Development of a Stability-Indicating HPLC Method for Simultaneous Determination of Olanzapine and Fluoxetine in Combined Dosage Forms

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Abstract

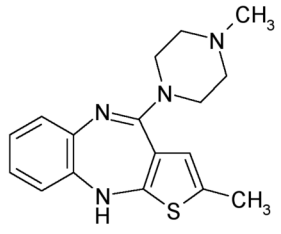
A stability-indicating high-performance liquid chromatography method is developed for analysis of olanzapine and fluoxetine in the presence of their degradation products generated from forced decomposition studies as prescribed by the International Conference on Harmonization. Hydrolysis, oxidation, photolysis, and thermal degradation are evaluated by subjecting the drug substances to stress conditions. Successful separation of drugs from degradation products is achieved on a reversed-phase C_{18} column using 75 mM potassium dihydrogen phosphate buffer (pH 4.0)–acetonitrile–methanol (55:40:5, v/v/v) as the mobile phase. The flow rate is 0.8 mL/min, and the detection wavelength is 227 nm. The method is validated with respect to linearity, precision, accuracy, system suitability, and robustness. The utility of the procedure is verified by its application to marketed formulations that are subjected to accelerated stability studies. Good separation of the drugs and their degradation products is observed using this method. The products formed in marketed tablet dosage forms are similar to those formed in standard drug solutions under similar stress conditions.

Introduction

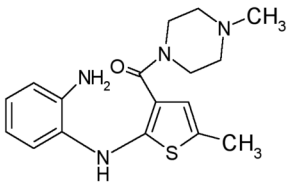
Stability testing and stress testing (forced degradation studies) are critical components of drug development strategy (1). The studies help us understand the mechanism of a drug's decomposition, which further helps in obtaining information on physical and chemical factors that result in instability (2). These factors are then controlled in order to stabilize the drug or drug formulation, resulting in increased shelf-life or improved efficacy. Stress testing is defined as the stability testing of drug substances and drug products under conditions exceeding those used for accelerated testing. These studies are undertaken to elucidate the intrinsic stability of the drug substance. According to International Conference on Harmonization (ICH) guideline Q1A (R2), the stability testing of drug substances should be carried out under different stress conditions (hydrolysis, oxidation, photolysis, and thermal degradation) to validate the stability-indicating supremacy of analytical methods used for the analysis of stability samples (3). The standard conditions for photo stability testing are described in ICH guideline Q1B (4).

These tests allow accurate and precise quantification of drugs and their degradation and interaction products.

Olanzapine (OLANZ, Figure 1) is an antipsychotic drug, chemically a thienobenzodiazepine described as 2-methyl-4-(4-methyl-1-piperazinyl)-10*H*-thieno[2,3-*b*][1,5]benzodiazepine (5), and fluoxetine (FLUOX) (Figure 2) is an antidepressant agent, selective serotonin re-uptake inhibitor, chemically described as (±)-N-methyl-3-phenyl-3-[α,α,α,-trifluoro-*p*-tolyl)oxy]propylamine (6). The combination of OLANZ and FLUOX is beneficial for treatment-resistant depression (7),



OLANZ



[2-(2-Amino-phenylamino)-5-methyl-thiophen-3-yl]-(4-methylpiperzin-1-yl)methanone

Figure 1. Chemical structure of OLANZ and its possible degradation product.

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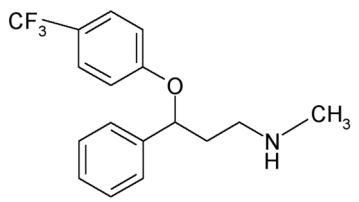
psychotic depression (8), and bipolar depression (9).

There are several high-performance liquid chromatography (HPLC) methods for the determination of OLANZ (10–14) and FLUOX (15–21), individually. HPLC (22) and HPLC–tandem mass spectrometry (23) methods have been reported recently for the combination of the two drugs. So far, to our present knowledge, no stability-indicating assay method has been reported for the simultaneous determination of OLANZ and FLUOX in the presence of their degradants using the ICH approach of stress-testing. The focus of the present study was to develop a simple, rapid, precise, and accurate isocratic reversed-phase stability-indicating HPLC method for the simultaneous determination of OLANZ and FLUOX in tablet dosage form.

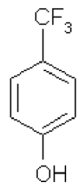
Experimental

Materials and chemicals

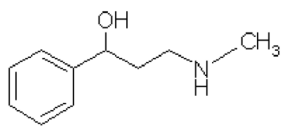
Pure OLANZ and FLUOX were procured as gift samples from Dr. Reddy's Laboratories Limited (Hyderabad, India). Olanex F tablets (containing OLANZ 5 mg and FLUOX 20 mg per tablet) were manufactured by Ranbaxy Laboratories Ltd. (Secunderabad, India). HPLC-grade acetonitrile, methanol, and water were purchased from Spectrochem Pvt. Ltd. (Mumbai, India). Potassium dihydrogen phosphate (Rankem, Mumbai, India) and ortho phosphoric acid (Qualigens Fine Chemicals, Mumbai, India) were analytical reagent grade.



FLUOX



p-Trifluoromethylphenol



α-[2-(Methylamino)ethyl]benzene methanol

Figure 2. Chemical structure of FLUOX and its possible degradation products.

Apparatus

Chromatography was performed on a Shimadzu (Shimadzu Corporation, Kyoto, Japan) chromatographic system equipped with an isocratic HPLC pump (Shimadzu LC-20AT) and a UV-visible detector (Shimadzu SPD-20AV) with a Rheodyne syringe-loading sample fixed loop (20 µL) injector (7725). The LC separations were performed at ambient temperature on a Phenomenex Luna C18 (250 × 4.6 mm, 5 µm) column (Torrance, CA). Data were acquired and processed by the use of Spinchrom (CFR version 2.4.1.93) software. Degassing of the mobile phase was done by sonication in an Ultrasonic bath (Ultrasonics Selec, Vetra, Italy). The standard substances were weighed on a Precisa (205 ASCS Swiss Quality, Switzerland) analytical balance. Photostability studies were carried out in a photostability (NEC-103R Newtronic, Mumbai, India) chamber, which was set at 25ºC ± 1ºC. The photostability chamber was equipped with an illumination bank on inside top as defined under option 2 in the ICH guideline Q1B (4). The light bank consisted of a combination of one black light UV lamp set at UV 200 watt/sq. meter and four white fluorescent lamps set at 1200 Klux Hrs. The samples were placed at a distance of 9 inches from the light bank. Both fluorescent and UV lamps were found on simultaneously. The samples were exposed for a total period of 15 days.

Peak purity analysis was carried out on another HPLC system (all equipment from Waters, Milford, MA), equipped with a 2996 photo-diode array (PDA) detector. Thermal stability study was carried out in a hot air oven (Sedko Laboratory Equipments, Ahmedabad, India).

Chromatographic separations

HPLC studies were individually carried out for all the reaction solutions, and in a mixture of the solutions in which decomposition was observed. The separation was carried out under isocratic elution with potassium dihydrogen phosphate buffer (75 mM, pH 4.0 adjusted with 0.5% orthophosphoric acid)–acetonitrile–methanol (55:40:5 v/v/v) as the mobile phase. The mobile phase was filtered through a 0.45-µm nylon filter and degassed before use. The flow rate was 0.8 mL/min, and the detection wavelength was 227 nm.

Preparation of standard solutions

Individual standard stock solutions of OLANZ (250 µg/mL) and FLUOX (1000 µg/mL) were prepared in methanol. These stock solutions were further diluted to obtain concentrations in the range of 5–80 µg/mL and 20–320 µg/mL for OLANZ and FLUOX, respectively. All dilutions from the stock solutions were made using the mobile phase.

Method validation

Linearity was established by triplicate injections of solutions containing standard OLANZ and FLUOX in the concentration ranges of 5–80 µg/mL and 20–320 µg/mL, respectively. The limit of detection (LOD) and limit of quantification (LOQ) values were calculated from the calibration curves as k = SD/b where k = 3 for LOD and 10 for LOQ. SD is the standard deviation of the response of the minimum detectable drug con-

centration and b is the slope of the calibration curve (24). The intra- and inter-day precisions of the proposed method were determined by estimating the corresponding responses 3 times on the same day and on 3 different days using 3 different concentrations of OLANZ (5, 25, and 75 µg/mL) and FLUOX (20, 100, and 300 µg/mL). From the area obtained, concentration was calculated, and the results were expressed as % relative standard deviation (RSD). Intermediate precision was established through separation studies on two different columns, and % RSD of retention time was calculated. Accuracy of the method was evaluated by spiking the three different concentration levels of standard solutions of OLANZ (20, 25, and 30 µg/mL) and FLUOX (80, 100, and 120 µg/mL) in a mixture of stressed sample. The % recovery of the added drugs was determined. The specificity of the method was established through a study of the resolution factors of the drug peaks from the nearest resolving peak and also among all other peaks. Specificity of the method towards the drugs was also established through determination of the purity of OLANZ and FLUOX peaks in a mixture of stressed samples through the study of purity plots using a PDA detector.

Robustness of the method was determined by deliberately varying certain parameters like flow rate (mL/min), concentration of acetonitrile (mL) in the mobile phase, and manufacturer of acetonitrile. Each parameter was studied at three levels (–1, 0, and 1), except for the acetonitrile, where two different manufacturers were studied. One factor at a time was changed to estimate the effect. The assay was carried out in triplicate (n = 3) at three different concentration levels: 5, 25, and 75 µg/mL and 20, 100, and 300 µg/mL for OLANZ and FLUOX, respectively. In the system suitability tests, six replicate injections of freshly prepared working standard solutions of OLANZ and FLUOX (50 µg/mL each) and two injections of the solutions prepared for the specificity procedure were injected into the chromatograph, and the % RSD of peak areas, resolution factor, tailing factor, and theoretical plates were determined.

Forced degradation studies

All drug solutions used in forced degradation studies were prepared by dissolving pure drugs or drug products in small volumes of methanol and later diluted with either 3% H_2O_2, distilled water, 0.1 M HCl, or 0.1 M NaOH to achieve a concentration of 500 µg/mL each of OLANZ and FLUOX. Solutions in water, 0.1 M HCl, and 0.1 M NaOH were heated at 80ºC for 12 h and 24 h, respectively. For oxidative degradation, drugs were stored at room temperature in 3% H_2O_2 for 24 h. Degradation was also carried out in solid state by exposing pure drugs and drug products to dry heat at 80ºC for 24 h. For photolytic studies, drug solutions in water, 0.1 M HCl, and 0.1 M NaOH were exposed in a photostability chamber for 15 days. Also, solid drugs and drug products were spread in a petri plate in 1-mm thickness and exposed in the photostability chamber for the same time period. Suitable controls were maintained under dark conditions. Samples were withdrawn periodically and diluted with mobile phase to yield starting concentrations of 50 µg/mL for both OLANZ and FLUOX. The samples of acid and base hydrolysis were neutralized suitably.

Identification of major degradation products

OLAZ mainly degrades under acidic hydrolysis and acidic photolysis. Though no efforts have been made to identify the degradation product, the expected product is [2-(2-aminophenylamino)-5-methyl-thiophen-3-yl]-(4-methylpiperzin-1-yl)methanone, shown in Figure 1.

The literature has suggested that α-[2-(methylamino) ethyl] benzene methanol and *p*-trifluoromethylphenol are the main degradation products (Figure 2) under acidic stress and acidic photolytic conditions, and their formation was confirmed by spiking the degraded sample with the standard (25).

Analysis of marketed tablet subjected to stress testing

Olanex F tablets containing OLANZ (5 mg) and FLUOX (20 mg) were subjected to accelerated test conditions which were similar to those applied to the standard mixture of OLANZ and FLUOX, as mentioned previously in "Forced degradation studies." The contents of the tablets were transferred to a 100-mL volumetric flask and the volume was made up to 100 mL with methanol. The resulting solution was filtered through the 0.45-µm nylon filter paper and subjected to analysis.

Results and Discussion

HPLC method development and optimization

Different parameters like buffer, organic modifier ratio, and pH were optimized to achieve good separation between OLANZ and FLUOX, and the degradation products formed under various conditions. Initial studies on individual reaction solutions were carried out using buffer–acetonitrile (50:50 v/v) as the mobile phase. Several studies were carried out by decreasing the percentage of acetonitrile from 50% to 35%, until satisfactory resolution was obtained. Another attempt was made by substituting acetonitrile with methanol. The advantages observed were smoothening of baseline and well-resolved peaks. It was found that good resolution was obtained with 55% buffer, 40% acetonitrile, and 5% methanol. Decreasing the pH resulted in shorter retention times and sharper peaks. A pH of 4 gave sufficient separation as well as symmetrical peak shape. Finally, the mobile phase comprising of potassium dihydrogen phosphate buffer (75 mM; pH 4.0 adjusted with 0.5% orthophosphoric acid)–acetonitrile–methanol (55:40:5 v/v/v) was used to analyse individual stressed samples. It was then applied to a mixture of those stressed samples in which there was recognizable degradation and/or different degradation products were formed. The method worked well with the mixture of degradation solutions and was even applicable to degraded formulations. Figures 3, 4A–4F, and 5 show the chromatographic resolution of the standard synthetic mixture, mixture of stressed samples, and a degraded formulation, respectively.

Validation of the method

The method was validated with respect to following parameters given in the following.

Linearity. Linear calibration plots of each drug for the pre-

viously mentioned method were obtained over the calibration ranges 5–80 µg/mL and 20–320 µg/mL for OLANZ and FLUOX, respectively; the correlation coefficient obtained was greater than 0.999 for both drugs (Table I). The results show that good correlation existed between the peak area and concentration of the analyte.

LOD and LOQ. The LOD values for OLANZ and FLUOX were 0.0345 µg/mL and 0.5736 µg/mL, and the LOQ values for OLANZ and FLUOX were 0.1151 µg/mL and 1.9121 µg/mL, respectively (Table I).

Precision. Data obtained from analysis of the samples on the same day (n = 3) and on consecutive days (n = 3) are given in Table II. As evident, the % RSD values of the data obtained were well below 2% (i.e., in the range of 0.18–0.71% and 0.12–0.79% for intra- and inter-day, respectively). The % RSD values indicate that the method was sufficiently precise. An intermediate precision was established for the method. It showed that similar resolution was possible on repeating the experiment on two different reversed-phase HPLC columns (Table III).

Accuracy. Percentage recovery was calculated from differences between the peak areas obtained for fortified and unfortified solutions. As shown from the data in Table IV, good recoveries were made at each added concentration, confirming that the method was accurate.

Specificity. Good resolution was obtained between the drugs and the degradation products formed under the various stress conditions, indicating the specificity of the method

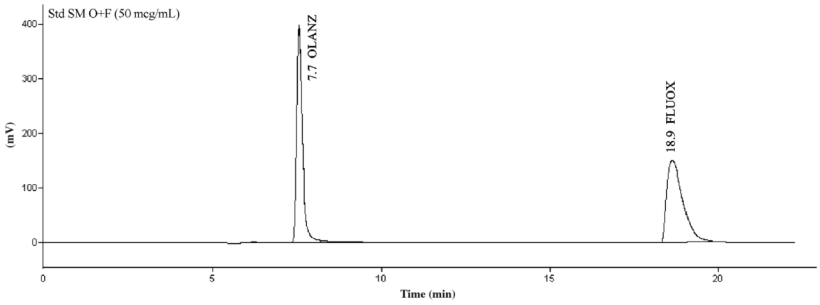


Figure 3. Chromatograms showing the separation of OLANZ (50 µg/mL) and FLUOX (50 µg/mL) in synthetic mixture on a C_{18} column, using 75 mM potassium dihydrogen phosphate buffer (pH 4)–acetonitrile–methanol (55:40:5 v/v/v) as the mobile phase.

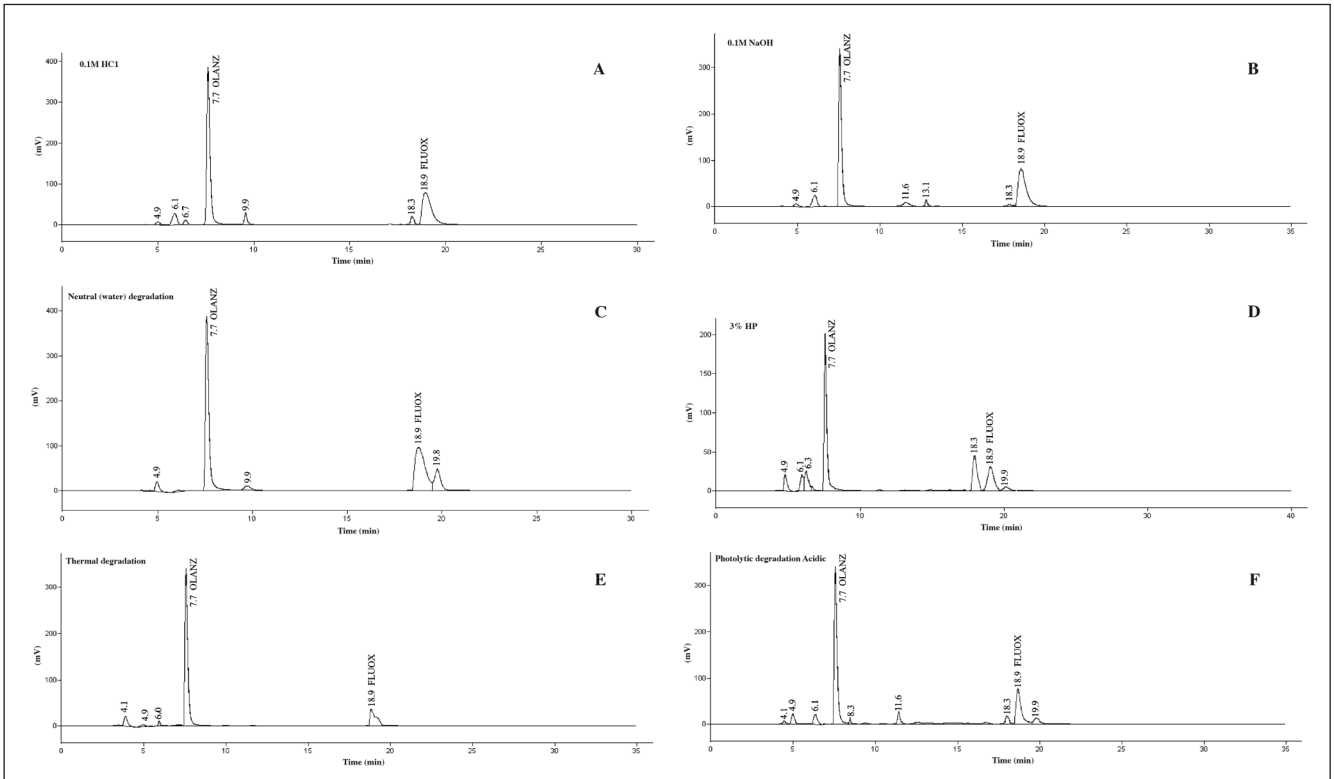


Figure 4. Chromatogram showing the acid hydrolysis (0.1 M HCl) of OLANZ and FLUOX (50 µg/mL each) at 80°C: mobile phase, 75mM potassium dihydrogen phosphate buffer (pH 4)–acetonitrile–methanol (55:40:5 v/v/v) and flow rate, 0.8 mL/min (A). Chromatogram showing the base hydrolysis (0.1 M NaOH) of OLANZ and FLUOX (50 µg/mL each) at 80°C: mobile phase, 75mM potassium dihydrogen phosphate buffer (pH 4)–acetonitrile–methanol (55:40:5 v/v/v) and flow rate, 0.8 mL/min (B). Chromatogram showing the hydrolysis under neutral condition (distilled water) of OLANZ and FLUOX (50 µg/mL each) at 80°C: mobile phase, 75mM potassium dihydrogen phosphate buffer (pH 4)–acetonitrile–methanol (55:40:5 v/v/v) and flow rate, 0.8 mL/min (C). Chromatogram showing the oxidative degradation (3% H_2O_2) of OLANZ and FLUOX (50 µg/mL each) at room temperature: mobile phase, 75mM potassium dihydrogen phosphate buffer (pH 4)–acetonitrile–methanol (55:40:5 v/v/v) and flow rate, 0.8 mL/min (D). Chromatogram showing the thermal decomposition of OLANZ and FLUOX (50 µg/mL each): mobile phase, 75mM potassium dihydrogen phosphate buffer (pH 4)–acetonitrile–methanol (55:40:5 v/v/v) and flow rate, 0.8 mL/min (E). Photolytic decomposition of OLANZ and FLUOX (50 µg/mL each) in acidic condition: mobile phase, 75mM potassium dihydrogen phosphate buffer (pH 4)–acetonitrile–methanol (55:40:5 v/v/v) and flow rate, 0.8 mL/min (F).

(Figures 4A–4F). The resolution factor (Rs) from acidic, alkaline, neutral, oxidative, and thermal degradation products was always ≥ 1.8, which ensured complete separation of OLANZ and FLUOX from their degradation products.

Studies performed to determine the purity of OLANZ and FLUOX peaks using a PDA detector showed purity angle (PA) values of 0.074 and 0.063 and purity threshold (TH) values of 0.256 and 0.272 for OLANZ and FLUOX, respectively. The PA value was found to be less than the TH value, indicating that the OLANZ and FLUOX were free from any co-eluting peak.

Robustness. The results presented in Table V indicate that the selected factors remained unaffected by slight variation of these parameters. It was also found that acetonitrile from the different manufacturers does not have significant influence on the determination. Insignificant differences in peak areas and less variability in retention times were observed.

System suitability. The results (Table VI) obtained from system suitability tests are in agreement with the United States Pharmacopoeia requirements (24). The variation in retention times among six replicate injections of OLANZ and FLUOX standard solutions was very low, rendering RSD of 0.49% and 0.34 %, respectively.

Forced degradation studies

Conditions used for forced degradation were attenuated to achieve degradation in the range of 20–80%. The following degradation behavior of the drugs was observed during the previously mentioned mentioned HPLC studies.

Acidic conditions. The combination of drugs was subjected to heating in 0.1N HCl for 12 h. Approximately 20% degradation was observed in OLANZ, and ~ 15% degradation was observed in FLUOX. On further heating up to 24 h, no new degradation peaks were observed. There was no rise in the proportion of the already degraded peaks. Among the drugs in the combination, OLANZ was found to be more acid labile in comparison to FLUOX. The major degradation products formed in combination of OLANZ were at retention times (RTs) 4.9, 6.1, and 9.9 min, while for the FLUOX a degradation peak was found at RT 18.3 min (Figure 4A).

Degradation in alkali. The degradation patterns of OLANZ as well as FLUOX in alkaline conditions were found to be similar to a great extent to the acidic condition, along with few more peaks at different RTs. The major degradation products of OLANZ were at RTs 4.9, 6.1, and 11.6 min, while for the FLUOX a major degradation peak was found at RT 13.1 min and a small peak was at 18.3 min (Figure 4B).

Neutral (water) conditions. Upon refluxing the drug combination in water at 80ºC for 24 h, sufficient degradation was observed in both drugs. The degradation products of OLANZ appeared at RTs 4.9 and 9.9 min, whereas mild degradation was seen in FLUOX, with the appearance of a single peak at 19.8 min (Figure 4C).

Oxidative conditions. Both drugs were found to be highly labile to oxidative hydrolysis in 3% H_2O_2 at room temperature after 24 h. FLUOX was comparatively more labile than OLANZ. Approximately 30% degradation was observed in the case of OLANZ, while the degradation of FLUOX was found to be more than 60%. The major degradation products of OLANZ were at RTs 4.9, 6.1, and 6.3 min, while for the FLUOX, degradation peaks were found at RTs 18.3 and 19.9 min (Figure 4D).

Solid-state study. The thermo-labile property of FLUOX was clearly observed when it was exposed to dry heat at 80ºC for 24

Table I. Statistical Results of Calibration Graphs Obtained by HPLC Method

Drug	Range (µg/mL)	Regression equation	*r*	SD		LOD (µg/mL)	LOQ (µg/mL)
				Slope	Intercept		
OLANZ	5–80	$A = 74.62C_{OLANZ} + 5.7372$	0.9999	1.9860	0.2751	0.0345	0.1151
FLUOX	20–320	$A = 46.728C_{FLUOX} + 781.86$	0.9994	0.6874	1.1254	0.5736	1.9121

Table II. Intra- and Inter-Day Precision Studies (*n* = 3)

Drug	Added (µg/mL)	Intra-day		Inter-day	
		Measured (µg/mL) ± SD; RSD (%)	Standard error	Measured (µg/mL) ± SD; RSD (%)	Standard error
OLANZ	5	5.01 ± 0.03; 0.69	0.015	4.98 ± 0.07; 0.14	0.005
	25	24.74 ± 0.13; 0.53	0.058	24.42 ± 0.17; 0.71	0.122
	75	74.70 ± 0.50; 0.67	0.225	73.93 ± 0.16; 0.21	0.112
FLUOX	20	19.84 ± 0.14; 0.71	0.099	19.97 ± 0.16; 0.79	0.071
	100	99.18 ± 0.69; 0.75	0.308	98.92 ± 0.65; 0.66	0.463
	300	299.16 ± 0.53; 0.18	0.235	298.60 ± 0.35; 0.12	0.248

Table III. Intermediate Precision Studies (*n* = 3)

Column	Retention time (min) ± RSD (%)	
	OLANZ	FLUOX
Phenomenex Luna—C18	7.55 ± 1.712	18.73 ± 0.912
Waters—C18	7.21 ± 1.134	18.3 ± 0.446

Table IV. Recovery Studies of OLANZ and FLUOX

Drug	Added concentration (µg/mL)	% Recovery ± SD; RSD (%)	Standard error
OLANZ	20	98.71 ± 0.31; 1.58	0.221
	25	99.41 ± 0.34; 1.38	0.243
	30	102.15 ± 0.59; 1.92	0.415
FLUOX	80	100 ± 0.13; 0.16	0.221
	100	99.88 ± 0.36; 0.38	0.253
	120	99.96 ± 0.40; 0.39	0.225

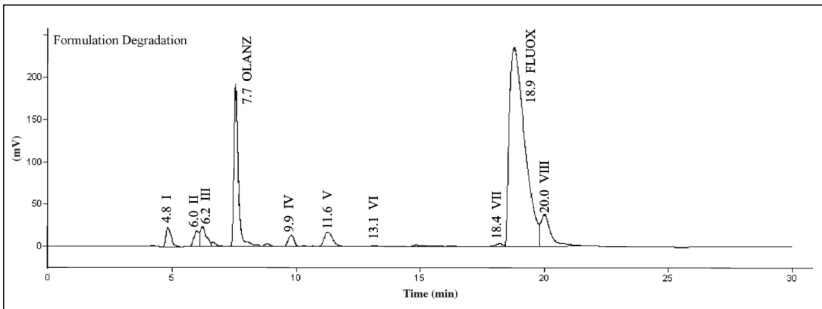


Figure 5. Chromatograms showing separation of OLANZ and FLUOX in degraded formulation. I: formed in acidic, alkali, neutral, oxidative, thermal and photolytic conditions; II: formed in acidic, alkali, oxidative and photolytic conditions; III: formed in oxidative condition; IV: formed in acidic and neutral condition; V: formed in alkali and photolytic conditions; VI: formed in alkali; VII: formed in acidic, alkali, oxidative and photolytic conditions; VIII: formed in oxidative and neutral condition.

Table V. Robustness Studies of OLANZ and FLUOX (*n* = 3)

		Retention time (Mean ± % RSD)		Asymmetric factor (Mean ± % RSD)	
Factor	Level	OLANZ	FLUOX	OLANZ	FLUOX
A: Flow rate (mL/min)					
0.7	–1	7.82 ± 1.25	19.56 ± 0.92	1.319 ± 0.90	1.391 ± 0.79
0.8	0	7.65 ± 0.32	18.8 ± 0.52	1.321 ± 0.22	1.395 ± 0.28
0.9	1	7.43 ± 0.99	18.54 ± 1.18	1.328 ± 0.37	1.413 ± 0.38
B: % of ACN in mobile phase					
50	–1	7.83 ± 1.11	19.19 ± 0.46	1.324 ± 0.45	1.393 ± 0.40
55	0	7.61 ± 0.72	18.82 ± 0.19	1.321 ± 0.61	1.381 ± 0.65
60	1	7.47 ± 0.64	18.43 ± 0.18	1.318 ± 0.30	1.381± 0.42
C: ACN of different companies					
RANKEM		7.7 ± 0.91	18.87 ± 0.11	1.322 ± 0.23	1.384 ± 0.29
SPECTROCHEM		7.8 ± 0.77	18.9 ± 0.16	1.324 ± 0.15	1.382 ± 0.22

Table VI. System Suitability Parameters

	Drug	
Parameters	OLANZ	FLUOX
RT (min ± SD)	7.64 ± 0.011	18.83 ± 0.015
Resolution factor	–	11.191
Tailing factor ± SD	1.33 ± 0.004	1.36 ± 0.003
Theoretical plates ± SD	48613 ± 1.43	68397 ± 1.03
% RSD	0.49	0.34

Table VII. Analysis of Tablets Containing OLANZ and FLUOX in Combination (*n* = 3)

Tablet	Drug (mg/tab)	% Drug obtained ± SD	Std error of estimation
Olanex F	OLANZ (5 mg)	97.31 ± 0.16	0.114
	FLUOX (20 mg)	99.38 ± 0.15	0.109

h. Profound degradation in FLUOX was seen from its diminished peak, but no degradation peaks were observed in the chromatograms. The degraded products could be hydrophobic in nature, as no peak could be observed even after increasing the acetonitrile concentration or after changing the detection wavelengths. Thus degraded products were non-chromatographic. On the other hand, OLANZ was found to be relatively stable in the study, as only minute degradation peaks were observed at RTs 4.1, 4.9, and 6.0 min (Figure 4E).

Photolytic conditions. OLANZ was found to be labile on exposure to light in acid, alkali, or neutral conditions. The decomposition pattern of OLANZ was found to be similar in all these conditions, whereas in FLUOX sufficient degradation was observed. The major degradation peaks of OLANZ were at RTs 4.1, 4.9, 6.1, 8.3, and 11.6 min, while for FLUOX, degradation peaks were found at RTs 18.3 and 19.9 min. The rate of photolysis was in the order of acid > base > water (Figure 4F).

Applicability of the developed method to marketed formulation

The developed method was successfully applied to analyze OLANZ and FLUOX in marketed tablet formulation. A clear separation of the drugs and degradation products was achieved in the tablet with no interference from excipients. In almost all the cases, chromatographic pattern was similar to the one shown in Figure 5, which indicates that the method could be extended for the study of available drug content in commercial products. The data in Table VII indicates that OLANZ is more prone to degradation, as the assay was less than the label claim in tablet. In comparison, FLUOX was less affected.

Conclusion

In this study, OLANZ and FLUOX were subjected to stress studies under various ICH-recommended conditions. The additional findings in this study show that the drugs undergo extensive degradation under acidic, photolytic, and oxidative stress conditions, degrade to a mild extent in basic and neutral conditions, and are stable to thermal stress. The drug can be analysed specifically in the presence of different excipients and degradation products by using the developed chromatographic conditions. The method was validated for parameters like linearity, precision, accuracy, specificity, robustness, and system suitability. Application of this method for the analysis of OLANZ and FLUOX in tablet dosage form shows that there is no interference of excipients or degradation products in the

analytical determination. Thus, the proposed method could be used as a stability-indicating method for the simultaneous determination of OLANZ and FLUOX either in bulk drug or in pharmaceutical formulations.

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