Journal of Pharmaceutical and Biomedical Analysis 54 (2011) 667–673

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Development of a stability-indicating UPLC method for determining olanzapine and its associated degradation products present in active pharmaceutical ingredients and pharmaceutical dosage forms

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ARTICLE INFO

Article history:
Received 10 June 2010
Received in revised form 12 October 2010
Accepted 17 October 2010
Available online 28 October 2010

Keywords:
UPLC
Olanzapine
Forced degradation
Validation
Stability-indicating

ABSTRACT

A simple, sensitive and reproducible ultra performance liquid chromatography (UPLC) coupled with a photodiode array detector method was developed for the quantitative determination of olanzapine (OLN) in API and pharmaceutical dosage forms. The method is applicable to the quantification of related substances and assays of drug substances. Chromatographic separation was achieved on Acquity UPLC BEH 100-mm, 2.1-mm, and 1.7-μm C-18 columns, and the gradient eluted within a short runtime, i.e., within 10.0 min. The eluted compounds were monitored at 250 nm, the flow rate was 0.3 mL/min, and the column oven temperature was maintained at 27 °C. The resolution of OLN and eight (potential, bi-products and degradation) impurities was greater than 2.0 for all pairs of components. The high correlation coefficient ($r^2 > 0.9991$) values indicated clear correlations between the investigated compound concentrations and their peak areas within the test ranges. The repeatability and intermediate precision, expressed by the RSD, were less than 2.4%. The accuracy and validity of the method were further ascertained by performing recovery studies via a spike method. The accuracy of the method expressed as relative error was satisfactory. No interference was observed from concomitant substances normally added to the tablets. The drug was subjected to the International Conference on Harmonization (ICH)-prescribed hydrolytic, oxidative, photolytic and thermal stress conditions. The performance of the method was validated according to the present ICH guidelines for specificity, limit of detection, limit of quantification, linearity, accuracy, precision, ruggedness and robustness.

1. Introduction

Ultra-performance liquid chromatography (UPLC) is a new category of separation based on well-established principles of liquid chromatography, which utilizes sub-2-μm particles for the stationary phase. These particles operate at elevated mobile phase linear velocities to drastically increase the resolution, sensitivity and speed of analysis. Because of its speed and sensitivity, this technique has gained considerable attention in recent years for pharmaceutical and biomedical analysis. In this present work, the technology has been applied for the method development and method validation study of related substances and the assay determination of OLN bulk drug and dosage forms.

There have been several improvements in the treatment of psychiatric disorders in the last few years. In particular, many new drugs other than classic neuroleptics (phenothiazines and butyrophenones) are now available for the treatment of schizophrenia. These new drugs, called atypical antipsychotics, such as risperidone, clozapine and OLN, seem to be more effective than earlier drugs because they can suppress both positive and negative symptoms of schizophrenia. Classic neuroleptics are only active against positive symptoms of the illness. Furthermore, the newer drugs cause fewer extra-pyramidal side effects than the older ones. The most recent commercially available atypical antipsychotic drug is OLN (2-methyl-4-(4-methyl-1-piperazinyl)-10*H*-thieno[2,3b][1,5]benzodiazepine).

In the literature, limited LC methods were reported for the determination of OLN in pharmaceutical preparations. The isolation and characterization of process-related impurities of OLN and degradation impurities under stress conditions were performed by LC and ESI-MS/MS [1–4]. Mass spectrometry and LC methods have been reported for the determination of OLN assays in dosage forms and human plasma [5–8]. Other methods include some derivative spectrometry and voltammetry of combined dosage forms [9–11]; simple protein precipitation of LC mass, LC with coulometric detection and LC electrochemical detection [12–14]; UV spectrom-

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doi:10.1016/j.jpba.2010.10.013

etry and non-aqueous titration [15]; and the HPTLC method [16], Hiriyanna et al. described possible oxidative degradants. In addition to oxidative degradants, other possible degradants were studied in the present work and were found to have shown separation of all the degradants from the process-related impurities, but a stability-indicating method has not been reported in any of the cited literature [1–16]. Currently, the determination of impurities is one of the most difficult tasks for pharmaceutical analysis during method development, especially if increasing numbers of impurities are required to be determined. OLN and its impurities should be monitored together with their degradation compounds, preferably in a single chromatographic run.

The United States Pharmacopoeia (USP) monograph method for OLN-related compounds cannot separate all the potential impurities and degradation compounds of OLN. However, the USP monograph method (monitored only two impurities: Imp-4 and Imp-7) can resolve Imp-4- and Imp-7-related compounds of OLN, and the total run time is approximately 40 min. According to our research, none of the currently available analytical methods, including the USP Method, can separate and quantify all the known related compounds and degradation impurities of OLN, API and the dosage forms. Furthermore, there is no stability-indicating HPLC/UPLC method reported in the literature that can conduct an accurate and quantifiable analysis of OLN, API and the dosage forms. It is, therefore, necessary to develop a new stability-indicating method for the determination and quantitative estimation of OLN and related substances.

Hence, a reproducible stability-indicating RP UPLC method was developed for the quantitative determination of OLN and its eight impurities, namely Imp-1, -2, -3, -4, -5, -6, -7 and -8 (Fig. 1). This method was successfully validated according to the ICH guidelines (Validation of Analytical Procedures: Test and Methodology Q2).

2. Experimental

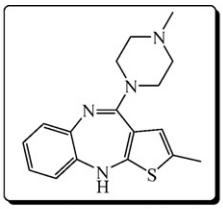
2.1. Materials and reagents

Active pharmaceutical ingredient standards and samples were supplied by Dr. Reddy's Laboratories Limited, IPDO, Hyderabad, India. Commercially available Zyprexa in 20-mg tablets was used for the dosage form analysis. Methanol, HPLC-grade acetonitrile, and analytical-grade ortho-phosphoric acid were purchased from Merck, Darmstadt, Germany. Triethylamine was purchased from JT Baker, Mallinckrodt Inc., Phillipsburg, NJ, USA. Water was prepared in-house by using a Millipore Milli-Q Plus water purification system (Millipore Corporate Headquarters, Billerica, MA).

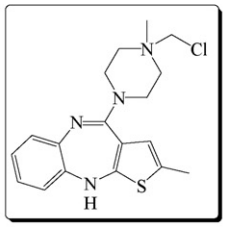
2.2. Chromatographic conditions and equipment

LC was carried out on a Waters Acquity UPLC with a photodiode array detector (DAD/PDA). The output signal was monitored and processed using empower software. The chromatographic column used Acquity UPLC BEH C-18 100-mm, 2.1-mm, and 1.7-μm particle sizes. Separation was achieved using a gradient method. Mobile phase A contained a mixture of 20 mM NaH_2PO_4, H_2O and 2.0 mL of triethylamine (TEA) buffer (pH of 6.8), acetonitrile, and methanol in a ratio of 50:20:30 (v/v/v), respectively. Mobile phase B contained a mixture of water and acetonitrile in a ratio of 10:90 (v/v), respectively.

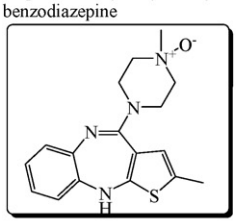
The flow rate of the mobile phase was 0.3 mL/min. The UPLC gradient program (*T*/*%B*) was set as 0.01/0, 5.0/20, 6.5/90, 8.0/100, 9.0/0 and 10.0/0. The column temperature was maintained at 27 °C, and the detection was monitored at a wavelength of 250 nm. The injection volume was 1.0 μL.



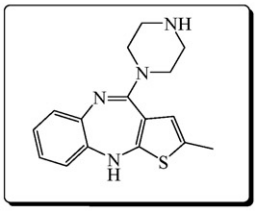
Olanzapine: 2-Methyl-4-(4-methyl-1-piperazinyl)-10H-thieno-[2,3-b][1,5]-benzodiazepine



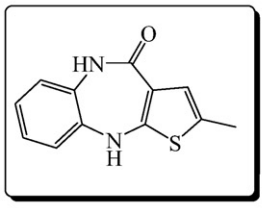
Imp-1:2-Methyl-4-(4-methyl-4-chloro methyl-1-piperazinyl)-10H-thieno-[2,3-b][1,5]-benzodiazepine



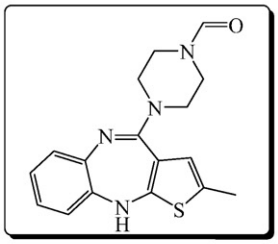
Imp-2:2-Methyl-4-(4- methyl-4-oxido-1-piperazinyl) 10H-thieno-[2,3-b][1,5]-benzodiazepine



Imp-3: 4-Methyl-4-(1-piperazinyl)-10H-thieno-[2,3-b][1,5]- benzodiazepine

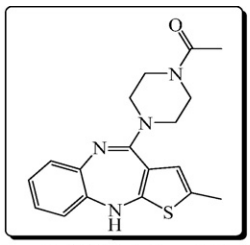


Imp-4: 2-Methyl-4-oxo-10H-thieno-[2,3-b][1,5]- benzodiazepine

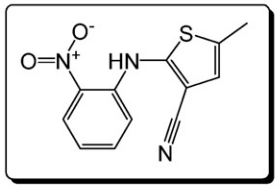


Imp-5:2-Methyl-4-(4-formyl-1-piperazinyl)-10H-thieno-[2,3-b][1,5]- benzodiazepine

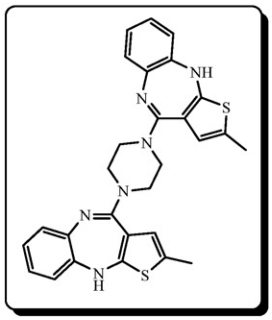
Fig. 1. Structures of olanzapine and its eight impurities.



Imp-6:2-Methyl-4-(4-acetyl-1-piperazinyl)-10H-thieno-[2,3-b][1,5]- benzodiazepine



Imp-7: 5-Methyl-2-((2-nitrophenyl) amino)-3-thiophenecarbonitrile



Imp-8:1,4 bis (2-Methyl-10H-benzo[b]thieno[2,3-e][1,4]diazepin-4-yl) piperazine

Fig. 1. *Continued*

2.3. LC–MS/MS conditions

An LC–MS/MS system (Agilent 1200 series liquid chromatograph coupled with Applied Biosystems 4000 Q Trap triple quadrupole mass spectrometer with Analyst 1.4 software, MDS SCIEX, USA) was used for the unknown compounds formed during forced degradation studies. A YMC pack ODS-A C18, 250 mm × 4.6 mm, 5-μm column (Kyoto, Japan) was used as the stationary phase. A 0.02-mM solution of ammonium acetate (Merck, Darmstadt, Germany) was used as a buffer. The ammonium acetate buffer, methanol and acetonitrile in a ratio of 50:30:20 (v/v/v) were used for solvent A, and water and acetonitrile in a ratio of 10:90 (v/v) were used for solvent B.

The gradient program (*T*/%*B*) was set as 0.01/0, 20/0, 25/30, 30/60, 35/80, 40/90, 45/90, 50/60, 55/0 and 60/0. Acetonitrile was used as a diluent. The flow rate was 1.0 mL/min. The analysis was performed in positive electro-spray/positive ionization mode, the ion source voltage was 5000 V, and the source temperature was 450 °C. GS1 and GS2 were optimized to 30 and 35 psi, respectively. The curtain gas flow was 20 psi.

2.4. Preparation of stock solutions

A stock solution of OLN (50 μg/mL) was prepared by dissolving an appropriate amount of the drug in acetonitrile and water at a ratio of 20:80 (v/v), respectively. Working solutions containing 50 μg/mL and 5 μg/mL were prepared from this stock solution for the determination of related substances and for the assay determination, respectively. Mixed and individual stock solutions (0.5 μg/mL) of the impurities (denoted Imp-1 to Imp-8) were prepared in acetonitrile and water in a ratio of 20:80 (v/v).

2.5. Preparation of sample solution

Twenty ($n = 20$) OLN 20-mg Zyprexa tablets were weighed, and the pellets were transferred into a clean, dry mortar. Pellets equivalent to 50 mg of the drug were dissolved in 100 mL of acetonitrile:water (20:80, v/v) to make a 500-μg/mL solution. An aliquot of 1.0 mL of this solution was diluted to 10 mL with the buffer solution, yielding 50-μg/mL solution that was filtered through a 0.22-μm nylon membrane filter. The resulting solution was analyzed by UPLC.

2.6. Stress studies

Specificity is the ability of the method to measure the analyte response in the presence of its potential impurities [17]. The specificity of the developed LC method for OLN was carried out in the presence of its eight impurities. Stress studies were performed at an initial concentration of 50 μg/mL of OLN in active pharmaceutical ingredients (API) and tablets to provide the stability-indicating property and specificity of the proposed method. Intentional degradation was attempted by the stress conditions of UV light (254 nm), heat (60 °C), acid (0.1-N HCl at 60 °C), base (0.5-N NaOH at 60 °C), hydrolysis (60 °C) and oxidation (1.0% H_2O_2 at 60 °C) to evaluate the ability of the proposed method to separate OLN from its degradation products. For heat and light studies, the study period was 10 days; whereas for the hydrolysis, base, acid and oxidation studies, the study periods were 1 h, 30 min, 10 min, and 10 min, respectively.

The purity of the peaks obtained from the stressed samples was verified using the PDA detector. The purity angle was within the purity threshold limit obtained in all the stressed samples and demonstrated the analyte peak homogeneity. An assay of stressed samples was performed by comparison with reference standards, and the mass (% assay + % impurities + % degradation products) for each of the stressed samples was calculated. An assay was also calculated for the OLN sample by spiking all eight impurities at the specification level (i.e., 0.15%).

3. Method validation

The described method has been validated for the assay and related substances by UPLC determination [18,19].

3.1. Precision

The repeatability of the method for the related substances was checked by a sixfold analysis of 50 μg/mL of OLN spiked with 0.075 μg/mL of each of the eight impurities. The RSD (%) of peak area was calculated for each impurity.

Inter- and intra-day variation and analyst variation were studied to determine the intermediate precision of the proposed method. Intra-day precision was determined by a sixfold analysis of 50 μg/mL of OLN spiked with 0.075 μg/mL of each of the eight impurities. The same protocol was followed for three different days to study inter-day variation ($n = 18$). Different analysts prepared different solutions on different days. The RSD (%) of the peak area was calculated for each impurity.

The precision of the assay was evaluated by performing six ($n = 6$) independent assays of a test sample of OLN and by comparison with a qualified reference standard. The RSD (%) of the six results was calculated.

3.2. Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the true value and the observed value. The accuracy of the assay method for OLN was evaluated in triplicate ($n = 3$) at the three concentrations of 2.5, 5.0 and 7.5 μg/mL (50%, 100% and 150%) of drug product, and the recovery was calculated for each added (externally spiked) concentration.

For all impurities, the recovery was determined in triplicate for 0.0375, 0.075 and 0.1125 μg/mL (50%, 100% and 150%) of the analyte concentration (50 μg/mL) of the drug product, and the recovery of the impurities was calculated.

3.3. Linearity of the response

The detector response linearities for all eight impurities and OLN were assessed by injecting eight separately prepared solutions covering the range of LOQ – 200% (LOQ, 0.015, 0.0375, 0.075, 0.1125, 0.15, 0.1875, 0.225, 0.2625 and 0.30%) of the normal sample concentration (50 μg/mL). The correlation coefficients, slopes and *Y*-intercepts of the calibration curve were determined.

3.4. Limit of detection (LOD) and limit of quantification (LOQ)

By injecting linear (0.05–0.5% with respect to the test concentration) solutions of known concentrations, based on the standard deviation (σ) of the response and the slope (S) of the calibration plot and using the formula LOD = $3.3\sigma/S$ and LOQ = $10\sigma/S$, the LOD and LOQ for OLN and for the eight impurities were estimated. The precision ($n = 6$) was also determined at the LOQ level, and the % RSD was calculated for the peak area for each impurity and for OLN.

3.5. Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

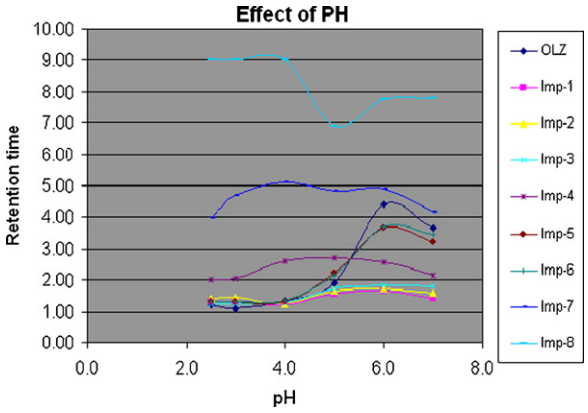
To determine the robustness of the method, the experimental conditions were deliberately changed. The resolution of OLN and the eight impurities was evaluated. The mobile phase flow rate was 0.30 mL/min; to study the effect of the flow rate on resolution, the flow rate was changed to 0.27 and 0.33 mL/min. The effect of the column temperature was studied at 22 °C and 35 °C (instead of 27 °C), and the mobile phase composition was changed +10% from the initial composition.

3.6. Solution stability and mobile phase stability

OLN-spiked solutions (with respect to the specification, i.e., 0.15% level) were prepared in the diluent by leaving the test solutions at room temperature. The spiked solutions were injected at 0-, 24- and 48-h time intervals. The impurity content (Imp-1, Imp-2, Imp-3, Imp-4, Imp-5, Imp-6, Imp-7 and Imp-8) was calculated, and the consistency in the % area of the principal peak at each interval was checked. The prepared mobile phase was kept constant during the study period.

The mobile phase study was demonstrated by injecting the freshly prepared sample solution at different time intervals (0 h, 12 h, 24 h, 36 h and 48 h).

Table 1
Effect buffer pH vs retention time.



4. Results and discussion

4.1. Method development and optimization

OLN drug substances have two reported pK_a values: 4.69 and 7.37. The main target of the chromatographic method is to achieve the separation of impurities (bi-products from the synthesis of OLN) and the main component OLN. The maximum absorption wavelength of the reference drug solution, process bi-products and forcibly degraded drug solutions is 250 nm, which is the intersecting value obtained from the UV absorption spectra; hence, 250 nm was selected as the detection wavelength for LC analysis. The blended solution containing 50 μg/mL of OLN and 1 μg/mL of each of the eight impurities was prepared in the diluent.

Initially, a mobile phase composed of ammonium dihydrogen orthophosphate (ADP) solution (20 mM) and acetonitrile (1:1) flowing at a rate of 0.3 mL/min over Inertsil ODS-3 (50-mm × 2.1-mm, 2-μm particles) and Zorbax XDB (C-18, 50-mm × 2.1-mm, 1.8-μm particles) columns was employed for OLN. The drug and its impurities eluted with highly asymmetric peak shapes (USP tailing was more than 2.0), and Imp-8 was highly retained. The variation in the organic modifier proportions in the mobile phase also not produced a pure, symmetrical peak from the Inertsil and Zorbax columns.

A change in a strength of the salt (buffer) in the aqueous phase exerted imperceptible effects on the retention time, resolution and peak shape of the drug, while the use of acetonitrile in different ratios (30–80%) resulted in peak-tailing proportional to the acetonitrile (organic modifier) concentration. Replacing acetonitrile with methanol resulted in peak-fronting. Hence, a mixture of acetonitrile and methanol was employed to obtain the optimum peak shape. To reduce the run time, the mode was changed to gradient HPLC mode after changing the elution mode as gradient. Mobile phase A contained a mixture of 20 mM NaH_2PO_4–H_2O buffer (pH 3.0), acetonitrile and methanol in a ratio of 50:20:30 (v/v/v), while mobile phase B contained a mixture of water and acetonitrile in a ratio of 10:90 (v/v), flowing at a rate of 0.3 mL/min through a Waters Acquity BEH C18, 100 mm × 2.1-mm, 1.7-μm column to analyze OLN. Imp-1 and Imp-2, Imp-5 and Imp-6 were merged, and Imp-8 was highly retained at all of the aforementioned conditions. The separation of impurities was not completed at a satisfactory level.

The criticality was observed via the buffer pH and was extensively studied for the buffer pH optimization (Table 1). A buffer pH of 6.8 was found to be satisfactory; increasing the pH from 3.0 to 6.8 while keeping the other chromatographic parameters unchanged increased the sharpness of the peaks due to the increased hydrophobic interactions between the stationary phase

Table 2
System suitability parameters.

Compound	RT (min)[a]	RRT[b] ($n = 6$)[a]	USP resolution[c] ($n = 6$)[a]	USP tailing factor ($n = 6$)[a]
Imp-1	1.476	0.32 ± 0.01	–	1.1 ± 0.10
Imp-2	1.629	0.36 ± 0.02	2.35 ± 0.15	1.2 ± 0.10
Imp-3	1.856	0.41 ± 0.02	3.10 ± 0.10	1.2 ± 0.10
Imp-4	2.215	0.48 ± 0.02	4.30 ± 0.30	1.2 ± 0.09
Imp-5	3.351	0.72 ± 0.03	11.60 ± 0.40	1.1 ± 0.10
Imp-6	3.628	0.78 ± 0.03	2.35 ± 0.05	1.05 ± 0.05
Imp-7	4.317	0.95 ± 0.02	6.40 ± 0.30	1.05 ± 0.05
OLN	4.525	1.00 ± 0.00	2.20 ± 0.30	1.1 ± 0.09
Imp-8	7.740	1.69 ± 0.05	42.0 ± 2.0	1.1 ± 0.10

OLN, olanzapine.
[a] Mean ± SD ($n = 6$).
[b] Relative retention times (RRT) were calculated against the retention time (RT) of olanzapine.
[c] Resolutions were calculated between two adjacent peaks.

Table 3
LOD, LOQ, regression and precision data.

Parameter	Olanzapine (OLN)	Imp-1	Imp-2	Imp-3	Imp-4	Imp-5	Imp-6	Imp-7	Imp-8
LOD (μg/mL)	0.00014	0.00014	0.00015	0.00012	0.00014	0.00015	0.00017	0.00030	0.00014
LOQ (μg/mL)	0.00044	0.00048	0.00046	0.00036	0.00050	0.00045	0.00055	0.00095	0.00044
Regression equation (y)									
Slope (b)	128118	120777	133970	167023	151664	137832	154624	132041	68583
Intercept (a)	−23	125	267	178	596	592	59	178	293
Correlation coefficient	0.9997	0.9993	0.9996	0.9998	0.9992	0.9991	0.9997	0.9997	0.9992
R^2 value	0.9995	0.9985	0.9991	0.9995	0.9983	0.9982	0.9993	0.9993	0.9984
Precision (% RSD)	0.1	1.8	0.9	1.6	0.8	0.7	0.7	2.4	0.7
Intermediate precision (% RSD)	0.2	1.2	1.2	0.9	1.3	2.1	0.8	1.4	1.1

Linearity range is LOQ – 150% with respect to 50 μg/mL of olanzapine for impurities.

and the less unionized analyte. Using 20 mM NaH_2PO_4–H_2O and 2.0 mL of triethylamine (TEA) buffer (pH of 6.8), acetonitrile, and methanol in a ratio of 50:20:30 (v/v/v), while mobile phase B contained a mixture of water and acetonitrile in a ratio of 10:90 (v/v) with the gradient elution (*T*/%*B*) set as 0.01/0, 5.0/20, 6.5/90, 8.0/100, 9.0/0 and 10.0/0, enabled separation between Imp-1 and 2; Imp-5 and 6; OLN and all other related compounds (Rs > 2.0). The eluted analyte retention time was approximately 4.4 min. Interference with the excipients (placebo) was also checked, and no interference was observed between the impurity peaks and the OLN peak. Several preliminary chromatographic runs were performed to investigate the suitability for drug content estimation and cost because of the increasing importance of rapid economic analysis in pharmaceutical analysis to increase the throughput. The system suitability parameters were evaluated for OLN and its eight impurities. The USP tailing factor for all eight impurities and OLN was found to be less than 1.4. The USP resolution (Rs) of OLN and the eight potential impurities was greater than 2.0 between all pairs of compounds (Table 2).

4.2. Validation of the method

4.2.1. Precision

The RSD (%) in the repeatability of the OLN assay was within 0.9%. The % RSD of the peak area for the eight impurities in the repeatability results is shown in Table 3. The RSD (%) results of OLN and its impurities for intermediate precision (intra- and inter-day repeatability) are within 2.4%. These results confirmed the high precision of the method.

4.2.2. Limits of detection and quantification

The determined limit of detection, limit of quantification and precision at LOQ values for OLN and its eight impurities are reported in Table 3.

4.2.3. Accuracy

The recovery of OLN from pharmaceutical dosage forms ranged from 98.2 to 101.6%. The recovery of the eight impurities from pharmaceutical dosage forms ranged from 91.5 to 108.6%.

4.2.4. Linearity of response

For all eight impurities and OLN, a linear calibration curve was obtained ranging from LOQ to 0.3% (LOQ: 25%, 50%, 75%, 100%, 125%, 150% and 200%). The correlation coefficient obtained was greater than 0.9991 in both cases (Table 3). Linearity was determined over three consecutive days, which confirmed the linear relationship between the peak areas and concentrations. The linearity range is LOQ – 150% with respect to 50 μg/mL of OLN for impurities. The results indicate clear linearity.

4.2.5. Robustness

In all the deliberately varied chromatographic conditions (flow rate, column temperature, mobile phase composition and pH variation), all of the analytes were adequately resolved, and the order elution remained unchanged. The resolution between Imp-7 and OLN was greater than 2.0, and the resolution between Imp-1 and Imp-2 was greater than 2.5 for the varied conditions of flow rate (0.27, 0.33 mL/min) and column temperature (22 °C, 33 °C).

4.2.6. Stability in solution and in the mobile phase

The RSD (%) values for the OLN assay during solution stability and mobile phase stability experiments were within 1.5%. No significant changes in the amounts of the eight impurities were observed during solution stability (on bench top) and mobile phase experiments when performed by the related substances method. The results from the solution stability and mobile phase stability experiments confirmed that the standard solutions and solutions in the mobile phase were stable for up to 48 h during the assay and determination of related substances.

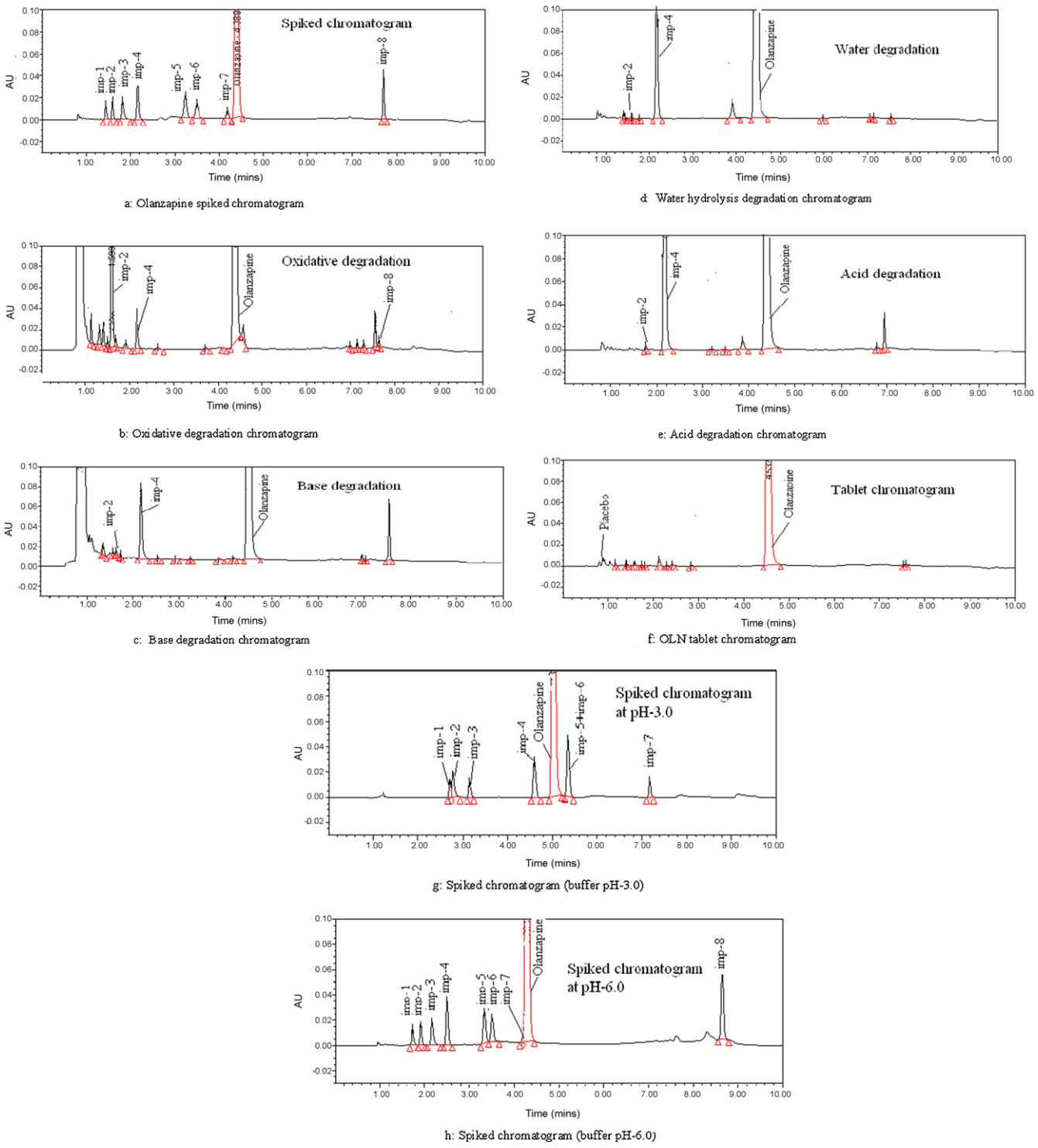


Fig. 2. Blend chromatogram of olanzapine and its impurities. (a) Olanzapine spiked chromatogram. (b) Oxidative degradation chromatogram. (c) Base degradation chromatogram. (d) Water hydrolysis degradation chromatogram. (e) Acid degradation chromatogram. (f) OLN tablet chromatogram. (g) Spiked chromatogram (buffer pH 3.0). (h) Spiked chromatogram (buffer pH 6.0).

4.2.7. Results from forced degradation studies

All forced degradation samples were analyzed at an initial concentration of 50 μg/ml OLN with the aforementioned UPLC conditions using a PDA detector to monitor the homogeneity and purity of the OLN peak. Degradation was not observed when OLN was subjected to light and heat conditions. Significant degradation was observed when the drug was subjected to hydrolysis (water at 60 °C for 1 h); base (0.5-N NaOH at 60 °C for 30 min); acid (0.1-N HCl at 60 °C for 10 min), leading to the formation of Imp-4; and oxidative hydrolysis (1.0% H_2O_2 at 60 °C for 10 min), leading to the formation of Imp-2 and Imp-4. All of the chromatograms for the force degradation studies are shown in Fig. 2. The impurities were confirmed by co-injecting Imp-2 and Imp-4 standards into the degraded samples and also by LC–MS/MS analysis. LC–MS/MS analysis was performed as per the experimental conditions (Section 2.3), and the masses of the impurities were 329 and 231, corresponding to the masses of

Table 4
Summary of forced degradation results.

Stress condition	% impurity formed									% assay	Mass balance[a]
	Imp-1	Imp-2	Imp-3	Imp-4	Imp-5	Imp-6	Imp-7	Imp-8	Total impurities		
Acid degradation	ND	0.03	ND	19.1	ND	ND	ND	ND	20.5	78.5	99.0
Base degradation	ND	0.10	ND	3.37	ND	ND	ND	ND	6.1	93.2	99.3
Water hydrolysis	ND	0.08	ND	6.22	ND	ND	ND	ND	7.7	91.0	98.7
Oxidative degradation	ND	8.26	ND	2.29	ND	ND	ND	0.28	17.1	81.0	98.1
Thermal degradation	ND	0.06	ND	0.07	ND	ND	ND	ND	0.20	99.5	99.7
Photolytic degradation	ND	0.07	ND	0.08	ND	ND	ND	ND	0.19	99.6	99.8

ND, not detected.
[a] Mass balance: (% assay + % sum of all compounds + % sum of all degradants).

Imp-2 and Imp-4, respectively. The results from the forced degradation studies are presented in Table 4.

Assay studies were carried out for the stress samples (at 5.0 μg/mL) against an OLN-qualified reference standard. The mass balance (% assay + % sum of all compounds + % sum of all degradants) results were calculated for all of the stressed samples and were found to be more than 98.1%. The purity and assay of OLN were unaffected by the presence of its impurities and degradation products, which confirms the stability-indicating power of the developed method.

5. Conclusion

The rapid gradient RP-UPLC method developed for quantitative analysis of OLN and related substances in pharmaceutical dosage forms is precise, accurate, linear, robust and specific. Satisfactory results were obtained from validation of the method. The retention time (10.0 min) enabled rapid determination of the drug. This method exhibited excellent performance in terms of sensitivity and speed. The method can indicate stability and can be used for the routine analysis of production samples and to check the stability of OLN samples, which may be useful for the study of *in vitro* dissolutions of pharmaceutical dosage forms.

Acknowledgement

The authors wish to thank the management of Dr. Reddy's group for supporting this work.

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