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Validated UV Spectrophotometric Methods for the Estimation of Olanzapine in Bulk, Pharmaceutical Formulations and Preformulation Studies

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Authors' contributions

This work was carried out in collaboration between all authors. Authors EJ and RNS designed the study. Authors EJ, GB, VN and SR managed the literature searches and experimental procedures. Author EJ wrote the first draft of the manuscript which was corrected by author RNS. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aims: To develop and validate two accurate and precise UV spectrophotometric methods for the estimation of widely prescribed anti psychotic drug-Olanzapine in bulk, formulations and preformulation studies.
Methodology: Two different solvent systems optimized for the analysis (100 mM hydrochloric acid with pH 1.2 and phosphate buffer saline with pH 7.4), were based on various criteria such as applicability of the method, sensitivity, ease of sample preparation, cost effectiveness, stability of drug in investigating medium, and reproducibility. Olanzapine was estimated at 258 nm in 100 mM hydrochloric acid and 252 nm in phosphate buffer saline.
Results: The linear regression equations obtained by applying least square regression analysis for Olanzapine were found to be: Absorbance = 0.0697 × concentration in $\mu g ml^{-1}$+ 0.008; r^2= 0.9999)

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in 100 mM hydrochloric acid; absorbance = 0.0571 × concentration in μgml^{-1}- 0.0052; r^2 = 0.9998) in the phosphate buffer saline. Sandell's sensitivity was found to be about 0.0144 μgcm^{-1} for hydrochloric acid and 0.0175 μgcm^{-1} for phosphate buffer saline. The apparent molar absorptivity was calculated to be $2.17x10^4$ $mol^{-1}cm^{-1}$ and $1.78x10^4$ $mol^{-1}cm^{-1}$ in hydrochloric acid and phosphate buffer saline respectively.

Conclusion: Two spectrophometric methods for the analysis of Olanzapine have been developed successfully and validated as per ICH guidelines. Developed methods were successfully applied for the determination of Olanzapine in commercial tablets of varying strengths and various preformulation parameters of the drug such as dissociation constant.

Keywords: Olanzapine; ultra violet spectrophotometry; validation; formulations; preformulation studies.

1. INTRODUCTION

Olanzapine (OLN) is considered as one of the widely useful psychiatric drug for effective treatment of schizophrenia and related disorders [1]. It is a very promising drug widely prescribed by physicians as an integral part of psychiatric treatment, due to its efficiency in controlling both positive and negative symptoms of schizophrenia with fewer side effects, which is a general quality observed with most of the atypical anti psychotics as compared to typical ones such as Haloperidol. OLN is a thienobenzodiazepine derivative which closely resembles another well known atypical antipsychotic-Clozapine. The IUPAC name of OLN is 2-methyl-4-(4-methyl-1-piperazinyl)-10H-thieno [2,3-b] [1,5] benzodiazepine. The mechanism of anti psychotic action of Olanzapine is still unclear, but it has been postulated that 5 HT receptor antagonistic action is primarily responsible for its antipsychotic effect. It is also believed that fewer extrapyrimidal adverse effects of atypical anti psychotics such as Olanzapine are due to its higher affinity towards 5 HT receptors than to dopaminergic receptors. Because of its significant use in psychiatry, numerous formulations are available in market and numbers are increasing day by day. Therefore, simple, less time consuming and cost effective analytical methods for the estimation of Olanzapine in bulk and various dosage forms are necessary for the routine analysis.

UV spectrophotometric method is among the top most cost effective analytical methods and extensive literature survey revealed few spectrophotometric methods for the estimation of Olanzapine [2,3]. One method [2] is developed in 100% organic solvent (methanol) and therefore is highly expensive for day to day analysis. Also, volatile nature of organic solvent would result in loss of medium, thereby change in concentration of the drug, thus affecting the accuracy of the method reported. Another method reported [3] gave more importance to the degradation study than validation and assay, even though degradation results obtained by UV spectrophotometric methods are less acceptable since identification of degradation products are highly difficult by these methods. Studies of significant importance for formulation scientists such as preformulation studies are not considered during the applicability of any of the reported methods. Further, various hplc methods in both aqueous and bio matrices have been reported for the estimation of Olanzapine elsewhere in literature [4-9]. These methods are very time consuming with long run times, use expensive organic solvents and require complicated treatment techniques for the analysis. Furthermore, sophisticated instruments and highly skilled persons are required for carrying out these reported analytical methods. All these factors make these methods less suitable for the routine analysis of Olanzapine in bulk, formulations or preformulation studies. The present study describes two simple, cost effective, reliable and less time consuming UV spectrophotometric methods for the estimation of Olanzapine in bulk, formulation and preformulation studies. The developed method was also validated as per International Conference on Harmonization (ICH) guidelines [10] and could be applied directly for the day to day analysis of Olanzapine. Suitable statistical tests were performed to check validity of the developed methods [11].

2. MATERIALS AND METHODS

2.1 Instruments

Ultra violet - visible - near infra red - spectrophotometer (Jasco, Japan, Model V-570) connected to computer, loaded with software (spectra manager) was used. The wavelength accuracy was 0.1 nm and for the analysis,

matched quartz cuvettes of 10 mm were used. For carrying out robustness study, a double-beam ultra violet - visible - spectrophotometer (Perkin Elmer, USA, model LAMBDA EZ210) connected to computer loaded with software (PESSW, Version 1.2 and Revision E) was used.

2.2 Materials

OLN was gifted by IPCA labs limited, Mumbai, India. Potassium dihydrogen phosphate, disodium hydrogen phosphate and sodium chloride were purchased from S.D. Fine Chemicals, India. Methanol and hydrochloric acid were purchased from Merck, India. High quality pure water was obtained using Millipore purification assembly (Millipore, Molsheim, France, Model Elix SA 67120).

2.3 Analytical Method Development

In order to develop a meaningful and sensitive analytical method for the estimation of Olanzapine, numerous media consisting of different buffers and organic solvents: Either alone or in combinations at different proportions was investigated. Absorbance of Olanzapine in various media at respective wavelengths was determined and apparent molar absorptivity and sandell's sensitivity were calculated and was also considered while selecting the optimized solvent system. Furthermore, the cost effectiveness and reproducibility of the solvent system was also considered during selection procedure.

2.4 Calibration Standards

Stock solutions of 100 μgml^{-1} of Olanzapine were prepared by dissolving 5 mg of the drug in 50 ml of 100 mM HCl and 100 mM phosphate buffer saline (PBS) in separate volumetric flasks. From these solutions, different aliquots were withdrawn and transferred to a series of 10 ml volumetric flasks. The volume was made up with respective medium to obtain six different concentrations in a range of 3-18 μgml^{-1} for hydrochloric acid medium (pH 1.2) and 4-24 μgml^{-1} for phosphate buffer saline medium (pH 7.4) respectively. The spectrum was recorded from 400 nm to 200 nm with scanning speed of 400 nm per min and optimum wavelengths were selected. These selected wavelengths were further set in fixed wavelength measurement mode of the spectrophotometer and absorbance was measured in order to obtain calibration curve data.

2.5 Analytical Method Validation

2.5.1 Specificity

In order to determine the specificity of the developed methods, Olanzapine solutions (10 μgml^{-1}) were prepared along with various pharmaceutical excipients such as lactose, micro crystalline cellulose, starch, hydroxypropyl methylcellulose (HPMC), methyl cellulose, dextrose, magnesium stearate, talc etc. and spectrum was recorded from 400 nm to 200 nm at a scanning speed of 400 nm per min. The spectrum of fresh Olanzapine solution with same concentration was also recorded and any change in the absorbance in the whole wavelength range was studied. The spectrum of fresh Olanzapine solution (10 μgml^{-1}) was overlaid with spectrum obtained from the same concentration of commercial dosage form (tablet) of Olanzapine.

2.5.2 Accuracy

The accuracy of the proposed methods was determined by standard addition method. Different concentrations of pure drug (2, 5 and 10 μgml^{-1}) were added to previously analyzed formulation samples of equal concentration (5 μgml^{-1}). The concentration of drug added was calculated after analyzing the final solution obtained using respective analytical method. The % analytical recovery was studied in each case using the formula: $R = [(Ct - Cf)/Ca] \times 100$, where R is % analytical recovery; Ct is the total drug concentration measured after standard addition; Cf is the initial drug concentration taken from formulation; and Ca is the drug concentration added to pre-analyzed formulation.

2.5.3 Precision

In order to study the precision of developed analytical methods, three different quality control standards (LQC, MQC and HQC) covering the whole calibration range were selected. For the method with 100 mM HCl medium, LQC, MQC and HQC selected were 3, 9 and 18 μgml^{-1} respectively whereas for PBS medium, these were 4, 12 and 24 μgml^{-1} respectively. With these selected QC standards, precision of the developed methods were studied at two levels: intra-day repeatability studies and inter-day intermediate precision studies. In intra-day repeatability studies, quality control standards in triplicate were analyzed at three different times in the same day and percent relative standard deviation (% RSD) was calculated to study the

repeatability of developed methods. Inter-day precision studies were performed in a similar way, on three consecutive days (n=27) instead of a single day and % RSD was determined.

2.5.4 Linearity

The linearity of the proposed methods were determined by analyzing six different concentrations (n=9) prepared from the stock solution and applying least square regression analysis on the obtained data. The concentration range selected for linearity study was 3 to 18 μgml^{-1} for HCl medium and 4 to 24 μgml^{-1} for PBS medium. One way ANOVA test was applied to the obtained absorbance data of the each concentration of the selected calibration range in replicates.

2.5.5 Detection and quantitation limit (DL & QL)

DL & QL of the developed methods were calculated as per standard formula, 3.3 σ/S and 10 σ/S, respectively, where the terms S and σ are slope of the standard calibration curve and standard deviation of the y-intercept of calibration equation respectively [10].

2.5.6 Robustness

Robustness of the proposed methods were studied by minutely changing an internal parameter such as pH (± 0.1 units) of the selected medium and measuring the absorbance in order to study the effect of these minor changes on the results obtained. Robustness was expressed as mean absolute recovery.

2.5.7 Ruggedness

Ruggedness of these proposed methods were studied with the help of another double-beam ultra violet - visible - spectrophotometer (Perkin Elmer, USA, model LAMBDA EZ210) connected to computer loaded with software (PESSW, Version 1.2 and Revision E). The concentrations determined for same solutions by both the instruments were compared during the ruggedness study.

2.5.8 Bench top stability study

The stock solution of Olanzapine was kept in room temperature for 24 hr. The concentration of drug was estimated after suitable dilutions and compared with fresh Olanzapine solutions of same concentration. Percentage analytical recovery was calculated and stability of the drug in proposed medium was determined.

2.6 Estimation from Formulations

The developed and validated spectrophotometric methods are applied for the estimation of Olanzapine from conventional, well established dosage form such as tablets of varying strengths. Twenty Olanzapine tablets of reputed brand were purchased from local market and pulverized with the help of a mortar and pestle into fine powder. From this, powder equivalent to 10 mg of Olanzapine was transferred to 100 ml volumetric flask sand extracted for 30 min with respective solvent systems along with methanol as co solvent. This was filtered using whatman filter paper (no - 40) and the filtrate obtained was diluted suitably to get a concentration of $10\mu gml^{-1}$ before analyzing by developed methods.

2.7 Preformulation Dissociation Constant (pKa) Studies

The dissociation constant (pKa) was also determined spectrophotometrically by the proposed method. Various buffers of different pH in the range of 1-12 were prepared using 0.2 M solutions of sodium dihydrogen phosphate, potassium dihydrogen phosphate and sodium chloride in order to obtain final buffer molarity and ionic strength of 0.01 M and 0.02 M respectively. The final pH of the resultant solutions was adjusted with 0.1 N NaOH/HCl as per the requirements. OLN primary stock solution of 100 μgml^{-1} was prepared. From this stock solution, an accurate volume was transferred into separate calibrated volumetric flasks and further made up the volume with media of varying pH to obtain a concentration of 10 μgml^{-1} which is in the calibration range of the proposed method. The spectrums of the obtained solutions were recorded for the UV wavelength range of 200-400 nm at a scanning speed of 200 nm/sec. Spectrums recorded were further overlaid and the wave length at which a largest change in absorbance in spectrums of Olanzapine at different pH was selected. The absorbance values obtained at this selected wavelength were plotted against pH of the prepared solutions. The pKa was determined by plotting a first derivative absorbance spectrum with ΔAbs/ΔpH Vs pH and the point of inflection in the curve was identified.

3. RESULTS AND DISCUSSION

3.1 Analytical Method Development

Numerous media consisting of different aqueous systems such as 100 mM hydrochloric acid, 100 mM sodium hydroxide, phosphate buffers of pH 5.8–8.0 and acetate buffers of pH 3.6 to 5.8 were investigated alone or in combination with organic solvents at different proportions. Presence of organic solvents such as methanol, acetonitrile etc. did not show any significant change in sensitivity of the methods investigated and therefore it was decided to minimize use of organic solvents and limit them as co-solvents with minimum usage, since it would result in reduction of analysis cost. Also, Olanzapine showed pH dependent absorbance and hence pH of the solvent is of important concern in the selection and preparation of media. Final selection of the optimized solvent systems, 100 mM HCl and PBS pH 7.4 were based on various criteria such as applicability of the method, sensitivity, ease of sample preparation; cost effectiveness, stability of drug in investigating medium, reproducibility and ability to cope with minute changes in the medium (e.g. pH) without affecting the results. The spectra of Olanzapine in the hydrochloric acid medium and phosphate buffer saline medium are shown in Fig. 1.

From the spectrum analysis, the λmax of Olanzapine in 100 mM hydrochloric acid medium and phosphate buffer saline were found to be 258 and 252 nm, respectively. Sandell's sensitivity of Olanzapine in hydrochloric acid medium and phosphate buffer saline medium were found to be 0.0144 and 0.0175 μgcm^{-2}/0.001 A respectively. It was also found that absorption spectrums of Olanzapine solutions after 24 hr were overlaying with fresh solutions in both the media without any significant change. Further, apparent molar absorptivity of the drug was calculated to be $2.17x10^4$ $mol^{-1}cm^{-1}$ in 100 mM hydrochloric acid medium and $1.78x10^4$ $mol^{-1}cm^{-1}$ in the phosphate buffer saline medium [Table. 1].

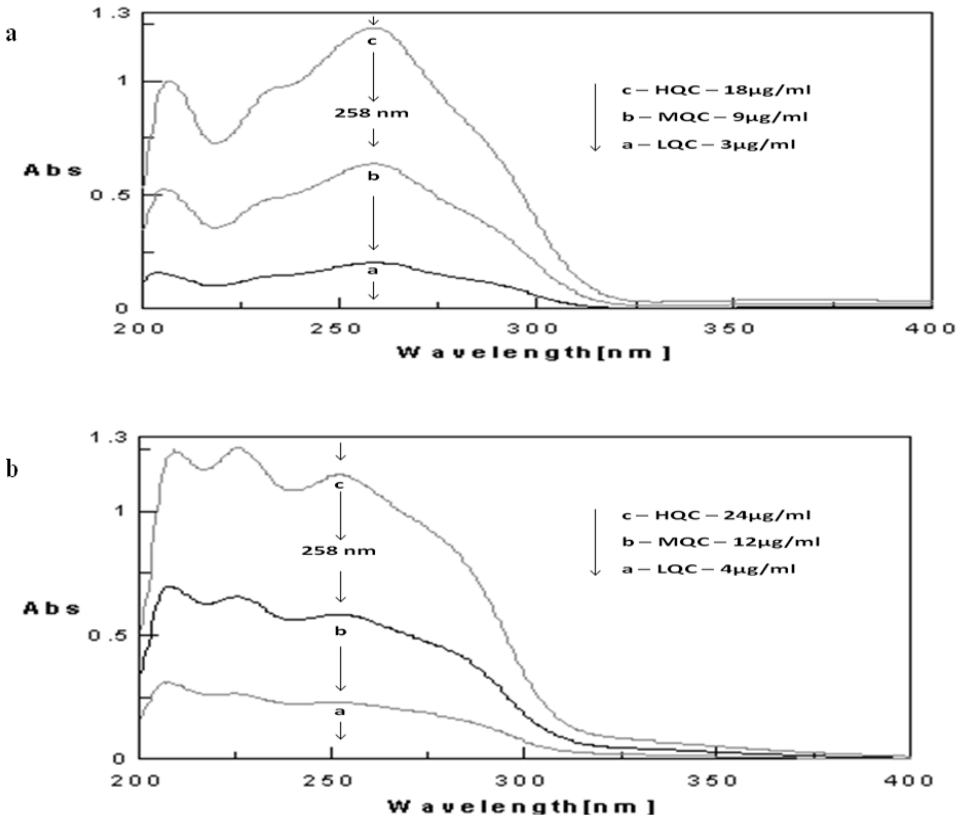

Fig. 1. Overlaid spectrum of pure Olanzapine at LQC, MQC & HQC levels in 100mM HCl buffer (a) and phosphate buffer saline (b)

3.2 Calibration Curve

The linear regression equations obtained by applying least square regression analysis for Olanzapine were found to be: Absorbance = 0.0697 × concentration in μgml^{-1}+ 0.008; r^2= 0.9999) in 100 mM hydrochloric acid medium; absorbance = 0.0571 × concentration in μgml^{-1}- 0.0052; r^2 = 0.9998) in the phosphate buffer saline medium.

3.3 Analytical Method Validation

3.3.1 Specificity and selectivity

The presence of commonly used excipients did not show any interference with the absorbance of Olanzapine. There was no change in the UV-spectra of drug in the presence of commonly used excipients in both the media investigated. Absorption spectrum obtained for pure Olanzapine was almost identical with the spectra of marketed formulation in both the media under investigation [Fig. 2].

All these results indicate that developed methods are highly specific and selective for Olanzapine in the presence various commonly used excipients, therefore can be applied for different dosage forms with wide range of excipients.

3.3.2 Accuracy

The accuracy of the proposed methods was studied by standard addition method by evaluating the recovery of the spiked pure drug samples [Table 2]. The mean percentage recoveries (% R.S.D.) obtained in the hydrochloric acid medium after spiking three different levels of pure drug were found to be between 99.86±0.78 and 101.21±0.54%. The mean percentage recoveries (% R.S.D.) in phosphate buffer saline were found to be between 100.26±1.07 and 101.51±0.83%. These results reveal that any minute addition in the drug concentration in the prepared solution can be determined accurately by both the developed methods and therefore both these methods are found to be highly accurate as per ICH guidelines.

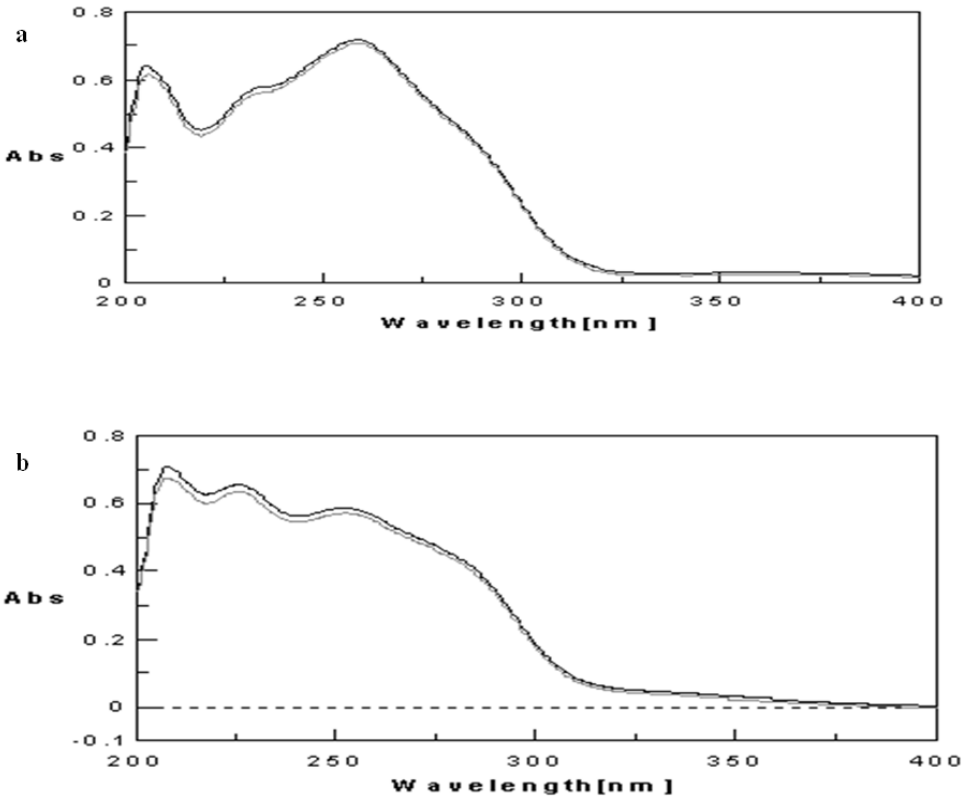

Fig. 2. Overlaid spectrum of pure olanzapine with marketed commercial sample in 100 mM HCl buffer with pH 1.2 (a) and phosphate buffer saline (b)

Table 1. Optical characteristics, summary of statistical data, and validation parameters for Olanzapine

Parameter	Hydrochloric acid medium (pH 1.2)	Phosphate buffer saline medium (pH 7.4)
Apparent molar absorptivity (l $mol^{-1}cm^{-1}$)	2.17 x 10^4	1.78 x 10^4
Sandell's sensitivity (µg cm^{-2}/0.001A)	0.0144	0.0175
Calibration range	3-18 μgml^{-1}	4-24 μgml^{-1}
Slope (S.E.[a])	0.0697 (1.6x10^{-4})	0.0571 (1.3x10^{-4})
95% confidence limits of slope	0.0693 to 0.0700	0.0568 to 0.0574
Intercept (S.E.[a])	0.0080 (8.04x10^{-3})	-0.0052 (2.01x10^{-3})
95% confidence limits of intercept	0.0044 to 0.0116	-0.0092 to -0.0011
Regression coefficient (r^2)	0.9999	0.9998
Detection Limit (µg ml^{-1})	0.1680	0.2018
Quantitation Limit (µg ml^{-1})	0.5091	0.6115

[a]*Standard error of mean*

Table 2. Data of accuracy studies by standard addition method

Medium	Con of drug in formulations (µg/ml)	Con of pure drug added (µg/ml)	[a]Con of drug recovered (µg)	% Analytical recovery (%)
HCl	5.02	2	2.01	100.5±1.18
	5.02	5	4.99	99.86±0.78
	5.02	10	10.12	101.21±0.54
PBS	5.03	2	2.02	100.9±1.68
	5.03	5	5.07	101.51±0.83
	5.03	10	10.02	100.26±1.07

[a]*Each value represents the average of three separate determinations*

3.3.3 Precision

Intraday and inter day repeatability were studied to investigate the precision of the developed methods. Percentage R.S.D of intraday repeatability studies ranged from 0.69 to 2.4% in the hydrochloric acid medium and 0.39 to 1.04% in phosphate buffer saline medium, at all the levels of LQC, MQC, and HQC [Table 3]. These results demonstrate inter-assay precision and precision under identical operating conditions over small time intervals. The R.S.D. values of intermediate precision study were found to be less than 2% in both the selected media. Inter day precision study evaluates precision mainly within laboratory variations in three consecutive days. The R.S.D. values obtained for both the methods at all the three different levels were well within the acceptable range demonstrating excellent precision of the selected methods.

3.3.4 Linearity

The linearity range of Olanzapine in hydrochloric acid medium was found to be 3–18 μgml^{-1} at 258 nm. In phosphate saline medium, the linearity range observed was 4–24 μgml^{-1} at 252 nm. The proposed methods demonstrated very low standard error of slope and intercept [Table 1] which indicated very high precision of both the developed methods. The values of mean slope and intercept are well within the 95% confidence interval which indicated good linearity. Also, there were high levels of goodness of fit of regression equations demonstrated by both the methods. Furthermore, regression coefficient values obtained were high, near to unity indicating the linearity of the proposed methods [Table 1].

3.3.5 Detection Limit and Quantitation Limit (DL and QL)

The detection limit and quantitation limit in hydrochloric acid medium, calculated as per standard formulae were 0.1680 and 0.5091 μgml^{-1}, respectively, and in phosphate buffer saline these were found to be 0.2018 and 0.6115 μgml^{-1}, respectively. The values of both these limits in both the media show that developed methods are sufficiently sensitive for the estimation of Olanzapine in very low concentrations. Also DL and QL obtained theoretically for both the methods were cross checked practically and found to be similar with negligible changes.

Table 3. Repeatability and intermediate precision data in 100 mM HCl and phosphate buffer saline

Medium	QC levels	Repeatability (Intra-day) (n=9)						Intermediate precision (Inter-day) (n=27)	
		Day (1)		Day (2)		Day (3)			
		Mean	%RSD	Mean	%RSD	Mean	%RSD	Mean	%RSD
HCl	LQC	4.03	2.40	3.98	1.60	4.04	0.98	4.08	1.49
	MQC	12.43	0.69	12.09	0.96	12.18	1.11	12.49	0.68
	HQC	24.33	1.60	24.16	1.21	24.23	0.92	24.28	1.51
PBS	LQC	2.97	0.98	3.02	0.87	2.99	1.04	2.96	1.14
	MQC	9.09	0.39	9.04	0.76	9.08	0.61	9.08	0.78
	HQC	17.91	0.49	18.16	0.88	18.08	0.96	18.04	0.68

3.3.6 Robustness

The results obtained for robustness study demonstrated that minor changes of internal parameters such as pH of the investigating medium (±0.1) did not show any significant change on absorbance of Olanzapine. The mean percentage recovery with S.D were calculated and found to be 99.97±1.07 and 100.76±1.34 in the hydrochloric acid medium and phosphate buffer saline medium respectively. Therefore both the methods are sufficiently robust enough to estimate Olanzapine even with minor pH changes in both the media.

3.3.7 Ruggedness

The concentrations determined for the same solution by both Ultra violet - visible - spectrophotometers (Jasco, Japan, Model V-570 and Perkin Elmer, USA, model LAMBDA EZ210) were similar and percentage recovery obtained was well within the limits; 100.95±0.592% for HCl medium and 100.58±1.230% for PBS medium, indicating the ruggedness of both the methods. These findings allow analysts to perform the estimation of Olanzapine in both media in a different instrument under identical conditions.

3.3.8 Bench top stability study

The Olanzapine solution in selected medium did not show any significant changes in concentration after 24 h when stored at room temperature. The percentage recovery of 99.12±0.43% obtained was well within the limits, indicating the stability of Olanzapine in both the media for 24 h.

3.4 Estimation from Formulations

The applicability of the developed methods is of major concern while deciding the success of any analytical method. Therefore, these proposed methods were applied for the estimation of Olanzapine in commercial dosage forms such as tablets of various strengths, procured from the drug market. In HCl medium, % assay for Oleanz 2.5, 5 & 10 were found to be 101.12±0.98, 100.76±0.84 and 101.69±0.46% respectively; in PBS medium, % assay for Oleanz 2.5, 5 & 10 were found to be 99.52±1.04, 101.15±0.59 and 100.54±0.63% respectively. Both the methods estimated Olanzapine successfully in marketed formulations (within the assay limits), proving the utility of the proposed methods. These results further prove that both the methods are unaffected by the presence of numerous excipients used during the formulation of these commercial dosage form. Furthermore, the amount of drug estimated with very low standard deviation demonstrated the precision of these proposed methods.

3.5 Preformulation Dissociation Constant (pKa) Studies

The dissociation constant of Olanzapine by spectrophotometric method was found to be 4.4. The wave length for studying pKa was selected based on the distinguishable absorbance values at each pH solutions. The first derivative graph was carefully studied to find out the point of inflection which corresponds to the largest change in the absorbance [Fig. 3].

Dissociation constant (pKa) is the pH at which drug molecules exist as ionized and unionized in equal proportions. Ionized species have a separate behavior of absorbance as compared to unionized species and at different pH buffers drug exists in both the form at different proportions based on the ionization of the drug molecules at that particular pH. When pH is pKa, where both the forms are equal present, the trend of absorbance changes to the maximum which is evident by the inflection point in first derivative graph; a graph between minor changes in absorbance with pH vs. pH.

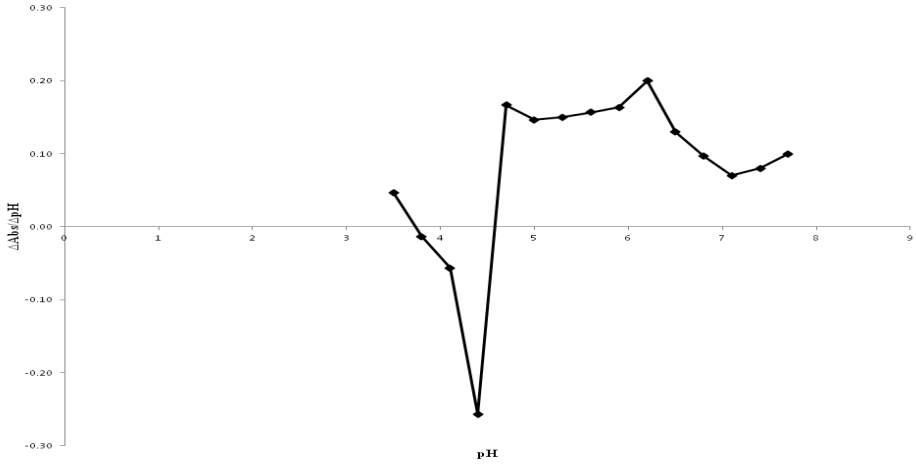

Fig. 3. Preformulation study profiles for pKa determination

4. CONCLUSION

The proposed methods are highly accurate, precise, robust, less time consuming and rugged. Both the methods have very good linearity and can be routinely used for the estimation of Olanzapine in bulk and various pharmaceutical dosage forms. The analysis techniques involved in these methods are very inexpensive and no complicated extraction steps are involved in any stages of analysis. The recovery values were very high and the presence of excipients did not interfere with the absorbance of Olanzapine. Furthermore, in assay studies, practical estimations of Olanzapine from formulations were in good agreement with their respective label claims with selective detection of Olanzapine. The developed methods were successfully applied for the determination of preformulation parameter of Olanzapine such as pKa, whose results would be highly useful during the formulation development stages and for predicting the ionization behavior in biological systems.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Stefania B, Giuseppe C, Meri DA, Caterina F, Vito N, Isabella F. Novel antipsychotic agents: Recent advances in the drug treatment of schizophrenia. Expert Opin Ther Patents. 2003;13(4):425-48.
2. Firdous S, Aman T, Nisa AU. Determination of olanzapine by UV Spectrophotometry and non-aqueous titration. J Chem Soc Pak. 2005;27(2):163 – 67.
3. Pradhan KK, Kumari S, Samanta R. Development and validation of a stability indicating uv spectroscopic method for olanzapine in bulk and pharmaceutical dosage forms. Int J Pharm Parm Sci. 2014;6(4):67-72.
4. Boulton DW, Markowitz JS, DeVane DL. A high-performance liquid chromatography assay with ultraviolet detection for olanzapine in human plasma and urine. J Chromatogr B. 2001;759(2):319-23.
5. Patel DS, Sharma N, Patel MC, Patel BN, Shrivastav PV, Sanyal M. LC–MS/MS assay for olanzapine in human plasma and its application to a bioequivalence study.

Acta Pharmaceutica Sinica B. 2012;2(5):481-94.
6. Chiu JA, Franklin RB. Analysis and pharmacokinetics of olanzapine (LY170053) and two metabolites in rat plasma using reversed-phase HPLC with electrochemical detection. J Pharm Biomed Anal. 1996;14(5):609-15.
7. Krishnaiah C, Murthy MV, Kumar R, Mukkanti K. Development of a stability-indicating UPLC method for determining olanzapine and its associated degradation products present in active pharmaceutical ingredients and pharmaceutical dosage forms. J Pharm Biomed Anal. 2011;54(4):667-73.
8. Josefsson M, Roman M, Skogh E, Dahl ML. Liquid chromatography/tandem mass spectrometry method for determination of olanzapine and N-desmethylolanzapine in human serum and cerebrospinal fluid. J Pharm Biomed Anal. 2010;53(3):576-82.
9. Berna M, Ackermann B, Ruterbories K, Glass S. Determination of olanzapine in human blood by liquid chromatography–tandem mass spectrometry. J Chromatogr B. 2002;767(1):163-68.
10. International Conference on Harmonization, ICH, Q2A validation of analytical procedure: Methodology. Geneva; 1994.
11. Bolton S. Pharmaceutical Statistics: practical and clinical application. 3rd ed. Marcel Dekker New York; 1997.

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