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RESEARCH ARTICLE

Assay of Olanzapine in Pharmaceutical Formulations by Visible Spectrophotometry using Cadmium (II)

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ABSTRACT

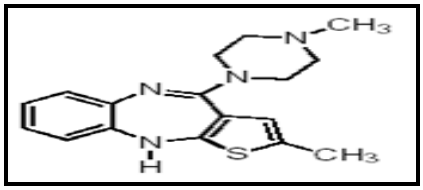
Cadmium (II) reacts with olanzapine in the pH range 8.0-12.0 forming a yellow colored complex solution which has sufficient absorbance at 420 nm. Studies were carried at pH 10.0. The colour intensity attains a maximum value after 30 minutes at 60° C. The straight line relations between absorbance and amount of olanzapine obeys the equation A = 0.0141 C - 0.00004. The linear plot indicates that Beer's law is obeyed in the range 4.0-40.0 µg/ml of olanzapine. The molar absorptivity and sandell's sensitivity are 4.375×10^3 l mol^{-1} cm^{-1} and 0.0714 µg cm^{-2} respectively. The standard deviation of the method for ten determinations of 25 µg/ml olanzapine is 0.0016. The correlation coefficient (γ) of the experimental data of the calibration plot is 0.9999. The effect of various excipients was studied. The composition of the complex is established as 1:2 [Cd (II): olanzapine]. The stability constant of the complex is 9.822×10^{12}. The developed method was validated according to ICH guidelines and was found to be accurate and precise. The validation parameters such as, linearity, accuracy, precision, LOD, LOQ and ruggedness were studied. The proposed method for the quantitative estimation of olanzapine is accurate, precise, highly sensitive and selective, for the estimation of olanzapine in its pharmaceutical formulations. Hence the proposed method is successfully applied for the determination of olanzapine in pharmaceutical formulation.

KEYWORDS

Olanzapine, Cd (II), Visible Spectrophotometry, Method Validation

INTRODUCTION

Olanzapine is 2-methyl-4-(4-methyl-1-piperazinyl)-10H-thieno [2,3-b] [1,5] benzodiazepine. The molecular formula is $C_{17}H_{20}N_4S$ (MW = 312.44). It is a yellow crystalline solid, practically insoluble in water. The chemical structure is



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The thienobenzodiazepine derivative olanzapine a second generation (atypical) antipsychotic agent has proven efficiency against the positive and negative symptoms of schizophrenia. Compared with conventional antipsychotics, it seems to have greater affinity for serotonin 5-TH_{2A} than for dopamine D_2 receptors and an apparently improved tolerability profile.

Olanzapine is a typical antipsychotic drug recently introduced commercially by Eli Lilly. It was introduced in Italy at the end of 1998 and its use is now widespread for the therapy of schizophrenic patients, particularly those who do not respond to classical neuroleptics. Olanzapine is metabolized mainly in the liver,

the main metabolite being the 10-N-glucuronide. The cytochrome P450 system is involved in the formation of other metabolites; the isoforms CYP1A2 and CYP2D6 are involved in the formation of 4'-N-desmethylolanzapine and 2-hydroxymethylolanzapine, respectively. Further investigation of the metabolism of olanzapine would be very important because its bio availability and pharmacokinetis are not completely understood. Olanzapine is administered as Zyprexa tablets, usually at very low dosages (2– 20 mgday-1) and the resulting plasma concentrations of olanzapine and its metabolites are in the few nanograms per milliliter range, very sensitive monitoring methods are, therefore, needed.

An indirect batch spectrophotometric and direct flow injection (FI) visible spectrophotometric methods have been developed for the assay of the novel antipsychotic drug olanzapine[1]. Spectrophotometric determination of olanzapine is reported based on oxidation with N – bromosuccinimide and cerium(IV) sulfate by Anna Krebs et al[2]. The most widely used method for the assay of olanzapine in biological samples and pharmaceuticals are liquid chromatography with ultraviolet[3–4] or electrochemical detection[5–7]. Olanzapine is also determined by liquid chromatography (LC) - tandem mass spectrometry (MS)[8–11], LC – atmospheric pressure ionization MS[12], gas chromatography-MS[13]. Mohamed et al. reported kinetic absorbance methods for determination of olanzipine.[14] Assay of olanzapine by indirect spectrophoometric methods[15-18] are also reported.

The above survey of literature shows no report of a direct and simple visible spectrophotometric method for the assay of olanzapine. This prompted the author to develop simple and direct spectrophotometric method for the assay of olanzapine.

MATERIALS AND METHOD

All chemicals and solvents used were of analytical reagent grade.

Solutions

Cadmium (II) Solution

Requisite quantity of cadmium acetate (Loba Cheme Ltd.) to get 0.01M solution is dissolved in distilled water in a 100 ml standard flask and standardized[19]. Working solution is prepared by diluting the stock solution.

Olanzapine Solution

100 mg of olanzapine is dissolved in dimethyl formamide (DMF) and made up to the mark with same solvent into a 100 ml volumetric flask. The stock solution is diluted as required

Buffer Solutions

Buffer solutions are prepared by adopting the standard procedures reported in the literature[20]. The solutions employed for the preparation are given below.

pH	Constituents
0.5 – 3.0	1 M Sodium acetate + 1 M Hydrochloric acid
3.0 – 6.0	0.2 M Sodium acetate + 0.2 M Acetic acid
7.0	1.0 M Sodium acetate + 0.2 M Acetic acid
8.0 – 12.0	2.0 M Ammonia + 2.0 M ammonium chloride

Instruments Employed

UV-Visible Recording Spectrophotometer (UV – 160A)

Shimadzo Corporation Spectrophotometric Instrument Plant, Analytical Instruments Division, Kyoto, Japan developed a versatile and indigenous microprocessor based UV-Visible recording spectrophotometer (UV-160A).

ELICO Digital pH Meter

ELICO digital pH meter manufactured by M/s ELICO Private Limited, Hyderabad, India is used for measuring the pH of buffer solutions. The instrument has a temperature compensate arrangement. The reproducibility of measurements is within ± 0.01 pH.

Procedure

A known number of tablets are weighed and ground to a fine powder. A portion of the powder containing 100 mg of the active component is accurately weighed into a 100 ml calibrated flask; 60ml of distilled water are added and shaken thoroughly for about 20 minutes to extract the drug. The contents are diluted to the mark, mixed well and filtered using quantitative filter paper to remove the insoluble residue. The filtrate is diluted to get required concentration of drug.

Absorbance Spectrum

The absorption spectra of the cadmium(II)solution and olanzapine solution in buffer solution of pH 10.0 and that of the experimental solution containing solutions of the cadmium(II), olanzapine and the buffer (pH 10.0) against the buffer blank are recorded in the wavelength range 300-600nm. The spectra presented in Fig.1 show that the complex has maximum absorbance at 420 nm. Hence, analytical studies were carried out at 420 nm.

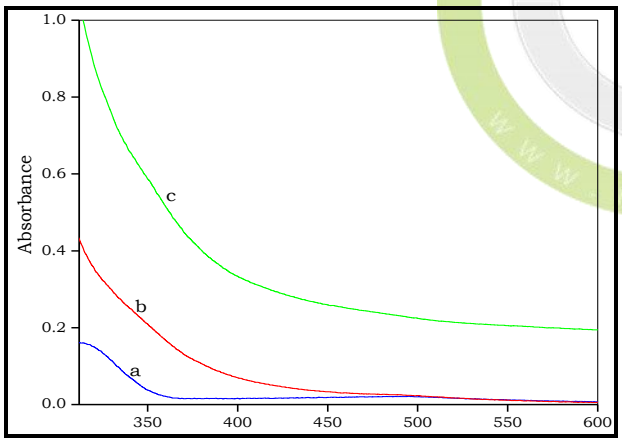

Figure 1: Absorption spectra of a) Cd (II) vs. buffer blank b) OZP vs. buffer blank; c) Cd (II) – OZP vs. buffer blank [Cd (II)] = 5.0 x 10^{-3}M; [OZP] = 1.0 x 10^{-4}M

Assay of Olanzapine

The present method for the determination olanzapine is applied for its determination in a pharmaceutical sample. A known aliquot of pharmaceutical sample solution of olanzapine is added to a 10ml volumetric flask containing 5 ml of buffer solution of pH 10.0 and 1 ml of cadmium (II) [5 x 10^{-3}M] solution. The contents are made up to the mark with distilled water. The absorbance is measured at 420 nm against the cadmium (II) blank after heating the experimental solution to 600C for 30 minutes and cooling it to room temperature. The amount of olanzapine is computed from the predetermined calibration plot at 420 nm.

Order of Addition of Constituent Solutions on the Absorbance of the Experimental Solution

The order of addition of the various constituent solutions, the buffer solution, cadmium (II) solution and olanzapine solution is studied by measuring the absorbance of the experimental solution at 420 nm. The result reveals that the absorbance remains unchanged irrespective of the order of addition of various constituent solutions. Hence, it is not necessary to follow a particular order of addition of the constituents of the experimental solution.

Effect of Surfactants on the Absorbance

To a series of experimental solutions containing buffer solution of pH 10.0, cadmium(II)solution and olanzapine solution, an aliquot of the known percentage of different surfactant (S.D.S, Triton – X 100, C.P.C) solutions is added and the absorbance is measured at 420 nm after heating the experimental solution to 60^0C for 30 minutes and cooling it to room temperature. The study reveals that the absorbance of the solution remained the same in all cases indicating that surfactants have no effect on the absorbance.

Effect of Temperature on the Absorbance of Experimental Solution

To a series of 10 ml volumetric flasks containing 5 ml of the buffer solution pH 10.0, requisite volumes of known concentrations of metal ion and drug solution are added and made up to the mark with distilled water. Sets of blank and the experimental solution are heated to different temperatures for 30 min and cooled and the absorbance measured at 420 nm wavelength. The results are presented in Table-1. The results in Table-1 indicate that the absorbance attains maximum value at 60^0 C.

Table 1: Effect of temperature on the absorbance of the experimental solution

[olanzapine] = 2 x 10^{-4}M pH = 10.0

[cadmium(II)] = 5 x 10^{-3}M λ = 420 nm

Temperature (^{0}C)	Absorbance
40	0.225
50	0.345
60	0.461
65	0.460
70	0.455

Hence, the absorbance is measured after heating the experimental solution to 60^{0}C for 30 minutes and cooling it to room temperature.

Effect of Excipients

Various amounts of excipients that are generally associated with the olanzapine in its pharmaceutical formulations are added to a fixed amount of olanzapine (25µg/ml) solution and the absorbance measurements are carried out under optimal conditions. The concentration (µg/ml) at which various ions do not cause an error of more than ± 4% in absorbance is taken as the tolerance limit and the results are given in Table-2.

Table 2: Tolerance limit of excipient

Amount of OZP = 25.0 µg/ml pH = 10.0

Excipient	Tolerance limit (µg/ml)
Fructose	1363
Glucose	952
Sucrose	1486
Lactose	1847
Gelatin	1964
Starch	1544
Sodium Alginate	1437
Boric Acid	2053
Magnesium stearate	1710

The results indicate that the excipients that are normally associated with olanzapine do not interfere even in large quantities in the determination of olanzapine making the method highly selective and direct.

RESULTS AND DISCUSSION

Olanzapine reacts with Cd (II) in the pH range 8.0-12.0 forming a yellow colored complex solution. The absorption spectra (Fig-1) of the yellow colored Cd (II) – Olanzapine complex and that of olanzapine alone show that the difference in absorbance between the two is maximum at 420 nm. At this wavelength either Cd (II) has no absorbance. The color intensity of the complex is maximum at pH 10.0 Hence studies were carried at pH 10.0. The color formation attains maximum intensity after 30 minutes at 60^{O}C. There after the color of the complex remains stable for more than 24 hours. A fivefold molar excess of Cd (II) is sufficient to produce maximum absorbance. The absorbance varied linearly with the concentration of olanzapine. Beer's law is obeyed in the range 4.0-40.0 µg/ml of olanzapine. The straight line plot obeyed the equation A = 0.0141 C - 0.00004. Optical characteristics and regression data presented in Table-3.

Table 3: Optical and regression characteristics of the Proposed method for Olanzapine

Parameter	Olanzapine
Analytical Wavelength (nm)	420
Beer's law limits (µg/ml)	5.0 – 40.0
Limits of detection (µg/ml)	0.3754
Limits of quantization (µg/ml)	1.1262
Molar absorptivity (l.mo1^{-1}cm^{-1})	4.375 x 10^3
Sandell's Sensitivity (µg/cm^2)	0.0714
Regression equation (y= a + b x)	
Slope (b)	0.0141

Intercept (a)	-0.0004
Correlation coefficient (γ)	0.9999
Standard deviation (Sd)	0.0016

The method was applied successfully for the assay of olanzapine in pharmaceutical formulation the data are presented in Table-4.

Method Validation and Statistical Analysis

The developed method was validated as per official specifications of ICH[21]. The validation parameters were found to be accurate and precise. Statistical results are expressed in terms of, mean ± SD, %RSD and student t-test values are calculated with aid of Excel-2007.

Table 4: Assay of olanzapine in pharmaceutical formulation

Sample (Manufacturer – Formulation)	Label Claim (mg)	Amount found *(mg)	Error (%)
BRAND-I (OLANDUS-Zydus cadila Health Care Ltd-Tablet)	2.50	2.48	-1.00
BRAND-II (OLEX-Cipla, Ltd, – Tablet)	2.50	2.55	2.00

*Average of Six determination

Table 5: Intra- and Inter- day precision studies of olanzapine (n=3, p=0.05)

Conc. (µg/ml)	Mean absorbance ± SD		% RSD		Calculated value of t
	DAY-1	DAY-2	DAY-1	DAY-2	
20	0.286±0.001	0. 283±0.002	0.70	0.54	0.091
30	0.430+0.002	0.428+0.001	0.48	0.36	0.125
40	0.570+0.001	0.568+0.002	0.27	0.44	0.851

Table 6: Recovery studies for tizanidine in tablets

Tablet	Amount of sample(µg/ml)	Amount of drug added (µg/ml) Amount	Amount Recovered(µg/ml)	%Recovery ±SD
BRAND-I (OLANDUS-Zydus cadila Health Care Ltd-Tablet)	20	20	39.20	98.00 ±0.002
	20	30	50.14	100.20±0.002
	20	40	59.20	98.6+0.003
BRAND-II (OLEX-Cipla, Ltd, – Tablet Tablet)	30	20	49.48	98.96 ±0.001
	30	30	60.10	100.31±0.002
	30	40	71.12	101.6±0.002

Table 7: Ruggedness studies for the tizanidine in tablets

Tablet	Analyst- I			Analyst- II	
	Label Claim (mg)	Amount found* (mg)	(%) Recovery ±SD	Amount found *(mg)	(%) Recovery ±SD
BRAND-I	2.50	2.48	99.20±0.001	2.51	100.40± 0.001
BRAND- II	2.50	2.52	100.80±0.001	2.49	99.60 ±0.002

*Average of Six determination

Differences were considered significant at the 95%confidence limit. Repeatability of the method was verified by intraday and inter day precision studies (Table-5).

Accuracy of the method was studied by recovery studies and the results are summarized in Table-6, Ruggedness studies were carried out by changing the analyst and the results were shown in Table-7.

CONCLUSION

The proposed method for the determination of olanzapine is a simple, visible spectrophotometric procedure which is not only fairly rapid, precise and sensitive but also is within the reach of an ordinary clinical laboratory.

The linearity parameter and the corresponding regression data indicated excellent linear relationship (γ =0.9999). Survey of literature shows no report of a simple, sensitive visible spectrophotometric procedure for the estimation of olanzapine.

Other methods reported for its determination either use costly instrumentation or suffer from interference of excipients.

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