Libyan Journal of Medical and Applied Sciences LJMAS
Online ISSN: 3006-1113
Volume 3, Issue 4, 2025, Page No: 115-122
Website: https://ljmas.com/index.php/journal/index

Using Giemsa Stain for Indirect Spectrophotometric Determination of Olanzapine

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Received: October 05, 2025 Accepted: December 16, 2025 Published: December 19, 2025

Cite this article as: S, A, Zakaria., N, S, Othman. (2025). Using Giemsa Stain for Indirect Spectrophotometric Determination of Olanzapine. Libyan Journal of Medical and Applied Sciences (LJMAS). 2025;3(4):115-122.

Abstract:
In this work, an indirect spectrophotometric method for estimation of olanzapine by oxidation and bleaching reaction in its pure and pharmaceutical form has been described. The principle of the method depended on the oxidation of olanzapine in an acidic medium by a known excess of sodium hypochlorite. The residual amount of oxidant (sodium hypochlorite) is consumed in the decolorization of the Giemsa stain. The absorbance of unreacted Giemsa stain at 622 nm is directly proportional to the amount of olanzapine. Beer's law was obeyed over the concentrations from 0.5 to 7.5 µg .ml-1with a good determination coefficient(R2=0.9973). The molar absorptivity was 4.4579x 104 l.mol-1.cm-1and Sandell´s index was 0.007 µg.cm-2. As for RSD% values, they fall within the range 0.56 to 0.69% depending on the concentration level. The values of LOD and LOQ have been assessed and found to be 0.04204 µg. mL-1and 0.1261 µg. mL-1 respectively, while the range of recovery was found to be 99.14% to 100.20%. A standard addition method for the determination of olanzapine was carried out and the results indicated that there are no interferences were observed from common additives found in pharmaceutical preparation(tablets). The recommended method was successfully applied to assay olanzapine in tablets. A t-test has been studied and the results indicated that no significant difference between the t-test results and tabulated values at 95% confidence level.

Keywords: Giemsa Stain, Olanzapine, Sodium Hypochlorite, Spectrophotometric Method.

استخدام صبغة الجيمسا في التقدير الطيفي غير المباشر للأولانزابين

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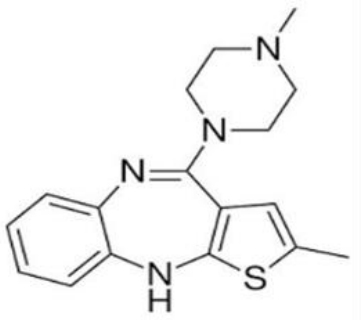
الملخص
في هذا العمل، وُصفت طريقة طيفية ضوئية غير مباشرة لتقدير تركيز أولانزابين في صورته النقية والصيدلانية، وذلك عن طريق تفاعل الأكسدة والتبييض. يعتمد مبدأ هذه الطريقة على أكسدة أولانزابين في وسط حمضي باستخدام كمية فائضة معروفة من هيبوكلوريت الصوديوم. تُستهلك الكمية المتبقية من المؤكسد (هيبوكلوريت الصوديوم) في إزالة لون صبغة جيمسا. يتناسب امتصاص صبغة جيمسا غير المتفاعلة عند 622 نانومتر تناسبًا طرديًا مع كمية أولانزابين. وقد لوحظ تطبيق قانون بير ضمن نطاق تركيز يتراوح بين 0.5 و7.5 ميكروغرام/مل، مع معامل تحديد جيد (R^2 = 0.9973). بلغ معامل الامتصاص المولي 4.4579 × 10^4 لتر/مول. سم، بينما بلغ مؤشر ساندل 0.007 ميكروغرام/سم². أما بالنسبة لقيم الانحراف المعياري النسبي (RSD%)، فهي تتراوح بين 0.56% و0.69% تبعًا لمستوى التركيز. وقد تم تقييم قيم حد الكشف (LOD) وحد الكمية (LOQ)، حيث بلغت 0.04204 ميكروغرام/مل و0.1261 ميكروغرام/مل على التوالي، بينما تراوحت نسبة الاسترداد بين 99.14% و100.20%. تم تطبيق طريقة الإضافة القياسية لتحديد تركيز أولانزابين، وأشارت النتائج إلى عدم وجود تداخلات من الإضافات الشائعة في المستحضرات الصيدلانية. وقد طُبقت الطريقة الموصى بها بنجاح لتحليل أولانزابين في الأقراص. أُجري اختبار t، وأشارت النتائج إلى عدم وجود فرق معنوي بين نتائج اختبار t والقيم الجدولية عند مستوى ثقة 95%.

الكلمات المفتاحية: صبغة الجيمسا، الاولانزابين، هيبوكلوريت الصوديوم، الطريقة الطيفية.

Introduction

Olanzapine (OlAN) is chemically known as 2-Methyl-4-(4-methylpiperazin-I-yl)-1OH-thieno [2,3-b] [1,5] benzodiazepine (Figure1). Slightly soluble in 96% ethanol, practically insoluble in water, and it is solubility in methylene chloride without restriction [1].



$C_{17}H_{20}N_4S$

M.wt: 312.4 g/mol.

Figure1. Chemical structure of olanzapine

OLAN is used to treat depressive episodes associated with bipolar disorder and schizophrenia. Also, it has been used as an antiemetic for the control of chemotherapy-induced nausea and vomiting [2].

The literature review detected that many analytical methods have been reported for determining OLAN in pure and dosage form, including spectrophotometric methods such as diazo coupling method using p-nitroaniline[3], charge–transfer complex with acceptor chloranilic acid[4] and DDQ[5], condensation reaction with p-dimethyl amino benzaldehyde [6], oxidation and bleaching colour of leuco crystal violet[7], and alkali blue 4B dye[8], ion-pair complexes with two sulphone phthalein acid dyes[9], Q-Absorbance Ratio[10], derivative of ratio spectra[11], Also, other types of techniques have been used such as: spectrofluorimetric determination[12], RP-HPLC[13], LC-MS/MS[14], cyclic voltammetry and square wave voltammetry[15], sensor of carbon paste electrode modified with glutamine and gold nanoparticles[16].

Giemsa stain (GES) was named after its discoverer, the German chemist and bacteriologist Gustav Giemsa. GES was used to demonstrate parasites in malaria, and employed in histology due to the high-quality staining of the chromatin and the nuclear membrane [17]. Through the literary survey, pharmaceutical formulations were not identified using GES as a reagent.

The present work involved an indirect spectrophotometric method for the assay of OLAN via an excess of oxidized reagent sodium hypochlorite, then bleaching the colour of GES by residual sodium hypochlorite, the absorbance of a bleached GES is directly proportional to the amount of OLAN solution.

Experimental

Instrumentation

A SHIMADZU UV-VIS spectrophotometer (UV-1900i) with a 1.0 cm light path cuvette has been employed for spectrum analyses, and a BEL-Sensitive balance was used for accurate and precise weight; also, the pH of solutions was measured using a BP3001 pH meter.

Chemicals and Materials

In this research, all chemicals used were of analytical reagent grade. The standard of OLAN was obtained from (SDI, Iraq); Giemsa stain from Sigma-Aldrich, and hydrochloric acid from Scharlau.

Standard Solutions

Standard solution of OLAN, 50 µg/mL, was prepared by dissolving 0.0100 g of pure OLAN in 2 mL of 0.1 M HCl then diluted to 200 mL with distilled water in a volumetric flask.

Giemsa stain solution (GES), 100 µg/mL, was prepared by dissolving 0.01 g of pure dye gradually in 50 ml of absolute ethanol, then completing the volume to 100 mL with distilled water.

Hydrochloric acid approximately, 1M was prepared by diluting 8.26 ml of hydrochloric acid 12.1 M with 100 ml of distilled water.

Sodium hypochlorite solution was prepared by transferring 1 mL of the liquid sodium hypochlorite 6% to a 100 mL volumetric flask, then completing the volume with distilled water to obtain a concentration, which is equal to $3x10^{-3}$M (the correct concentration was detected iodometrically).

Pharmaceutical Preparation Solutions, 50 µg/mL

Ten tablets of each type are weighed, crushed well into powder, and a specific quantity of powdered drugs equivalent to 0.0100g of pure drug is dissolved in 2 mL of 0.1 M HCl, then diluted to 200 mL with distilled water and filtered.

Results and discussion

General procedure

Different volumes of OLAN solution 0.1-1.5 ml of 50 µg/ml were added to a series of 10 mL volumetric flasks then followed by adding 1ml of hydrochloric acid (1 M), then 2 ml of the oxidizing agent sodium hypochlorite was added and waited for 5 minutes, then followed by adding 2mL of GES and wait for 15 minutes, then the absorbance was measured at 662 nm. Fig. 2 explains that the calibration curve follows Beer's law within the range from 0.5 to 7.5 µg/mL, and the value of the molar absorptivity was 4.4579x 10^4 l/mol. cm and Sandell index was 0.007 µg/cm^2.

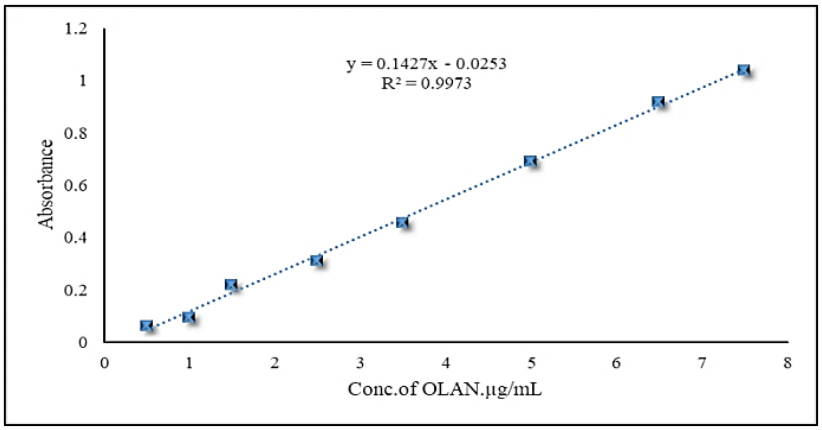


Figure 2. Calibration curve of OLAN

The principle of the method

The method depends on the oxidation of OLAN by adding an excess of sodium hypochlorite in an acidic medium in the presence of hydrochloric acid (1M) as following equations:

The blue colour of GES was bleached by unreacted sodium hypochlorite, and the absorbance of residual GES was measured at 662 nm. The values of absorbance are proportional to the amounts of OLAN (Figure 3).

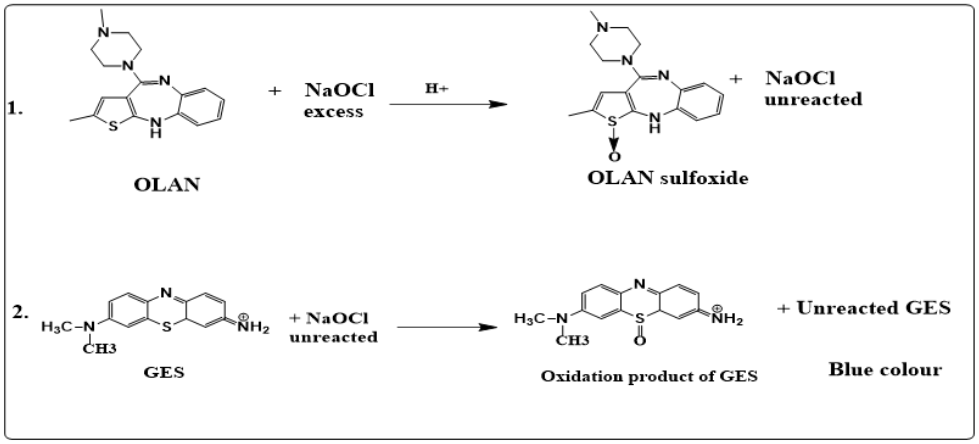


Figure 3. The equations of oxidation of OLAN and GEM.

Optimization of experimental parameters

All variables that affected the intensity of unreacted Giemsa stain were studied to select the optimal results.

Amount of GES

Different volumes of 0.1-2.3 ml of GES (100 µg.ml^{-1}) solution were added to a series of volumetric flasks, which contain 1mL HCl (1M), and the volume was diluted to 10 mL with distilled water (Figure 4).

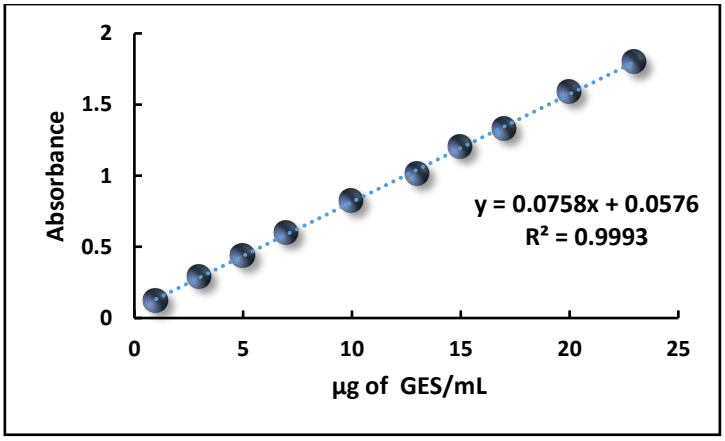


Figure 4. Calibration curve of GES

A 2 mL has been selected as a useful amount for the reaction in the subsequent experiments.

Effect of various types of oxidant reagents

Different types of oxidant reagents such as potassium periodate, potassium dichromate, N-bromosuccinimide, sodium hypochlorite, and potassium permanganate had been tested (see Figure5).

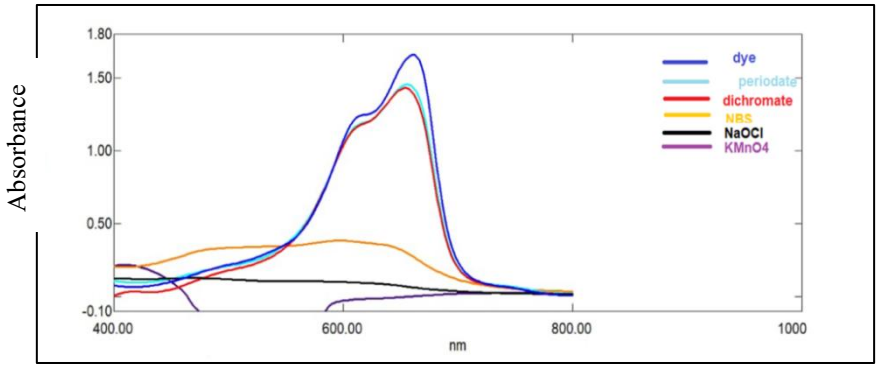


Figure 5. Effect of oxidant type on bleaching colour of GES

The results in Fig.5 showed that potassium permanganate gave the highest bleaching of GES, but it was excluded to give a blue shift in the presence of OLAN, therefore, sodium hypochlorite was suitable oxidant agent and depended on the subsequent experiments.

Selection of the best amount of sodium hypochlorite

0.5-3.0 mL of 0.8 x 10^{-2} M of NaOCl has been added to 2mL of GES in acidic medium without OLAN as shown in Figure 6.

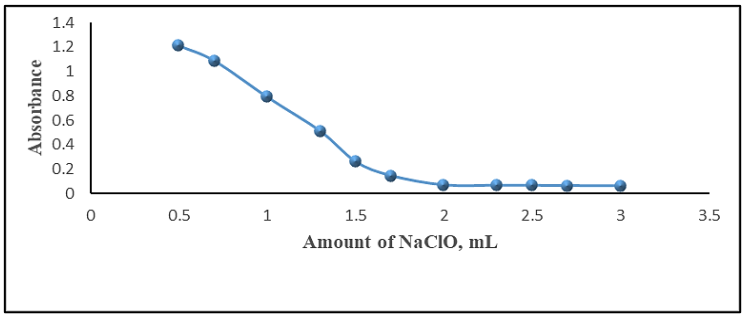


Figure 6. Effect of the oxidant amount

Effect of acid types and amounts

The research results show that an acidic medium is required for the oxidation of GES and OLAN by NaOCl. In order to achieve high sensitivity, the effects of varying acids (1 M) have been studied. HCl proved to be the best acid, as seen in Figure 7.

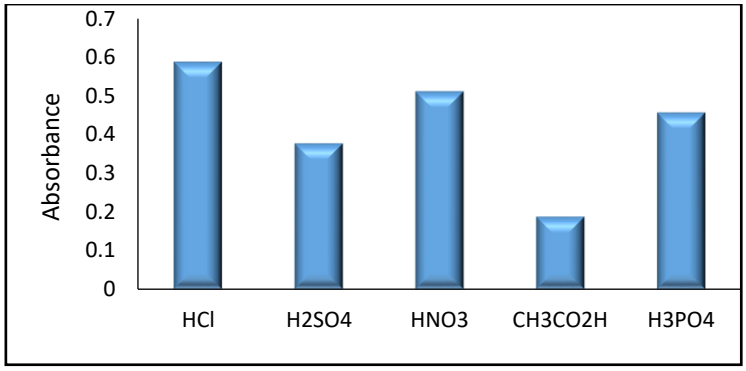


Figure 7. Effect of acid types

In addition,1 mL of 1M HCl was selected as an optimum amount in the subsequent experiments to give the highest absorbance, as shown in Figure 8.

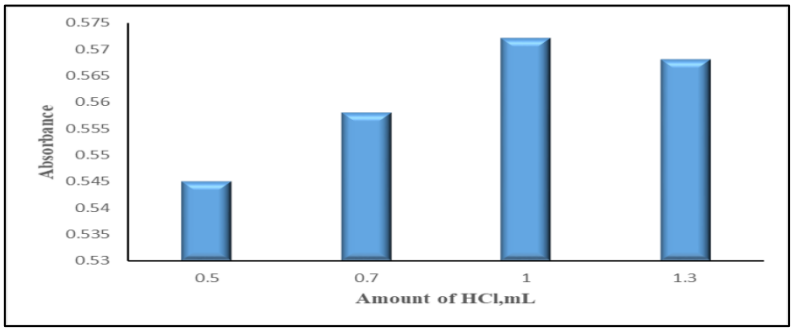


Figure 8. Effect of HCl amount

Effect of Oxidation time

The oxidation time was studied by adding 2 mL of 3 x 10^{-3} M NaOCl to 50 µg/mL of OLAN and 1 mL of 1N HCl. The solution was left for different periods at room temperature. Then 2.5 mL of 100 µg/mL GES was added to the solution and left for different periods, and then diluted with distilled water to the mark, and then the absorbance of GES at a wavelength of 662 nm was measured shown in Table 1.

Table 1. Effect of time on oxidation of OLAN and bleaching colour of GES

Standing time(min.) after adding GES	**Standing time(min.) after adding NaOCl**			
	5	**10**	**15**	**20**
2	**0.540**	**0.546**	**0.528**	**0.495**
5	**0.577**	**0.660**	**0.685**	**0.656**
10	**0.558**	**0.548**	**0.525**	**0.497**
15	**0.488**	**0.521**	**0.515**	**0.482**

The results showed that 15 minutes was selected as a suitable time for oxidizing OLAN and 5 minutes for bleaching the colour of GES.

The effect of additions sequence

Different addition sequences have been done, to select the best order of the addition reaction. The results were shown in Table 2.

Table 2. Effect the sequence of addition

Reaction components	**Order number**	**Absorbance**
OLAN+HCl+NaOCl+GES	**I**	**0.688**
HCl+OLAN+NaOCl+GES	**II**	**0.597**
OLAN+NaOCl+HCl+GES	**III**	**0.680**
OLAN+GES+HCl+NaOCl	**IV**	**0.403**
OLAN+GES+NaOCl+HCl	**V**	**0.607**

The optimal sequence (I) was selected to give the highest absorbance, and it was the same as that determined in previous experiments.

Absorption spectrum

Absorption spectrum at optimum conditions for GES in the presence of 5µg/mL of OLAN and without it (only GES). The results were shown in Figure 9.

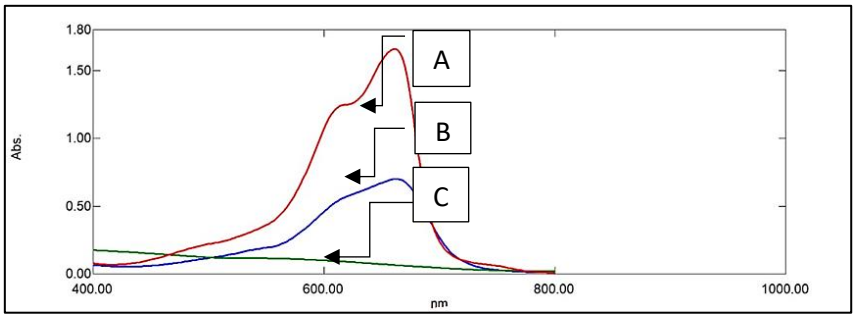


Figure 9. Absorption spectrum of 25 µg/mL GES(A), residual GES after oxidation 5 µg/mL OLAN (B), reagent blank agents distilled water (C)

The results in Figure 8 indicated that the presence of OLAN caused bleaching of GES colour partially depending on the residual NaOCl from oxidized OLAN. The statistical and analytical values for determining OLAN can be summarized in Table 3.

Table 3. Statistical values for determining OLAN

Amount taken (µg/mL)	Amount found (µg/mL)	Recovery*, %	Relative error, %	Relative standard deviation*, %
3.5	**3.47**	**99.14**	**-0.86**	**0.69**
5.0	**5.01**	**100.20**	**+0.20**	**0.56**

Accuracy and precision of the proposed method

A good accuracy and compatibility of the suggested method were obtained by calculating the recovery ratio and the relative standard deviation value, using four replicates of two different concentrations of OLAN solution, as in Table 4.

Table 4. Accuracy and precision of the suggested method

Parameter	Value
Linearity range, µg\ml	**0.5-7.5**
Slope	**0.1427**
Intercept	**0.0253**
Molar Absorptivity, l.mol $^{-1}$.cm^{-1}	**4.4579x 10^{4}**
Sandell index, µg .cm^{-2}	**0.007**
Determination coefficient R^{2}	**0.9973**
LOD, µg.ml $^{-1}$	**0.01261**
LOQ, µg.ml $^{-1}$	**0.04204**

* Average of four determinations

Application part

The current spectrophotometric method was applied effectively for the determination of OLAN in the commercially available pharmaceutical preparation. Drug concentration was evaluated using a direct calibration curve, as in Table 5.

Table 5. Determination of OLAN in tablet

Pharmaceutical Preparation	Amount of OLAN taken, µg.ml^{-1}	Amount of OLAN Found, µg.ml^{-1}	Recovery, *	t-exp.
Rexapin®,5mg/tablet (Abdi Ibrahim Ilac San, Turkey)	3.5	3.47	99.14	2.88
	5.0	4.97	99.40	2.32
Olan®,5mg/tablet (MICRO LABS LIMITED, India)	3.5	3.49	99.71	0.77
	5.0	4.99	99.80	1.09

*Average of four determinations, t-exp.: t-experimental.

According to the above results, the values of t-experimental at the 95% confidence level for four replicates were less than the theoretical value (t=3.18), showing that there is no difference between the certified value and the determined amount. Also, the standard addition method has proven successful in the determination of 0.5 and 1.5 µg/ml of OLAN in tablet solution, as shown in Figure 10 and Table 6.

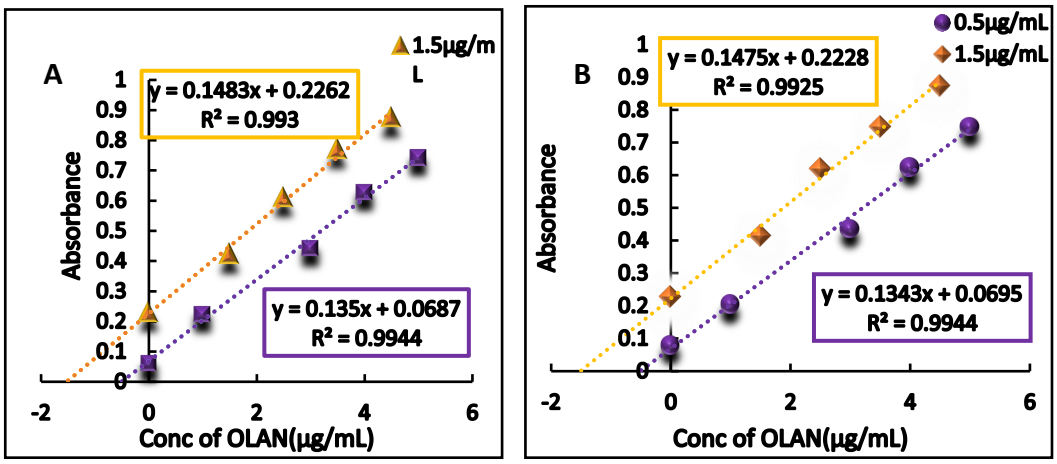


Figure 10. Standard addition plot for determining OLAN in tablet. (A) Rexapin ,(B) Olan

Table 6. Standard addition method

Drug	µg of OLAN present	µg of OLAN measured	Recovery, %	Drug contain measured, mg
Rexapin 5mg/tablet	0.5	0.50	100.00	5.00
	1.5	1.52	101.33	5.06
Olan-5 5mg/tablet	0.5	0.51	102.00	5.10
	1.5	1.51	100.66	5.03

Conclusion

The present study has provided an easy, precise, and sensitive spectrophotometric technique for the evaluation and estimation of olanzapine in its pure form and pharmaceutical product by reacting with Giemsa stain in a bleaching reaction. This reaction does need buffer or surfactant or heating steps and the suggested method was unaffected by the typical interferences found in pharmaceutical formulae. As a result, it might be safely suggested for regular olanzapine analysis.

Acknowledgments

We thank the Head of the Chemistry Department, and the Deanship of the College of Science / Mosul University-Mosul-Iraq for providing the necessary supplies to complete the research.

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