block the 5-HT_{2A} receptors from serotonin, the dopamine release is increased.[3,5] Since its introduction in 1996 in over 84 countries[6] (OLN.) was determined by using several methods that have been reported for the analysis in pure form, dosage forms or in combination with other drugs. These methods include spectrophotometry,[7-10] HPLC,[11,12] flow injection analysis,[13] electro-analytical method,[14,15] and capillary electrophoresis [16] .

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The aim of the present study is to develop an accurate, simple and sensitive spectrophotometric procedure for the determination of olanzapine in pure and tablet dosage forms.

The method is based on the coupling of olanzapine with diazotized p-nitroaniline in basic medium to form a colored complex. In addition, the reaction conditions were studied univariatly one-factor-a time to provide an optimized analytical response.

Experimental

Apparatus

A PG instrument, UV- visible spectrophotometer model T80 (U.K) with 1 cm matched quartz cells was used for the absorbance measurements.

Sartorius BL 210S electronic balance was used for weighing the samples.

Hot plate with magnetic stirrer, Ijlassco, India.

Materials and methods

All chemicals used were of analytical reagent grade and were obtained from BDH and Panreac. Olanzapine standard powder was kindly provided by the State Company for Drug Industries and Medical Appliances (SDI), Samara-Iraq. The pharmaceutical preparation; Zyprexa® tablet (5 mg) Lilly (Spain), Zyprexa® tablet (10 mg) Lilly (Spain) and Olan® tablet (5 mg) Micro (India) were purchased from local markets.

Olanzapine stock solution [1000 µg.mL^{-1}]

The stock solution of (OLN.) was prepared by dissolving an accurately weighed 0.1000 g of pure drug in 5 ml of 0.1 M HCl and the volume was made up to the mark in a 100 mL volumetric flask with distilled water. The stock solution was protected from light and stored at 5 ºC.

Olanzapine working solution [100 µg.mL^{-1}]: prepared by diluting 10 mL of the stock solution to 100 mL in a volumetric flask with distilled water.

Diazotized p-Nitroaniline reagent solution, (DPNA), [0.01 M]

p-Nitroaniline (PNA) (0.2762) g was dissolved in 50 ml distilled water and 6.7 ml of concentrated HCl was then added to this solution with stirring. The mixture was heated to obtain a clear solution, transferred to 200 ml volumetric flask, and cooled to (0-5) °C in an ice-bath. $NaNO_2$ (0.1380) g was then added and the mixture was stirred vigorously. Five minutes later, the solution was made up to the mark with cold distilled water. The solution is kept in a brown bottle into refrigerator and used after three hours of preparation. It is stable for at least 72 hours.

Sodium hydroxide solution (~2 M):

prepared by dissolving (8.000) g of NaOH in a suitable volume of distilled water and the volume was made up to the mark in 100 mL volumetric flask.

Olanzapine tablets solution [1000 µg.ml^{-1}]

Seven tablets of both strengths of Zyprexa and ten tablets of Oln-5 were accurately weighed and separately, grinded into fine powder and mixed well then the average weight was calculated for each brand. An amount of the powder equivalent to (0.5928) g, (0.2972) g and (0.5848) g (containing 0.0200 g of the drug olanzapine) of Zyprexa-5 mg, Zyprexa- 10 mg and Oln-5 mg respectively was accurately weighed, dissolved in 5 ml of 0.1 M HCl and stirred for 10 min to ensure complete dissolution of the drug, then transferred into 20 mL volumetric flask and diluted to the mark with distilled water to get 1000 µg.mL^{-1} of (OLN.). The solution was filtered by using Whatman filter paper No.41 to avoid any suspended or un-dissolved material before use.

Working solution 100 µg.mL^{-1} was freshly prepared and analyzed by the recommended procedure.

General recommended procedure for calibration

In a series of 10 mL volumetric flasks, 1 mL of 0.01 M of the diazotized p- nitroaniline solution, aliquots of working drug solution (100 µg.mL^{-1}) in the range (0.05, 0.1, 0.3, 0.5……4.5) mL were added to each flask followed by 1 ml of 2 M sodium hydroxide with shaking and allowed to stand for 5 min. The contents were diluted to the mark with distilled water and mixed well. After 5 min, the absorbance of the brown colored azo-dye was measured at 405 nm against the reagent blank prepared in the same manner without the analyte.

Results and Discussion

Absorption Spectra for primary test

The primary test for the present method involved diazotization of p-nitroaniline followed by coupling with olanzapine (OLN.). The test was done by adding 1 ml of 0.005 M diazotized p-nitroaniline in 10 mL volumetric flask, followed by the addition of 1 ml of 100 µg.ml^{-1} (OLN.) with shaking. 1 ml of 1 M NaOH was then added to the above mixture. The contents were diluted to the mark with distilled water. The absorbance and λmax of the colored azo-dye was measured against the reagent blank prepared in the same manner without the analyte. (Figure 1) shows that the maximum absorption was obtained at a wavelength of 405 nm.

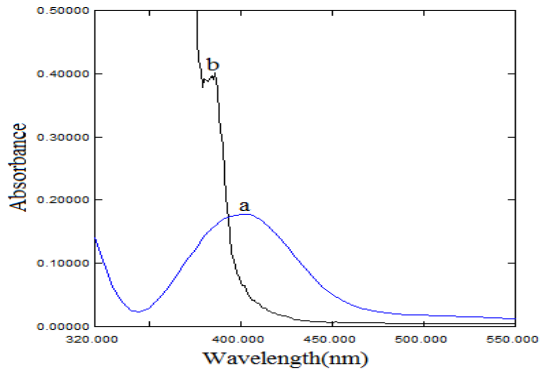


Figure (1): Absorption spectra of: (a) the complex of 10 µg.mL^{-1} (OLN.) with diazotized p-nitroaniline against reagent blank, (b) blank solution against distilled water under the primary test conditions.

Optimization of reaction variables

The various parameters related to the colored product formation have been studied by varying the parameters one at a time and controlling all others fixed and the optimum conditions have been selected

1. Effect of the diazotized p-nitroaniline concentration

The optimum diazotized p-nitroaniline concentration was estimated by adding 1mL of various concentrations [0.005- 0.030] M of diazotized p-nitroaniline reagent solution, The results showed that 0.01 M of the reagent solution is sufficient for production of maximum and reproducible color intensity (Figure 2). Therefore, the recommended concentration of diazotized p-nitroaniline was chosen to be 1 mL of 0.01 M and used for all subsequent measurements.

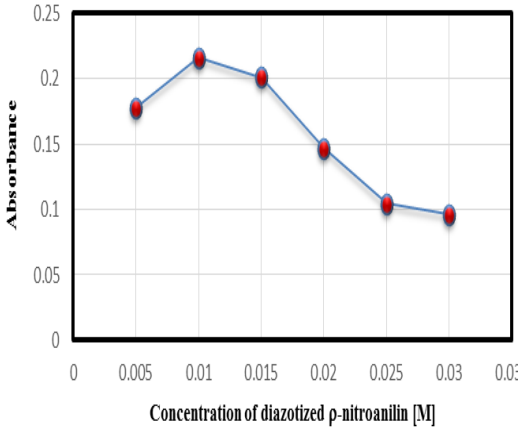


Figure (2): Effect of diazotized p-nitroaniline concentration on the color development in the determination of 10 µg.mL^{-1} (OLN).

*2. **Effect of type of the base***

The effect of different alkaline solutions with concentration of 1 M on the absorption intensity of the colored azo-dye formed was investigated. It was found that sodium hydroxide gave the maximum absorption intensity of the colored product which is used for the subsequent work, (Table 1).

Table (1): Effect of different bases.

Alkaline medium [1M]	Absorbance
NaOH	0.216
KOH	0.188
Na_2CO_3	0.104
NH_4OH	0.025

*2. **Effect of sodium hydroxide concentration***

The stability of the formed azo dye product depends upon the nature of reaction medium.[17] The formed azo dye was found to have a reasonable stability when the reaction medium was rendered alkaline via the addition of 1ml of 2 M sodium hydroxide solution which was optimum and recommended for the subsequent work, (Figure 3).

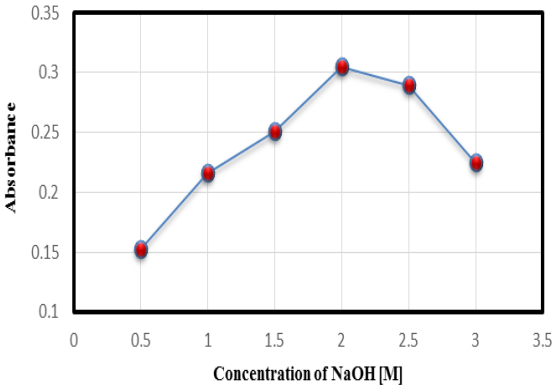


Figure (3): Effect of sodium hydroxide concentration on the color development in the determination of 10 µg.mL^{-1} (OLN.).

4. Effect of coupling reaction time

The optimum time of coupling reaction was determined by choosing different time periods (0-20) min for development the color of azo-dye at room temperature, it was found that 5 minutes period was required for full color development as shown in (Table 2).

Table (2): Effect of coupling reaction time.

Time (min)	Absorbance
0	0.305
5	0.392
10	0.362
15	0.314
20	0.288

5. Effect of order of mixin

The effect of different orders of component addition on chromogen formation was studied by changing the order for three times as

shown in (Table 3). Results shows that mixing order number one was recommended and thus was followed in the subsequent experiments, since it resulted in obtaining maximum absorbance.

Table (3): Variation of absorbance with reactants addition order on the determination of 10 µg.mL^{-1} (OLN.).

No	Sequence	Absorbance
1	Diazotized reagent + Drug + Base	0.392
2	Diazotized reagent + Base+ Drug	0.330
3	Drug + Base + Diazotized reagent	0.327

6. Stability

Under the aforementioned optimum condition, the effect of time on the formation of the azo-dye product was investigated by allowing the reaction to proceed by varying periods. It was found that the absorbance reach to a maximum constant value after 5 min, and the color of the azo product was nearly stable for at least 60 min as shown in (Figure 4).

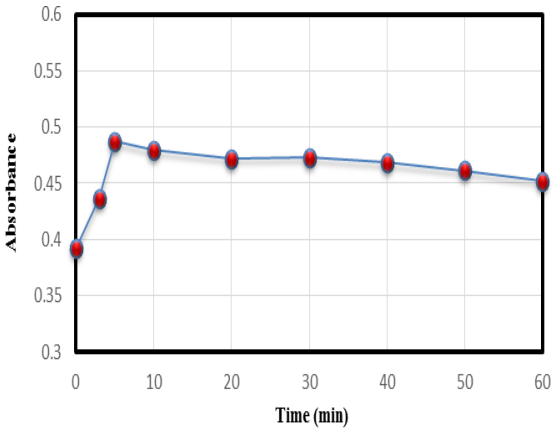


Figure (4): The stability of colored reaction product with time.

Final absorption spectra

When 10 µg. mL^{-1} of (OLN.) is treated with diazotized p- nitroaniline reagent, under the aforementioned optimum conditions, an absorption peak is obtained showing intense brown azo - dye absorption at 405 nm, the reagent blank showed almost nil absorption at this maximum wavelength as shown in (Figure 5).

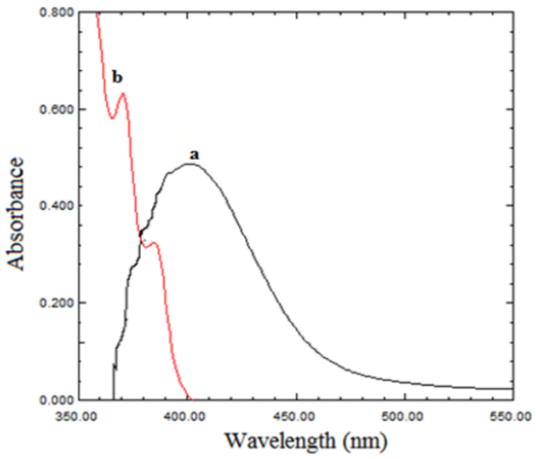


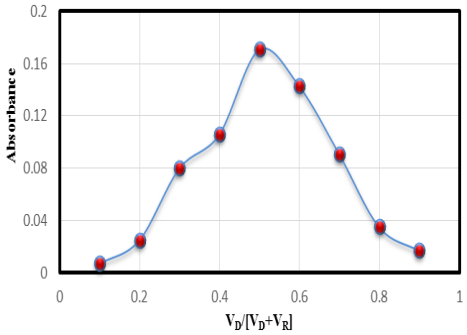
Figure (5): Absorption spectra of: (a) the complex of 10 µg.mL^{-1} (OLN.) with iazotized p-nitroaniline against reagent blank, (b) blank solution against distilled water under the optimum conditions.

Calibration curve and analytical data

According to the optimum experimental conditions, linear calibration graph for (OLN.) was obtained (Figure 6), which shows that Beer's law was obeyed in the concentration range of (0.5- 45.0) µg.mL^{-1}. The regression equation, correlation coefficient, molar absorptivity, Sandell's sensitivity, limit of detection (LOD) and limit of quantification (LOQ) were given in (Table 4).

Table (4): Optical characteristics and statistical data for the determination of (OLN.).

Parameter	Value
λ_{max} (nm)	405
Color	Brown
Regression equation	Y=0.0505[(OLN.) µg.mL^{-1}]-0.0119
Linearity range (µg.mL^{-1})	0.5 - 45
Calibration sensitivity (mL.µg^{-1})	0.0505
Correlation coefficient (r)	0.9994
Correlation of linearity (R^2)	0.9989
Molar absorptivity (L.mol^{-1}.cm^{-1})	1.5777x10^4
Sandell's sensitivity (µg.cm^{-2})	0.0198
L.O.D. (µg.mL^{-1})	0.3148
L.O.Q. (µg.mL^{-1})	1.0495



Figure(6): Calibration curve for the determination of (OLN.) under optimum conditions.

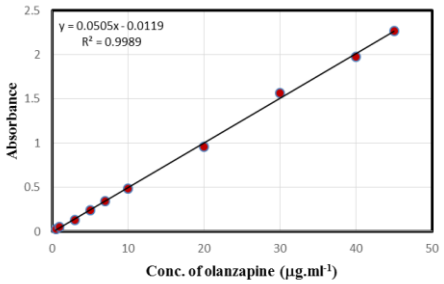


Figure (7): Continuous variation method for reaction (OLN.) with diazotized p-nitroaniline

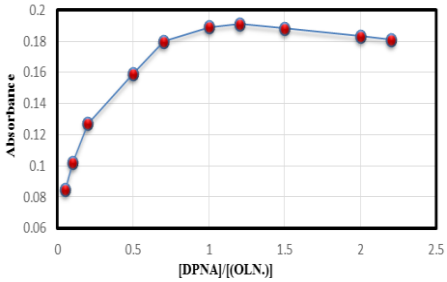
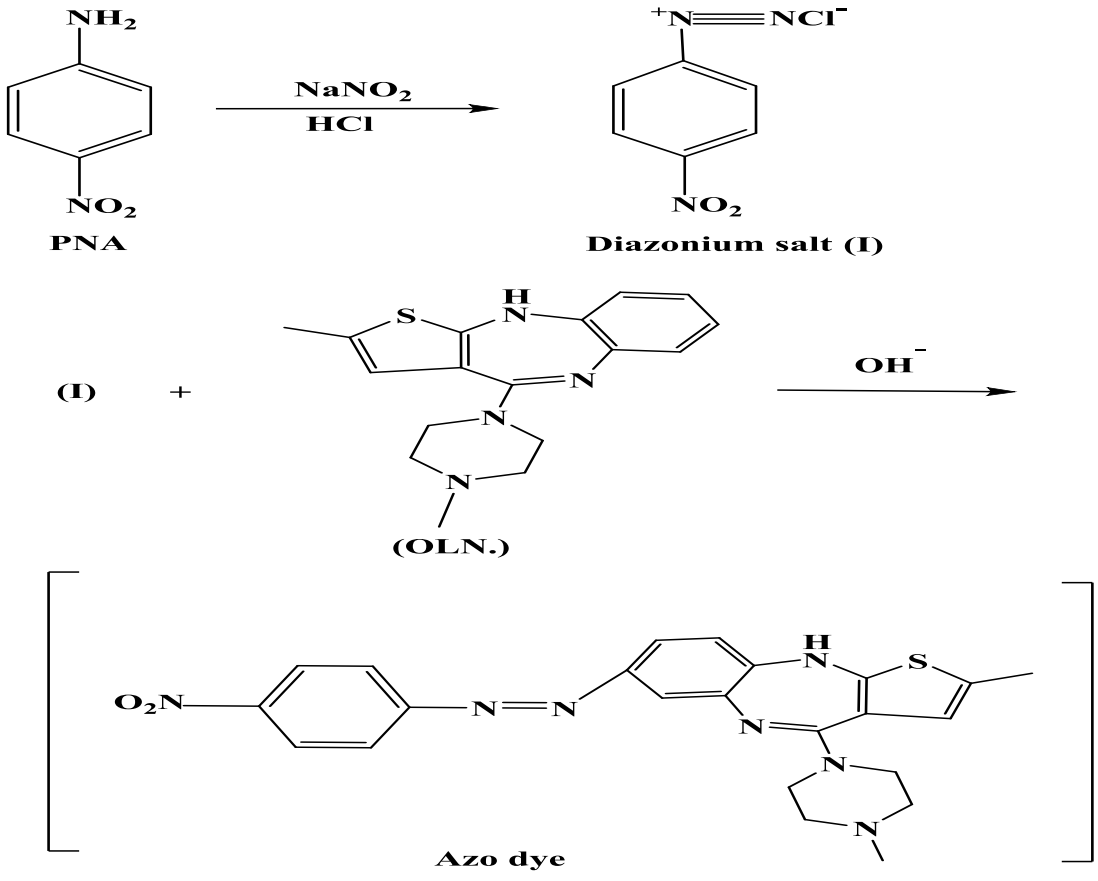


Figure (8): Mole ratio method for (OLN.) with diazotized p-nitroaniline.

Nature of the dye product

Job's method[18] and mole ratio method[19] have been used in the determination of the reaction ratio of (OLN.) with p-nitroaniline reagent. The obtained results in (Figures 7 and 8) showed that 1:1 (OLN.) to diazotized p-nitroaniline reagent ratio is obtained. Hence the azo-dye may have the proposed mec hanism illustrated in (Scheme 2).



Scheme (2):- The suggested reaction mechanism between DPNA and (OLN.).

Comparison of the methods

(Table 5), shows the comparison between some analytical variables of the present method with another spectrophotometric methods in literature.

Precision and accuracy

The precision and accuracy for the determination of (OLN.) via the proposed method were studied by calculating the values of coefficient of variation (C.V%) and percentage of relative error (Er %), for three replicates at three different concentration levels of (OLN.) drug. The results in (Table 6) show acceptable values for accuracy and precision.

Interference study

The effect of various excipients, which may be present in pharmaceutical products and affecting the reaction between (OLN.) and diazotized p-nitroaniline, was studied. Optimum experimental conditions, were employed to determine 10µg.mL^{-1} concentration of (OLN.). (Table 7) shows that the studied excipients did not interfere in the present method.

Application in pharmaceutical preparation

The application of the method for the assay of olanzapine in drugs has been applied successfully, and the results obtained were listed in (Table 8) for each sample in three replicates

Table (5): Analytical parameters for the analysis of olanzapine by the proposed ethodcomparing to the methods.

Methods	Linear range µg.mL^{-1}	(ε) L.mol^{-1}.cm^{-1}	Correlation Coefficient (R)	C.V% range	Ref.
Proposed method	0.5-45.0	1.5777x10^4	0.9994	0.033-0.080	…
Spectrophotometric	4.0-20.0	1.74×104	0.9998	0.355-0.822	6
Spectrophotometric	5.0-160.0	0.60 x 10^3	0.9999	0.820–0.910	7
Spectrophotometric	5.0 -40.0	----	0.9980	0.120-0.590	9
Spectrophotometric	0.4 -8.0	2.08 × 10^4	0.9994	0.669-2.278	20
Spectrophotometric	0.4 -40.0	4.375 × 10^3	0.9999	0.270-0.700	21
RP-UPLC	25.0-150	----	0.9990	0.210-0.330	10
HPLC	2.5-20.0	----	0.9999	0.150-0.460	11

Table (6): Evaluation of accuracy and precision for the determination of (OLN.) by proposed method.

Conc. of (OLN.) µg.mL^{-1}		Er%	C.V%
Taken	**Found***		
10.000	9.951	-0.490	0.458
18.000	18.013	0.072	0.013
32.000	32.046	0.143	0.033

*Average of three measurements.

Table (7): Recovery values for 10 µg.mL^{-1} of (OLN.) in the presence of different excipients.

Excipients		Olanzapine Conc.		Recovery (%)
Name	**Conc. (µg.mL^{-1})**	**Taken (µg.mL^{-1})**	**Found (µg.mL^{-1})**	
Lactose	500	10.000	9.981	99.810
Glucose	500		9.839	98.390
Sucrose	500		9.990	99.900
Starch	1000		9.971	99.710
Magnesium Stearate	1000		9.952	99.520

Table (8): Application to the olanzapine concentration measurement in tablets.

Sample	Weight Found* (mg)	Concentration ($\mu g.mL^{-1}$)		Recovery %	C.V %
		Taken	Found*		
Zyprexa-5 mg	5.026	10.000	10.052	100.523	0.469
	5.027	30.000	30.166	100.553	0.285
Zyprexa-10 mg	10.145	10.000	10.145	101.450	0.514
	10.031	30.000	30.093	100.310	0.107
Olan-5 mg	4.882	10.000	9.947	99.473	0.280
	4.986	30.000	29.917	99.725	0.185

*Average of three measurements.

Conclusion

The proposed method permits rapid, precise, and accurate determination of olanzapine. It makes use of simple reagents, which an ordinary analytical laboratory can afford. The method was found to be free from interference by the excipients. The wide applicability of the new procedure for routine quality control was well established by the assay of olanzapine in pure form and in pharmaceutical preparations.

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