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Research Article

Simultaneous High-Performance Liquid Chromatographic Determination of Olanzapine hydrochloride in Pharmaceutical Dosage Form

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ABSTRACT

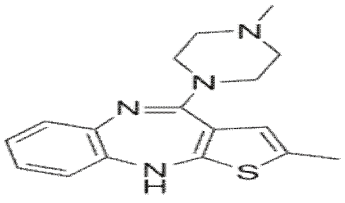
An isocratic reversed phase stability-indicating high-performance liquid chromatographic (HPLC) assay method was developed and validated for quantitative determination of Olanzapine hydrochloride in bulk drugs. An isocratic, reversed phase HPLC method was developed to separate the drug from the degradation products, using an Kromasil C18 (250 x 4.6) mm, 5u column and the mobile phase containing 0.1 M Potassium dihydrogen phosphate PH 6.0 with triethyl amine. Prepare a homogenous mixture of buffer, methanol and Acetonitrile (55:45, v/v). The detection was carried out at wavelength 229 nm. The developed method was validated with respect to linearity, accuracy (recovery), precision, system suitability, selectivity, robustness prove the stability indicating ability of the method.

Keywords: Olanzapine hydrochloride, Method Development, HPLC, Isocratic.

INTRODUCTION

Olanzapine, (2-methyl-4-(4-methyl-1-piperazinyl)-10*H*-thieno-[2,3*b*][1,5]benzodiazepine), is the most commonly prescribed second-generation neurloteptic for the treatment of psychiatric patients suffering from schizophrenia. Since its introduction in a therapy of psychiatric disorders in 1997, the need for reliable, sensitive and fast methods for its analysis in bulk samples and pharmaceutical preparations is obvious.

Chemical Structure



Literature survey

Literature survey reveal that Several methods have been already reported for the determination of Olanzapine, including hyphenated techniques: spectrophotometric [1-4], HPLC-MS [5-6], HPLC [7], Capillary zone electrophoresis and GC-MS[8].In the present work a report a simple, reliable and reproducible RP-HPLC method which was duly validated by statistical parameters precision, accuracy and recovery. The method has been satisfactorily applied to the determination of Olanzapine in pharmaceutical preparations.

EXPERIMENTAL

Material and reagents

Olanzapine hydrochloride bulk drug was made available from Merck Ltd. India (purity 99.8). Sodium dihydrogen phosphate, 1-octaneSulfonic acid was obtained from Qualigens fine chemicals, India Limited. Acetonitrile and methanol were obtained from Rankem laboratories, India. All chemicals and reagent were used as HPLC grades; Milli-Q-Water was used throughout the experiment.

Chromatographic Conditions

A chromatographic system (Systronic) consisting of quaternary solvent delivery pump, a degasser, an auto- injector, column oven and UV detector**.** The chromatographic column of 250 mm length and internal diameter of 4.6 mm filled with Octadecyl silane Inertsil ODS C18 stationary phase with particle size 5 micron and pore size 100A□ was used. The instrumental settings were a flow of 1 ml/min; the injection volume was 20 µl. And wavelength 229 nm.

Mobile Phase

The mobile phases containing 0.1 M Potassium dihydrogen phosphate pH 6.0 with triethyl amine were prepared. Homogenous mixture of buffer, methanol and acetonitrile (55:45, v/v) were also prepared.

Preparation of Standard stock solutions

Standard stock solutions of 1000 ppm of Olanzapine hydrochloride in mobile phase were prepared in volumetric flasks.

Sample solution

1000 ppm of Olanzapine hydrochloride in 100ml calibrated flask diluted with mobile phase [9-10]

Selectivity

Selectivity is the ability of the method to assess unequivocally the analyte in the presence of components, which may be expected to be present. Typically, these might include degradants, matrix etc. The selectivity of the developed LC method for Olanzapine hydrochloride was carried out in the presence of its degradation products. Stress studies were performed for Olanzapine hydrochloride bulk drug to provide an indication of the stability indicating property and selectivity of the proposed method. Intentional degradation was attempted to stress condition exposing it with acid (0.5 N Hydrochloric acid) Fig-3, alkali (0.1N NaOH) Fig-4, hydrogen peroxide (30%), heat (80 ^{o}C), to evaluate the ability of the proposed method to separate Olanzapine hydrochloride from its degraded products. For heat study, study period was 5days where as for acid, oxidation 48 hr and for base 2 hour. Assay studies were carried out for stress samples against Olanzapine hydrochloride reference standard and the mass balance (% assay + % sum of all impurities + % sum of all degraded products) was calculated.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions

The main target for the development of chromatographic method was to get the reliable method for the quantification of Olanzapine hydrochloride from bulk drug and which will be also applicable for the degradable products. Initially, we took the effort for the development of HPLC method quantification of standard Olanzapine hydrochloride from bulk. For this purpose, we have used Water nova pack C18(150X4.6)mm,5µ, Kromasil C18(150X4.6)mm,5µ, Inertsil ODS 3V C18(250X4.6)mm,5µ and Star ODS-II C18 (250X4.6)mm,5µ and Grace Alpha C18 (250mm x 4.6)mm,5u Out of these used HPLC column, Kromasil C18 (250 x 4.6)mm,5u found to comparatively better and gave the graph with better gaussian shape at retention time 23.48 min. To improve the shape and width of the graph, for the above columns different solvents and buffer taken for trials such as 0.1M KH_2PO_4 and Acetonitrile (60:40,v/v) in these trials peak shape is not good, another trials 0.01M Ammonium acetate P^H-5.9 and acetonitrile(20:80,v/v) peak shape not found well, trials Acetonitrile and water (80:20, v/v) column temperature 35 ^{o}C peak shape not found good, trials K_2HPO_4,Methanol and water (10:70:20,v/v/v)column temperature 35 ^{o}C, trials 1.0gm KH_2PO_4 and 0.45gm 1-Hexa sulphonic acid sodium salt make P^H-3.5 Ortho phosphoric acid and methanol(25:75, v/v) peak shape obtained but retention is not good, finally try for 0.1 M Potassium dihydrogen phosphate PH 6.0 with triethyl amine. Prepare a homogenous mixture of buffer, methanol and acetonitrile (55:45, v/v).

Result of forced degradation experiments

Considerable degradation was not observed in Olanzapine, bulk samples, under stress conditions such acid, thermal stress .Considerable degradation of Olanzapine, and was observed under stress condition such as base and oxidative hydrolysis leads to the formation of some unknown degradation peaks. The mass balance of Olanzapine, in stress samples was close to 100% and moreover, the unaffected assay of Olanzapine, in the Tablets confirms the stability indicating power of the method. The summary of forced degradation studies is given in Table I.

Table I: Summary of Forced degradation results

Stress condition	Time	Assay of active Substance%	Remarks
Acid Hydrolysis (0.5 N HCl)	48 Hrs	98.45	No Degradation
Base Hydrolysis (0.1 N NaOH)	2 Hrs	82.14	Degradation
Oxidation (30% H_2O_2)	48 Hrs	87.54	No Degradation
Thermal (80°C)	5 days	99.00	No Degradation
Photolytic degradation	1.2Lux million Hrs	97.89	negligible degradation

Method Validation

System suitability

For system suitability studies, five replicate injections of acid, base and oxidative degraded solutions were used and the RSD of peak area ratio, resolutions, tailing factor and number of theoretical plates of the peak were calculated. The system suitability results are shown in Table II.

Table II: System suitability reports

Compound (n=3)	Retention Time	% RSD	USP tailing	Theoretical plates
Olanzapine hydrochloride	23.48	0.67	1.06	12200

Precision

The precision of the method was studied by determining the concentrations of the drug Olanzapine hydrochloride in the tablet for six times.[11] Results obtained of the precision study (Table IV) indicate the reliability of the method (RSD %< 2).

Table IV. Results of the Linearity study and Precision

Ingredient	Precision (% RSD)	Linearity (µg/ml)	Slopes* (n= 3)	Coefficients of correlations
Olanzapine HCl	0.58	80-120	5421.23	0.99971

*Standard deviation shown in parentheses

Accuracy (Recovery test)

The accuracy of an analytical procedure expresses the closeness of agreement between the value, which is accepted either as a conventional true value or an accepted reference value and the value found. Accuracy of the method was studied by recovery experiments. The recovery experiments were performed by adding known amounts of the drugs in the placebo. The recovery was performed at three levels, 80%, 100% and 120%. The recovery samples were prepared as aforementioned procedure. The solutions were then analyzed, and the percentage recoveries were calculated from the calibration curve. The recovery values for Olanzapine hydrochloride ranged from 100.41% to 100.87% (Table V). The average recoveries of three levels nine determinations for Olanzapine hydrochloride were 100.33- 100.51%.

Table V: Results of the Recovery Tests for the Olanzapine HCl

Level of Addition (%)	Amount added (n = 3) (ppm)	% Recovery*	% Average recovery^
80	50	98.44	98.47
100	100	99.45	99.14
120	150	99.78	99.88

* RSD shown in parenthesis.

^ Average recovery = the average of three levels, nine determinations

Calibration and linearity

Linearity test solutions for the method were prepared from Olanzapine hydrochloride stock solutions at six concentrations levels from tested from 80% to 120% of the targeted level of the assay concentration Olanzapine hydrochloride. Standard solutions containing 80-120 µg/ml of Olanzapine hydrochloride in each linearity level were prepared. Linearity solutions were injected in triplicate. The calibration graphs were obtained by plotting peak area verses the concentration data was treated by least-squares linear regression analysis, the calibration graphs were found to be linear in the mentioned concentrations.

Robustness

To determine the robustness of the developed method experimental condition were purposely altered and the resolution between Olanzapine hydrochloride and acid degraded product were evaluated. The flow rate of the mobile phase was 1.0 ml/min. To study the effect of flow rate on the resolution, it was changed by 0.2 unit from 0.8 to 1.2ml/min while the other mobile phase component were held as stated in chromatographic conditions. The effect of percent organic strength on resolution was studied by varying acetonitrile from –10 to +10 % while other mobile phase components were held constant as stated in chromatographic condition. The effect of column temperature on resolution was studied at 25 and 35^{o}C instead of 30^{o}C while the other mobile phase components were held constant stated in chromatographic condition. The results are shown in table-VI

Table VI: Results of robustness study

Sr. No.	Parameters	Variations	Resolutions between Irinotecan HCl and base degraded product
1	Temperature	25 °C 35 °C	8.21 7.68
2	Flow rate	0.8 ml/min 1.2 ml/min	8.02 8.94
3	Mobile phase	40.5 ml 49.5 ml	3.7 3.3

LOD and LOQ (Sensitivity)

A series of solutions in the range 0.14–1.3% of the assay concentration (40 µg mL−1) were prepared by dilution of the standard solutions. Each solution (20 µL) was injected five times, the areas were measured for the drug peak, and the standard deviation for the five injections was calculated for each concentration. On the basis of data obtained, the standard deviation was calculated and these values were used for calculation of the LOD and LOQ. The results are shown in table-VII

Table VII: Results of the LOD and LOQ

Name	%LOD	%LOQ
Olanzapine HCl	0.18	0.26

Stability of analytical solution

The stability of the standard solutions and the sample solutions was tested at intervals of 24, 48 and 72 h. The stability of solutions was determined by comparing results of the assay of the freshly prepared standard solutions. The RSD for the assay results determined up to 72 h for Olanzapine hydrochloride was 0.35 %. The assay values were within ± 2 % after 72 h. The results indicate that the solutions were stable for 72 h at ambient temperature.

CONCLUSION

The method developed for quantitative determination of Olanzapine hydrochloride is rapid, precise, accurate and selective. The method was completely validated showing satisfactory data for all method-validated parameters tested. The developed method is stability indicating and can be used for assessing the stability of Olanzapine hydrochloride as bulk drugs. The developed method can be conveniently used for the assay determination of Olanzapine hydrochloride in bulk drugs and pharmaceutical dosage form.

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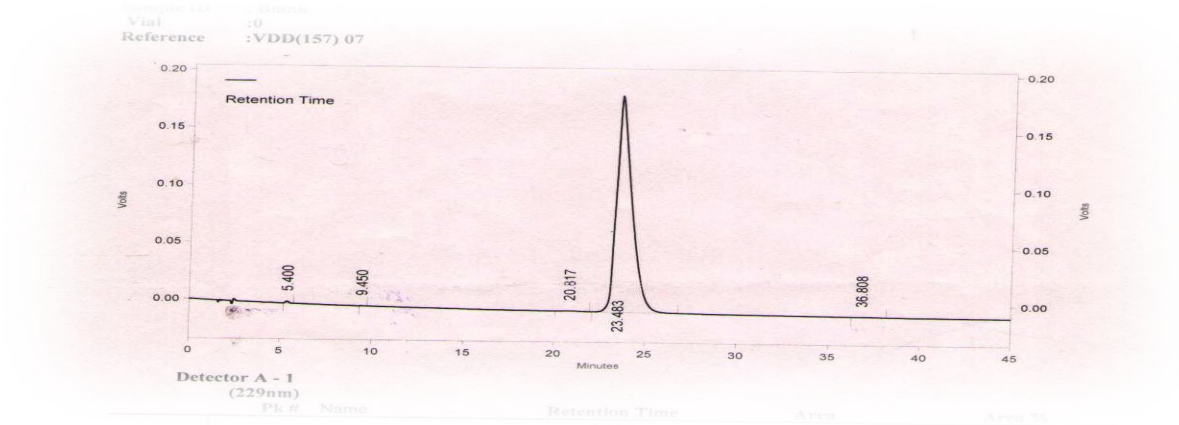

Fig. 1: A Typical Chromatogram of Olanzapine Sample Preparation

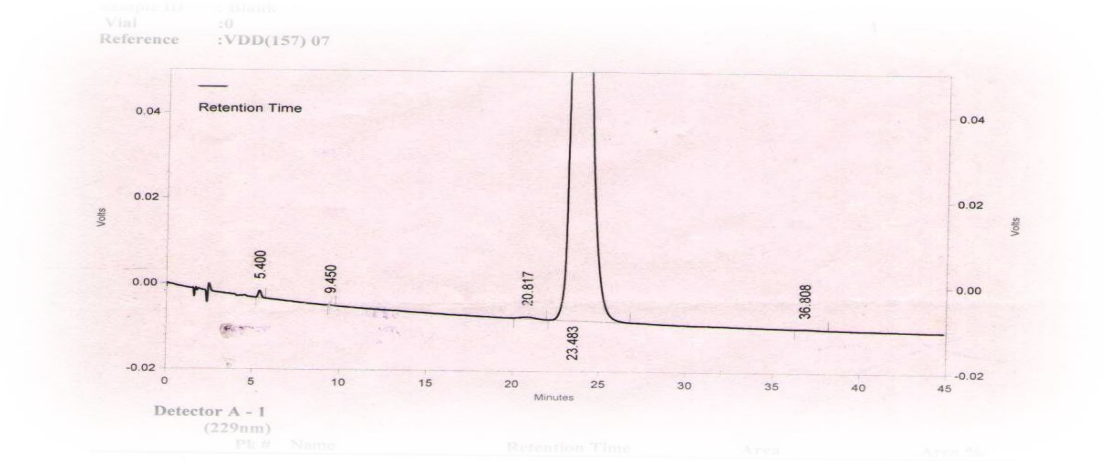

Fig. 2:A Typical Chromatogram of Olanzapine Standard Preparation

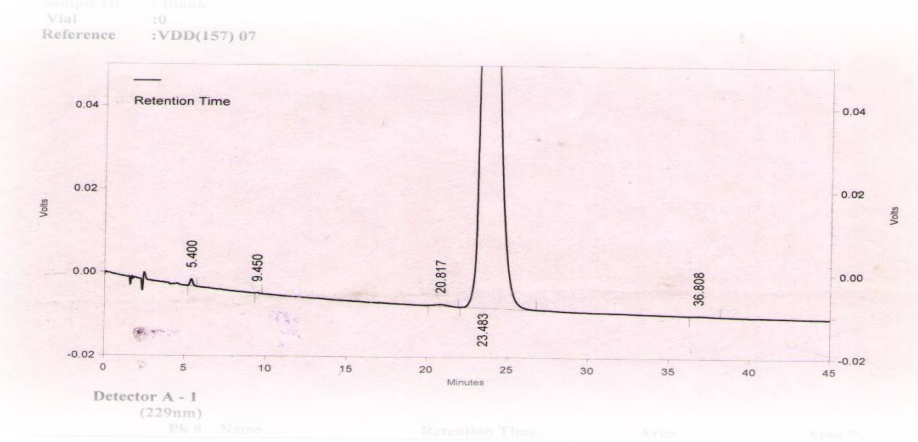

Fig. 3: A Typical Chromatogram of *Olanzapine* Acid Degradation

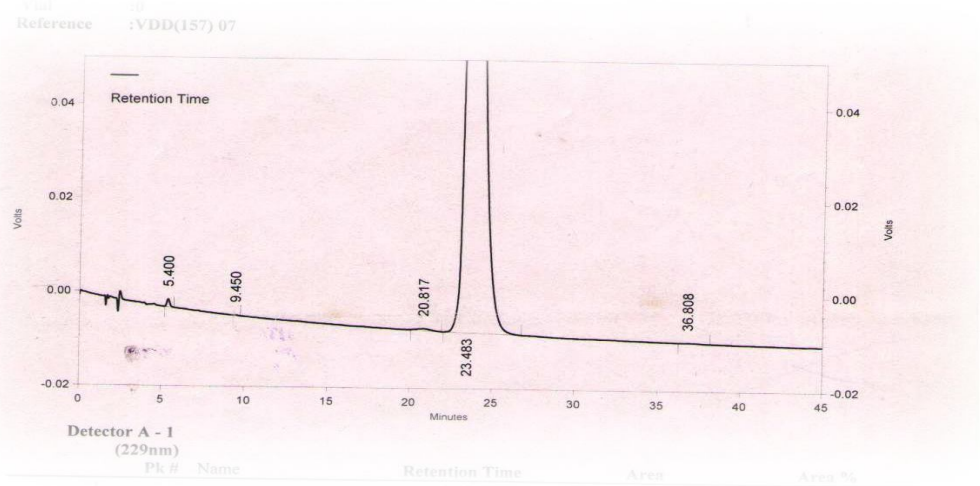

Fig. 4: A Typical Chromatogram of Olanzapine Alkali Degradation

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