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High-Performance Thin Layer Chromatography Determination of Olanzapine in Pharmaceutical Dosage Form

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A simple, selective, precise and stability-indicating high-performance thin layer chromatographic method of analysis of olanzapine in pharmaceutical dosage form was developed and validated. The method employed TLC aluminium plates precoated with silica gel 60F-254 as the stationary phase. The solvent system consisted of toluene-methanol-ethyl acetate-ammonia (8 : 2 : 1 : 0.1 v/v/v/v). This system was found to give compact spots for olanzapine (R_f value 0.25). Olanzapine was subjected to acid and alkali hydrolysis, oxidation, photochemical degradation and thermal degradation. Also, the degraded product was well separated from the pure drug. Densitometric analysis of olanzapine was carried out in the absorbance mode at 280 nm. The linear regression analysis data for the calibration plots showed good linear relationship with coefficient of regression value, $r^2 = 0.9991$ in the concentration range 10.8–108.0 ng/spot. The value of correlation coefficient, slope and intercept were 0.9995, 22.49 and 18.06, respectively. The method was validated for precision, recovery, ruggedness and robustness. The limits of detection and quantitation were 5.4 and 10.8 ng/spot, respectively. The drug undergoes degradation under acidic, basic, photochemical degradation and thermal degradation conditions. All the peaks of degraded product were resolved from the active pharmaceutical ingredient with significantly different R_f values. The samples degraded with hydrogen peroxide showed no additional peak. This indicates that the drug is susceptible to acid-base hydrolysis degradation, photochemical degradation and thermal degradation. Statistical analysis proves that the method is reproducible and selective for the estimation of said drug. As the method could effectively separate the drug from its degradation product, it can be employed as a stability-indicating one.

Key Words: Olanzapine, HPTLC, Determination.

INTRODUCTION

Olanzapine, chemically, 2-methyl-4-(4-methyl-1-piperazinyl)-10H-thieno[2,3-*b*][1,5] benzodiazepine (Fig. 1) is an antipsychotic drug[1]. Literature survey reveals that there are capillary electrophoresis method[2–4], HPLC method[5–19], for quantitation of olanzapine. Rarely any HPLC-electrospray tandem mass spectrometry[20–22] is reported for evaluation of olanzapine. A few methods for gas chromatography[23–25] and GCMS[26] are reported for determination of olanzapine. A solid phase extraction method[27] is reported for systematic toxicological analysis in biological fluid. An MS-MS library with triple quadrupole-tandem mass spectrometers[28] technique for drug identification and drug screening is reported. But there is

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no stability indicating HPTLC method for determination of olanzapine from its tablets, as its pharmaceutical dosage form.

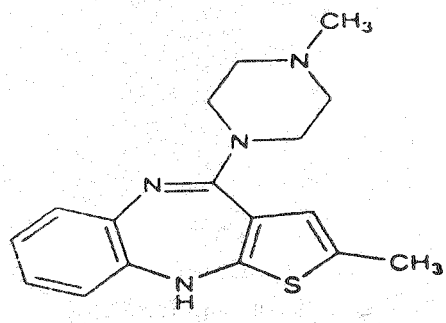

Fig. 1. Chemical structure of Olanzapine

The International Conference on Harmonization (ICH) guideline entitled 'Stability Testing of New Drug Substances and Products' requires a stress testing to be carried out to elucidate the inherent stability characteristics of the active substance. Susceptibility to oxidation is one of the required tests. The hydrolytic and the photolytic stability are also required. An ideal stability-indicating method is one that quantifies the drug *per se* and also resolves its degradation products. A very viable alternative for stability-indicating analysis of olanzapine is high-performance thin-layer chromatography (HPTLC). The advantage of HPTLC is that several samples can be run simultaneously by using a small quantity of mobile phase unlike HPLC, thus lowering analysis time and cost per analysis[29, 30]. The aim of the present work was to develop an accurate, specific, reproducible and stability indicating method for the determination of low levels of olanzapine in the presence of its degradation products and related impurities as per ICH guideline.

EXPERIMENTAL

Olanzapine was supplied by Sun Pharma India Ltd. and tablets (label claim: 5 mg/tablet, product name: Olanex 5 and manufacturer: Solus-A Division of Ranbaxy Laboratories Ltd.) were procured from the market. All chemicals and reagents used were of analytical grade and were purchased from Merck Chemicals, India.

The samples were spotted in the form of bands of width 6 mm with a Camag microlitre syringe on precoated silica gel aluminium plate 60F-254 (20 cm × 10 cm with 250 μm thickness, E. Merck, Germany) using a Camag Linomat V (Switzerland). A constant application rate of 150 μL/s was employed and the space between two bands was 5 mm. The slit dimension was kept at 5 mm × 0.45 mm and 20 mm/s scanning speed was employed. The mobile phase consisted of toluene-methanol-ethyl acetate-ammonia (8 : 2 : 1 : 0.1 v/v/v/v). Methanol was used as diluent for standard and sample preparations. Linear ascending development was carried out in twin trough glass chamber saturated with the mobile phase. The optimized chamber saturation time for mobile phase was 20 min at room temperature. The length of the chromatogram run was 7 cm. Subsequent to the development, TLC plates were dried in a current of air with the help of an air-dryer. Densitometric scanning was performed on Camag TLC scanner III in the absorbance mode at 280 nm. The source of radiation utilized was deuterium lamp.

Calibration Curves of Olanzapine

A stock solution of olanzapine (10.8 μg/mL) was prepared in diluent. Different volumes of stock solution 1, 3, 5, 7.5 and 10 μL, were spotted on TLC plate to obtain concentration of 10.8, 32.0, 54.0, 81.0 and 108.0 ng/spot of olanzapine, respectively. The data of peak area *vs.* drug concentration were treated by linear least-square regression analysis.

Method Validation

Precision: Precision was measured in terms of repeatability of application and measurement. Repeatability of standard application was carried out using five replicates of the same spot (50 ng/spot for standard application). Repeatability of sample measurement was carried out in six different sample preparations from same homogenous blend of marketed sample (50 ng/spot for sample application). It showed very low % relative standard deviation (% RSD) of peak area of olanzapine.

Ruggedness and robustness: Method ruggedness and robustness was determined by analyzing same sample blend at normal operating conditions and also by changing some operating analytical conditions such as development distance, mobile phase composition, injection volume, chamber saturation time and analyst.

Limit of detection and limit of quantitation: In order to estimate the limit of detection (LOD) and limit of quantitation (LOQ), blank diluent was spotted in replicates following the same method as explained in HPTLC instrumentation. The signal to noise ratio was determined.

Recovery studies: Recovery study was performed by spiking 30, 50 and 70% of olanzapine working standard to a pre-analyzed sample. The pre-analyzed sample is to be weighed in such a way that final concentration is half or 50% of the sample preparation before spiking. The percentage sum level of pre-analyzed sample and spiked amount of drug should be 80, 100 and 120% of simulated dosage nominal or target concentration of sample preparation. The accuracy of the analytical method was established in duplicate across its range.

Analysis of Marketed Formulation

Weighed and finely powdered not less than 20 tablets. Transferred blend equivalent to 5 mg of olanzapine to a 100 mL volumetric flask. Added about 60 mL of diluent and sonicated for 15 min and made up to volume with diluent. Mixed well and centrifuged the solution at 2500 rpm for 10 min. Spotted the clear supernatant solution in the form of bands on the specified TLC plate followed by development and scanning as described in HPTLC instrumentation. The analysis was repeated in triplicate. The possibility of excipient interference in the analysis was studied.

Forced Degradation of Olanzapine

Preparation of acid and base-induced degradation product: Tablet powder equivalent to 5 mg of olanzapine was transferred to 100 mL volumetric flask. To it, 25 mL of diluent was added and sonicated for 10 min with intermittent shaking. To it, 1 mL of 5 N HCl and 1 mL of 5 N NaOH were added separately. The sample was heated on a boiling water bath for 5 min. After cooling to room temperature it was diluted to volume with diluent and mixed. This solution was centrifuged at 2500

rpm for 10 min. This acidic and basic forced degradation was performed in the dark in order to exclude the possible degradative effect of light. The resultant supernatant solution was applied on TLC plate and the chromatograms were run as described in HPTLC instrumentation.

Preparation of hydrogen peroxide-induced degradation product: Tablet powder equivalent to 5 mg of olanzapine was transferred to 100 mL volumetric flask. To it, 25 mL of diluent was added and sonicated for 10 min with intermittent shaking. To it, 1 mL of 30.0% H_2O_2 was added. The sample was heated on a boiling water bath for 5 min. After cooling to room temperature it was diluted to volume with diluent and mixed. This solution was centrifuged at 2500 rpm for 10 min. The resultant supernatant solution was applied on TLC plate and the chromatograms were run as described in HPTLC instrumentation.

Photochemical degradation product: Tablet powder equivalent to 5 mg of olanzapine (previously kept in UV light for 24 h) was transferred to 100 mL volumetric flask. To it, 25 mL of diluent was added and sonicated for 10 min with intermittent shaking, diluted to volume with diluent and mixed. This solution was centrifuged at 2500 rpm for 10 min. The resultant supernatant solution was applied on TLC plate and the chromatograms were run as described in HPTLC instrumentation.

Thermal degradation product: Tablet powder equivalent to 5 mg of olanzapine was transferred to 100 mL volumetric flask. To it, 50 mL of diluent was added and sonicated for 10 min with intermittent shaking. This sample was heated on a boiling water bath for 5 min. After cooling to room temperature it was diluted to volume with diluent and mixed. This solution was centrifuged at 2500 rpm for 10 min. The resultant supernatant solution was applied on TLC plate and the chromatograms were run as described in HPTLC instrumentation.

Detection of the Related Impurities

Weighed and finely powdered not less than 20 tablets. Transferred blend equivalent to 5 mg of olanzapine to a 100 mL volumetric flask. Added about 60 mL of diluent and sonicated for 15 min and made up to volume with diluent. Mixed well and centrifuged the solution at 2500 rpm for 10 min. Spotted the clear supernatant solution in the form of bands on the specified TLC plate and the chromatograms were run as described in HPTLC instrumentation.

RESULTS AND DISCUSSION

Development of the Optimum Mobile Phase

TLC procedure was optimized with a view to develop in stability-indicating assay method. Both the pure drug and the marketed products were spotted on TLC plates and run in different solvent systems where bands closer to the solvent front and diffused bands were observed. Finally, the mobile phase of toluene-methanol-ethyl acetate-ammonia (8 : 2 : 1 : 0.1 v/v/v/v) gave good sharp and symmetrical peak with R_f value 0.25 for olanzapine. When the chamber was saturated with the mobile phase for 5 min at room temperature, well defined spots were obtained.

Calibration Curves: The linear regression data for the calibration curves indicate that the response is linear over the range 10.8–108.0 ng/spot for olanzapine with coefficient of regression r^2 value as 0.9991. The values of correlation coefficient, slope and intercept were 0.9995, 22.49 and 18.06, respectively.

Validation of the Method

Precision: The % RSD for repeatability of standard application is 0.97% whereas the % RSD for repeatability of sample preparation is 1.13%. This shows that precision of the method is satisfactory as % relative standard deviation is not more than 2.0% and mean recovery between 98.0–102.0%. Table-1 represents the precision of the method.

TABLE-1
METHOD PRECISION OF OLANZAPINE

Sample preparation	Assay (%)	Deviation from mean assay value (%)
1	98.55	–0.67
2	99.66	0.45
3	99.44	0.22
4	97.29	–1.94
5	100.08	0.87
6	100.27	1.06
Mean	99.22	
± SD	1.12	
% RSD	1.13	

Ruggedness and robustness of the method: The parameters and results of normal operating condition (original) against changed conditions are indicated in Table-2. The low value of % RSD obtained after introducing the deliberate changes in parameters alters the results of olanzapine to –1.37% of method precision study, which is not a significant change. The ruggedness and robustness of the method is established, as the % deviation from mean assay value obtained from precision study is less than ± 2.0%.

LOD and LOQ: The signal-to-noise ratios of 3 and 10 were considered as LOD and LOQ, respectively. The LOD and LOQ for olanzapine are 5.4 ng/spot and 10.8 ng/spot, respectively.

Recovery studies:

$$\% \text{ Recovery} = \frac{\% \text{ Amount recovered}}{\% \text{ Sum level}} \times 100$$

The results of recovery are shown in Table-3. The results indicate that the individual recovery of olanzapine ranges from 97.99–101.08% with mean recovery of 99.66% and % relative standard deviation of 1.30%. The recovery of

olanzapine by proposed method is satisfactory as % relative standard deviation is not more than 2.0% and mean recovery between 98.0–102.0%.

TABLE-2
RUGGEDNESS AND ROBUSTNESS OF OLANZAPINE

Parameter	Normal (Original)	Changed conditions
Development distance (from line of application)	70%	75%
Mobile phase composition (% v/v/v)	Toluene-Methanol-Ethyl acetate-Ammonia (8 : 2 : 1 : 0.1)	Toluene-Methanol-Ethyl acetate-Ammonia (7 : 3 : 1 : 0.1)
Injection volume	1 µL	2 µL
Chamber saturation time	20 min	30 min
Analyst	Vinay	Analyst II
% Assay, olanzapine	99.22	97.86

% RSD from mean assay value obtain in method precision studies is –1.37% of Olanzapine

TABLE-3
RECOVERY OF OLANZAPINE

Sample preparation	Simulated dosage nominal (%)	Sum level (%)	Amount recovered (%)	Recovery (%)
Pre-analysed samples				
1	80	80.2	81.07	101.08
2	80	74.6	74.60	100.00
1	100	91.4	92.14	100.81
2	100	87.8	87.69	99.88
1	120	104.0	101.91	97.99
2	120	99.4	97.63	98.22
Mean				99.66
± Standard deviation				1.29
% Relative standard deviation				1.30

Analysis of the marketed formulation

A single spot at R_f 0.25 was observed in the chromatogram of the drug sample extracted from tablets. There was no interference from the excipients commonly present in the tablets. The drug content was found to be 99.22% with a % relative standard deviation of 0.59%. It may therefore be inferred that degradation of olanzapine did not occurr in the marketed formulations that were analyzed by this

method. The low % relative standard deviation value indicates the suitability of this method for routine analysis of olanzapine in pharmaceutical dosage form.

Stability-indicating property

The % assay and % degradation with stress conditions are shown in Table-4 with respective figures in Fig. 2. The no treatment sample (as control) has been evaluated relative to the standard concentration whereas rest of the stressed condition samples (S. No. 2–6) are evaluated relative to the control sample with respect to the % assay and % degradation. The percentage degradation results are calculated by area normalization method. The chromatogram of the acid degraded sample for olanzapine showed additional peak at R_f value of 0.28. The chromatogram of the alkali degraded sample for olanzapine showed additional peak at R_f value of 0.19. The chromatogram of photochemical degraded sample for

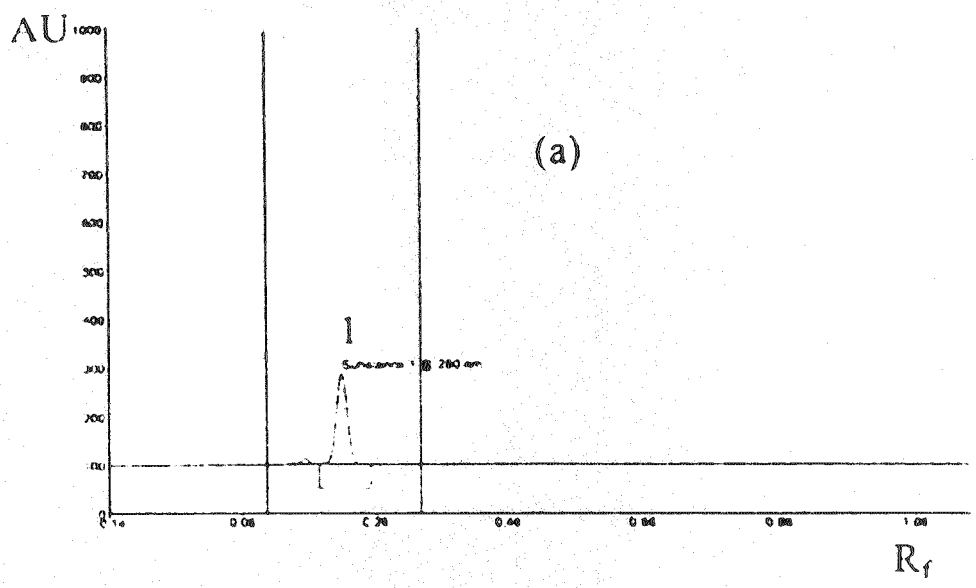

Fig. 2 (a)

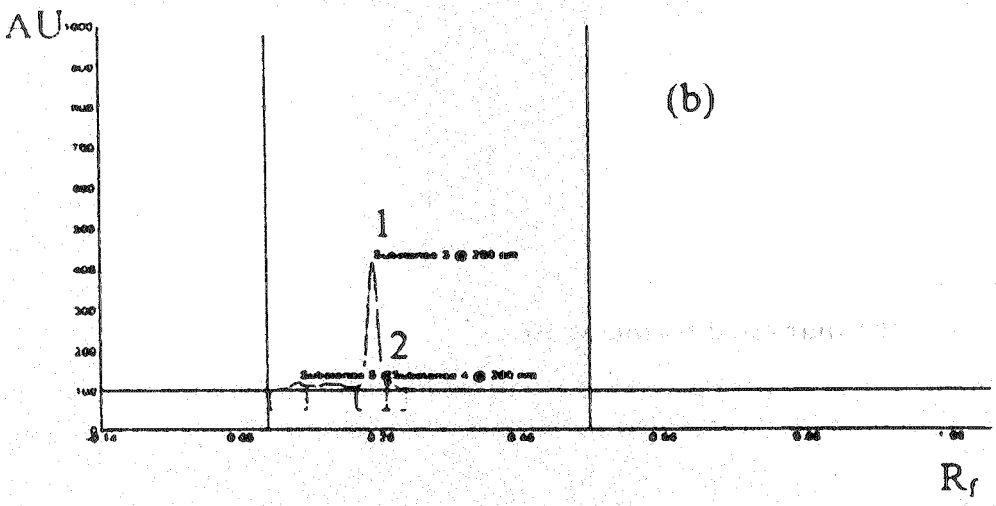

Fig. 2 (b)

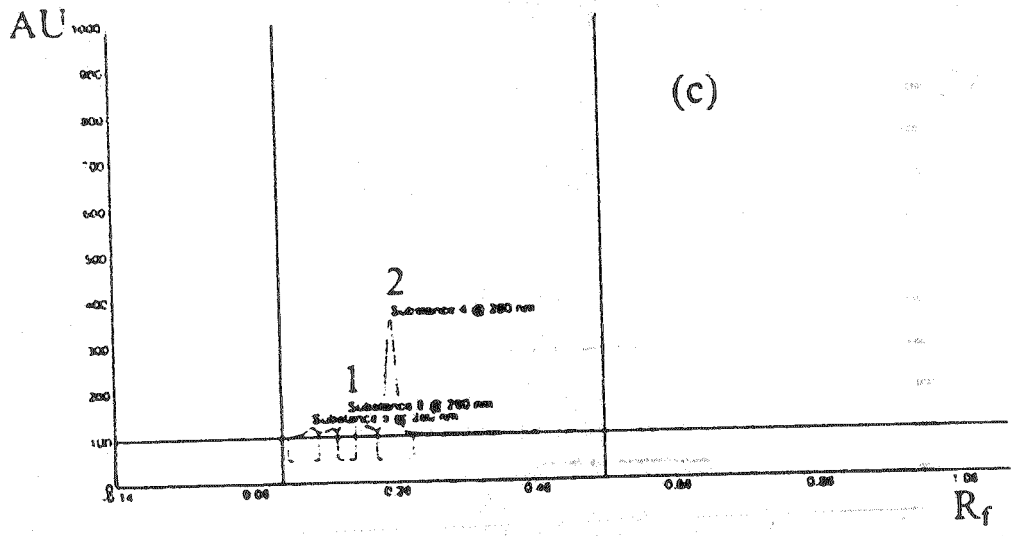

Fig. 2 (c)

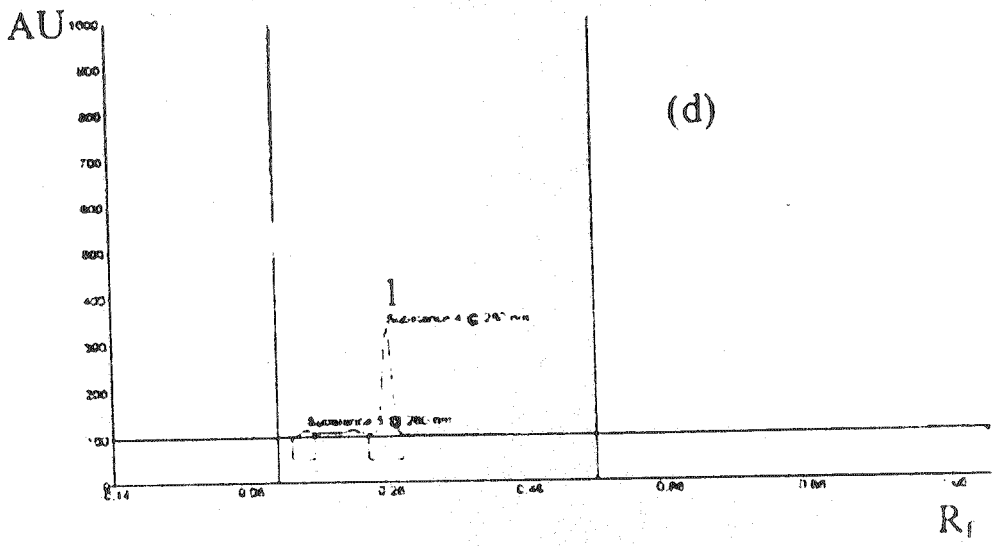

Fig. 2 (d)

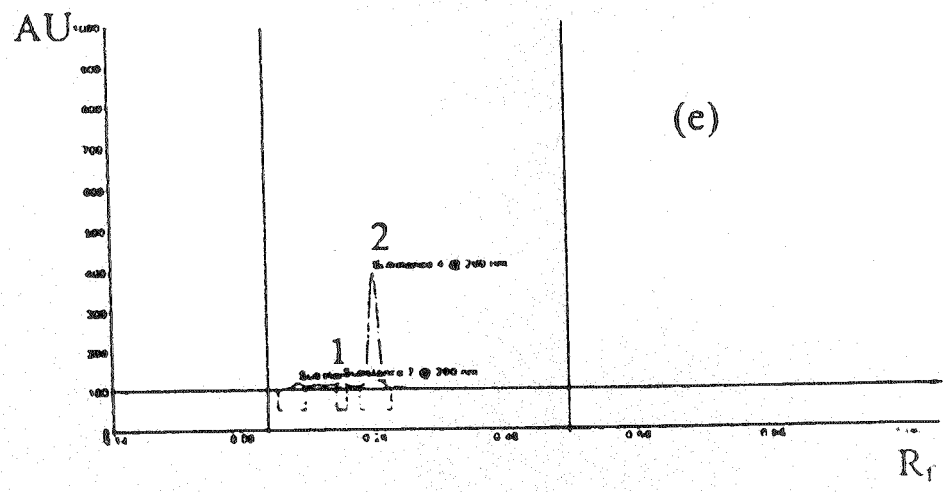

Fig. 2 (e)

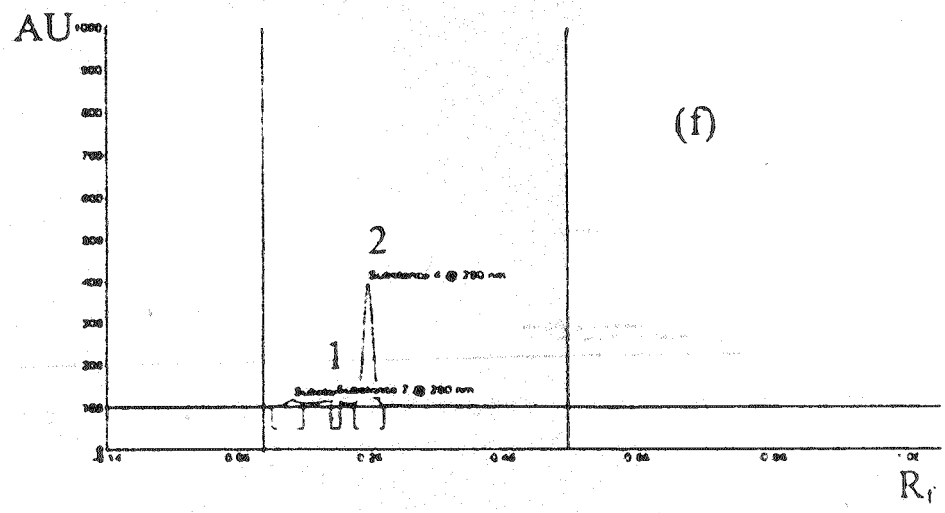

Fig. 2 (f)

Fig. 2. Chromatograms of olanzapine and its degraded products: (a) Pure drug: Peak 1 (R_f 0.25) is of olanzapine, (b) Acid induced: Peak 1 (R_f 0.25) is of olanzapine, peak 2 (R_f 0.28) is of degraded product, (c) Based induced: Peak 1 (R_f 0.19) is of degraded product, peak 2 (R_f 0.25) is of olanzapine, (d) Hydrogen peroxide induced: Peak 1 (R_f 0.25) is of olanzapine, (e) Photochemical degradation: Peak 1 (R_f 0.21) is of degraded product, peak 2 (R_f 0.25) is of olanzapine, (f) Thermal degradation: Peak 1 (R_f 0.21) is of degraded product, peak 2 (R_f 0.25) is of olanzapine.

olanzapine showed additional peak at R_f value of 0.21. The chromatogram of thermal degraded samples for olanzapine showed additional peak at R_f value of 0.21. The sample degraded with hydrogen peroxide showed no additional peak. In each forced degradation sample where additional peaks were observed, the response of the drug was changing from the initial control sample. This indicates that the drug is susceptible to acid-base hydrolysis degradation, photochemical degradation and thermal degradation. The lower R_f values of the degraded components indicate that they were less polar whereas higher R_f values of the degraded components indicate they were more polar than the analyte itself.

TABLE-4
STRESSED STUDY DATA OF OLANZAPINE

S. No.	Condition	Assay olanzapine (%)	Degradation (%)	
			Single maximum	Total
1.	No treatment (control sample)	99.22	Nil	Nil
2.	Acid	94.02	5.64	5.64
3.	Alkali	92.38	7.24	7.24
4.	H_2O_2	98.53	Nil	Nil
5.	UV	93.92	5.37	5.37
6.	Thermal	95.81	3.50	3.50

Detection of the related impurities

The sample solution shows no additional spots other then principal spot. Hence no related impurities are present in the market sample.

Conclusion

The developed HPTLC technique is precise, specific, accurate and stability-indicating. Statistical analysis proves that the method is reproducible and selective for the analysis of olanzapine in pharmaceutical dosage form. The method can be used to determine the purity of the drug available from various sources by detecting the related impurities. As the method separates the drug from its degradation products, it can be employed as a stability indicating one.

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