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DEVELOPMENT AND VALIDATION OF A NEW ANALYTICAL METHOD FOR THE ESTIMATION OF OLANZEPINE IN BULK AND IN FORMULATION BY UV SPECTROPHOTOMETRIC METHOD

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ABSTRACT
A simple precise and reliable UV-Visible spectroscopic process was developed for the quantitative determination or estimation of olanzepine an antipsychotic, neuroleptic. This drug was developed a easy, particular and dependable UV spectroscopic method from the quantitative estimation of olanzepine and its validation parameters like linearity, precision, ruggedness, robustness, limit of detection (LOD) and limit of quantification (LOQ) are conducted and standard truthfulness to International conference of harmonization guidelines. Water: HCl (9:1) was elected as the solvent system and UV spectroscopic method determination carried out and at a highest absorption at about 310 nm using above solvent system. In this process linearity of olanzepine was found to be 5-45µg/ml with co-relation coefficient 0.989 respectively. The percentage of relative standard deviation (RSD) value for both intraday and interday precision was less than 1%. The recovery of the drug from the sample was ranged between 99.6-101.1% while estimation the commercial formulation was no interference of excipients and other additives. Hence this method can be used for the routine determination of olanzepine in bulk and pharmaceutical dosage forms and proposed method for study of stability shows that there was applicable degradation found in stress condition of olanzepine.

Key words: UV Spectrometric Method, Olanzepine, Anti-Psychotic.

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INTRODUCTION

Analytical chemistry is a stand for measuring the chemical composition of natural and synthetic materials. The techniques of this science are used to recognize the substances which may be current in a material and to conclude the exact amounts of the notorious substances. Olanzapine and risperidone are two 'atypical' antipsychotics, but they differ in terms of chemical structures, overall receptor binding affinity, animal neuro-pharmacology, pharmacokinetics and

risk/benefit profiles. Olanzepine is second-generation antipsychotic and serotonin-dopamine-antagonist which is structurally related to clozapine, and is classified as a thieno benzodiazepine. Olanzapine is now most often prescribed in the healing of schizophrenia and bipolar affective disorder[1]. Bipolar disorder is a widespread psychiatric illness with a highly variable course and high rates of morbidity and mortality requiring permanent treatment. It has a projected prevalence of 1.6%–3.7%, and is an episodic illness interspersed with erratic cycles of mania and depression or mixed episodes. Substantial advances in the recent years have fostered an expansion in the pharmacological treatment options for bipolar disorder. Best clinical evidence with mood stabilizers including lithium, valproate, lamotrigine, and carbamazepine as well as the atypical antipsychotics are olanzapine, risperidone, quetiapine, ziprasidone and aripiprazole suggests that these agents are more effective for symptoms of mood elevation than for symptoms of depression[2]. Olanzapine is sometimes used to treat problem behaviours in some autistic people, including hyperactivity, aggression, and self-injurious behaviours. Clinical studies and trials suggest that olanzapine is superior to haloperidol and also maybe superior to risperidone in terms of efficacy and side-effect profiles. Final FDA approval was granted in year of October 1, 1996 for the treatment of schizophrenia.

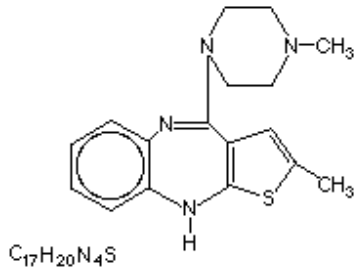

Fig-1 Structure of Olanzepine

IUPAC NAME: (2-methyl-4-(4-methyl-1-piperazynyl)-10*H*-thieno-[2,3-*b*][1,5]benzodiazepine) (Fig-1). It was first synthesized by Eli and Peer review under responsibility of Taibah University Lilly in the United Kingdom in 1982, and the United States Food and Drug Administration agreed olanzapine sold by Eli Lilly under the trademark Zyprexa in late 1996 (1, 2). The olanzapine molecule has high attraction for two receptors in the brain, D_2 dopamine receptors and the $5HT_2$ Serotonin receptor, which are important for maintaining chemical equilibrium in the brain.

Olanzapine exists in five probable polymorphic forms, the polymorphism can be restricted, and the drug remains stable (3). Olanzapine is easily oxidized, and several oxidizing agents have been used, occasionally in combination with chromogenic agents like dyes, to improve spectrophotometric quantification in the visible region. Now UV spectroscopic method was developed and validated as per ICH guidelines. Spectroscopic method is generally favored especially by small scale industries as the cost of the equipment is less and the preserve problems are nominal. Present work based on measuring the absorption of a monochromatic radiance by colourless compounds in the close to UV-path of spectrum (200-380nm).

Materials and methods:

- Apparatus: SHIMADZU S-90 D Digital Balance.
- ELICO SL 210 Double Beam: UV – Visible spectrophotometer with pair of 10mm matched quartz cells.
- Digital P^H Meter by Globe instruments Model: 011G.

METHOD (4-6)

I) Selection of Solvent

The solubility of olanzepine was determined in a variety of solvents as per Indian Pharmacopoeia standards. Solubility test for olanzepine was carried out in different polar and non-polar solvents. From the solubility studies, Distilled water was selected as suitable solvent for proposed method.

II) Preparation of Standard Stock Solution

100 mg of Olanzepine (USP) raw material was accurately weighed and transferred into the 100 ml volumetric flask contain 30ml of 0.1N HCl and sonicated for 15min made up to 100 ml with distilled water and the final concentration was made as 1000µg/ml.

III) Selection of λ_{max}

The standard stock solution was further diluted with distilled water to get 10 µg/ml concentration. The solution was scanned between 200 and 400 nm ranges using distilled water as blank. From the UV Spectra 310 nm was selected as λ_{max} for analysis of olanzepine **(**Fig-

2**).** Stability of the olanzepine in 0.1N HCl was studied by measuring the same solution at this λ_{max} in different time intervals. It was observed that olanzepine was stable for more than 4 hours.

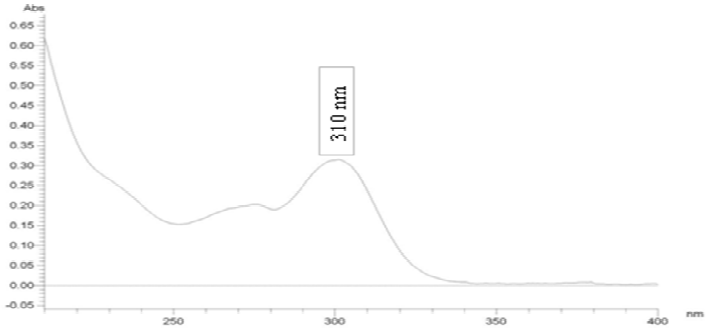

Fig-2 Absorption maxima λ $_{max}$ of OLANZAPINE

DRUG PROFILE

Some fundamental properties

Fundamental properties are given in table-1 and optical characters are given in table-2

Table-1 Fundamental properties

PROPERTIES	SPECIFICATIONS
Formula	$C_{17}H_{20}N_4S$
State and form	Solid and powder
Colour	Yellow
Molecular mass	312.43
Melting point	195^0C (383^0F)
Solubility in water	Practically insoluble in water and slightly soluble in chloroform
Storage and temperature	2-8^0C
P^H sensitivity	Water solubility increase in acidic P^H (<7)
Pk_a constant	Strongest acidic (14.17), Strong basic (7.24)
Protein binding	Approx. 93%
Metabolism	Hepatic
Half-life	12-54 Hrs
Clearance	12-47 L/H
Route	I.M and Oral
Toxicity	Coma (In overdose)
Route of elimination	First pass metabolism

Table-2 Optical characters

PARAMETERS	METHOD VALUES
λ_{max}(nm)	310
Beer's law limit(µg/ml)	5-45
Sandell's sensitivity (µg/cm^2/0.001 AU)	0.021855
Molar absorbtivity(L mol^{-1} cm^{-1})	$1.2810x10^2$
Correlation Co-efficient (R^2)	0.989
Regression equation (y = mx+c)	y=0.003x+0.004
Slope(m)	0.003
Intercept	0.004
% RSD	0.015492

VALIDATION PARAMETERS (7-9)

PREPARATION OF CALIBRATION CURVE:

Calibration Graph: In these aliquots of stock solution of Tizadine hydrochloride (0.5-3.0 ml of 100 µg /ml) were transferred in to 10 ml volumetric flask and made up to the mark with distilled water. The absorbance of different concentration solutions were measured at 268.5 nm against blank. The samples were found to be linear from 5-30 µg /ml. The calibration curve was plotted using concentration Vs absorbance. The curve obtained was linear in the concentration range of 5-30 µg /ml (Fig-3).

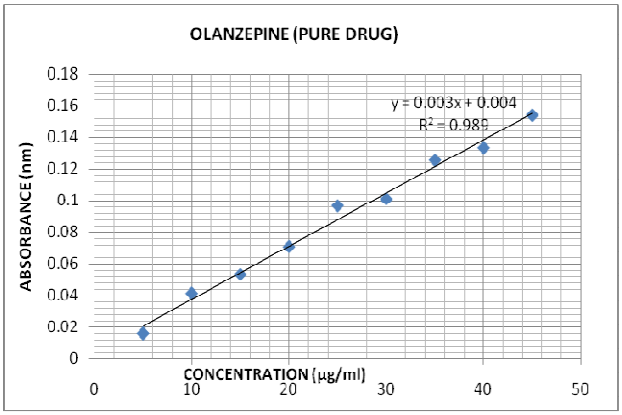

Fig-3 Linearity Curve of Olanzepine by UV Method

Precision Studies for Olanzapine by UV Method

Method precision was demonstrated by intraday and interday variation studies. In intraday variation studies three different solutions of same concentration 10, 20, 30, 40 and 50 µg/ml where analyzed three times in a day and the absorbance was noted. In the interday variation studies, solutions of same concentration 10, 20, 30, 40 and 50 µg/ml where analyzed three times for the three consecutive days and the absorbance result mean, standard deviation, % RSD were calculated (Table-3 and 4).

Table-3 Intra day Precision

Concentration (µg/ml)	Absorbance(nm)			Mean	Standard deviation	% RSD	Average of % RSD
	1	2	3				
10	0.1395	0.1397	0.1399	**0.1397**	0.0002	**0.14**	**0.137**
20	0.1655	0.1653	0.1658	**0.1655**	0.000252	**0.15**	
30	0.1863	0.1869	0.1865	**0.1865**	0.000306	**0.16**	
40	0.2086	0.2084	0.2088	**0.2086**	0.0002	**0.10**	

Table-4 Inter Day Precision

Concentration (µg/ml)	Absorbance(nm)			Mean	Standard deviation	% RSD	Average of % RSD
	1	2	3				
10	0.2150	0.2151	0.2154	**0.2151**	0.00021	**0.10**	**0.395**
20	0.2152	0.2154	0.2158	**0.2154**	0.0003055	**0.14**	
30	0.224	0.223	0.228	**0.225**	0.002645	**1.18**	
40	0.2213	0.2206	0.2208	**0.2209**	0.0003606	**0.16**	

Accuracy Studies of Formulation Olanzepine

Solutions were prepared in triplicate at levels, 50%, 100% and 150% of test concentration using olanzepine working standard as per the method and taken absorbance of each solution in triplicate (Table-5).

Table-5 Recovery Studies

S.No	Recovery	Target in µg/ml	Spiked in µg/ml	Total in µg/ml	Amount found in µg/ml	Recovery percentage
1	50%	30	15	45	44.89	99.6
2	50%	30	15	45	44.91	99.7
3	50%	30	15	45	45.06	100.1
4	100%	30	30	60	59.70	99.6
5	100%	30	30	60	60.09	100.1
6	100%	30	30	60	59.68	99.7
7	150%	30	45	75	74.98	99.9
8	150%	30	45	75	74.65	99.6
9	150%	30	45	75	75.16	101.01

Limit of Detection (LOD) and Limit Of Quantification (LOQ):

Preparation of calibration curve from the serial dilutions of standard was repeated for six times. The limit of detection and limit of quantification was calculated by using the average value of slope(s) and standard deviation of intercept (Table-6)

Table-6 LOD & LOQ of Olanzapine

Limit of detection (µg/ml)	0.33930
Limit of quantification (µg/ml)	1.02820

Quantification of Formulation- Veenat By UV Method

Table-7 Quantification of Formulation

S.No	Labelled Amount (mg/cap)	Amount found (mg/cap)	%Obtained	Average %	S.D	%RSD
1	2.5	2.51	100.40	**100**	**0.015492**	**0.015492**
2	2.5	2.52	100.8			
3	2.5	2.49	99.6			
4	2.5	2.48	99.2			
5	2.5	2.49	99.6			
6	2.5	2.51	100.40			

*Average of six estimations.

Analysis of marketed formulation by UV spectrophotometric method

The percentage of olanzepine in marketed formulation (olanze) was calculated from the calibration curve of olanzepine.

RESULTS AND DISCUSSION

The solubility was determined in a variety of solvent ranging from non polar to polar using essentially a method of Schefter and Higuchi. The drug was found to be freely soluble in distilled water, acetate buffer 4.6, and very soluble in phosphate buffer 6.8, 0.1N HCl Solubility profile is given in Table-1.

100 mg raw material was accurately weighed and transferred into the 100 ml volumetric flask and dissolved in minimum quantity of distilled water and made up to 100 ml with distilled water, resulting in 1000 µg/ml of drug concentration. It was scanned in the range of 200-400 nm and it shows constant λmax at 310 nm this is shown in Fig-2. Stability of the absorbance at their λmax was also checked for up to 2 hours. The linearity of the drug Olanzapine was found, its calibration curve was constructed and is shown in Fig-3 and the optical characteristics such as Beer's law limit (5-45µg/ml), Sandell's sensivity (0.021855), correlation coefficient (0.989), slope(0.003) and intercept(0.004) , molar absorbtivity ($1.2810x10^2$), were calculated and shown in Table-2.

The limit of detection and limit of quantification were determined from the linearity studies. The limit of detection was found to be 0.33930µg/ml and the limit of quantification was found to be 1.02820µg/ml shown in Table-6. Table-7 shows the result of formulation quantification on

olanze tablets repeatability also found to be within the limits 2.48- 2.52 (99.5 ± 1.5), %RSD value 0.015492. To evaluate the accuracy of the method, known amount of pure drug (2, 4 and 6 µg/ml solution) was added to the previously analyzed solution containing pharmaceutical formulation and the mixture was analyzed by the proposed method and the recoveries were calculated. The percentage recovery of Olanzapine sample was found within the limit (99.6-101.1%) and precision was carried from both intraday and interday studies and their % RSD values found to be 0.137 and 0.395 respectively and given in Table-3 & 4**.**

CONCLUSION

The proposed analytical methods are simple, reliable, rapid, sensitive, reproducible and accurate for the estimation of Olanzapine. The method adopted for our studies are Simple UV-Spectroscopic method. The drug samples were analyzed by UV spectroscopy using 0.1N Hcl as solvent and the average content of drug present in the formulation was found to be 2 mg (100 %).The above method do not suffer from any interference due to common excipients. Therefore it was shown that the proposed method could be successfully applied to estimate commercial pharmaceutical products containing Olanzapine. Thus the above studies and result will permit the quantification of the drug for future investigation in the field of analytical chemistry.

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