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Validated Stability Indicating UHPLC Method for the Quantification of Escitalopram and Flupentixol in Pharmaceutical Formulation

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Abstract: To assess Escitalopram and flupentixol simultaneously, a verified method for ultra-phase high-performance liquid chromatography (UHPLC) has been developed to indicate stability. The method was thoroughly evaluated and met satisfactory criteria for precision, linearity, accuracy, limits on detection, robustness, and quantitation. The quantitation wavelength of 235 nm was determined. Linearity was successfully demonstrated across concentration ranges of 1–5 µg/ml of Escitalopram and 20-100 µg/ml of Flupentixol. UHPLC separations were conducted employing a Phenomenex L. C18 column measuring 100 x 4.6 mm and containing particles as small as 2.5 µm. To create the mobile phase, the 1% OPA and methanol (a pH of 4.2 with TEA) were combined in a volumetric ratio of 65:35v/v. Escitalopram and Flupentixol were effectively eluted at retention durations of 3.044 and 4.118 minutes, respectively, with the flow rate adjusted at 1.0 ml. The stability-indicating nature of the method was established through validated forced degradation studies. Which included hydrolysis under acidic and basic conditions, exposure to H_2O_2, thermal degradation, and photo-degradation. Escitalopram and Flupentixol exhibited 10 to 20% degradation under the specified conditions. Importantly, the process evaluated the two prescription drugs in detail with all degradation products generated during the forced degradation experiments. This developed method is characterized as straightforward, specific, and cost-effective, making it suitable for simultaneous estimating Escitalopram and Flupentixol in tabs dose forms.

Introduction

Escitalopram oxalate (ESC) is utilized in the treatment of depression and anxiety, functioning by restoring the balance of serotonin, a natural substance in the brain. Flupentixol HCI (FLU) is employed to alleviate symptoms associated with Schizophrenia and other mental health disorders. Numerous analytical methods for evaluating pharmaceutical drugs in various formulations. A comprehensive literature review has disclosed a range of analytical methods for estimating ESC alone and in combination with other drugs. Similarly, different methods are available for determining FLU alone and in combination with other drugs. Although there is a UV-spectroscopic method for Simultaneous determination of ESC (escitalopram) and FLU (flupentixol) in a combined dosage form (Goulikar et al., 2022; Patel et al., 2016; Darthi et al., 2018; *Singh* et al., 2016; Sakhreliya et al., 2012) also it was achieved using RP-HPLC for Analysis of ESC and FLU in combined dosage form and with other drugs reported in the literature (Panchale et al., 2021; Sellappan et al., 2021; Damor et al., 2017; Kakde et al., 2013; Kadam et al., 2022; *Stefan* et al., 2022; Beula et al., 2022; Nagar et al., 2015; Bindusar et al., 2019; Kumar et al., 2022; Nagar et al., 2015). HPTLC method is also available for determining FLU and ESC in combined dosage form (Malathi et al., 2022). Validated analytical method development for other combination by Rp-HPLC (Gosavi et al., 2023) with stability by Rp-HPLC method for other drugs which is referred for stability parameters (Dey et al., 2020; Deshpande et al., 2023). None have encompassed complete validation according to ICH

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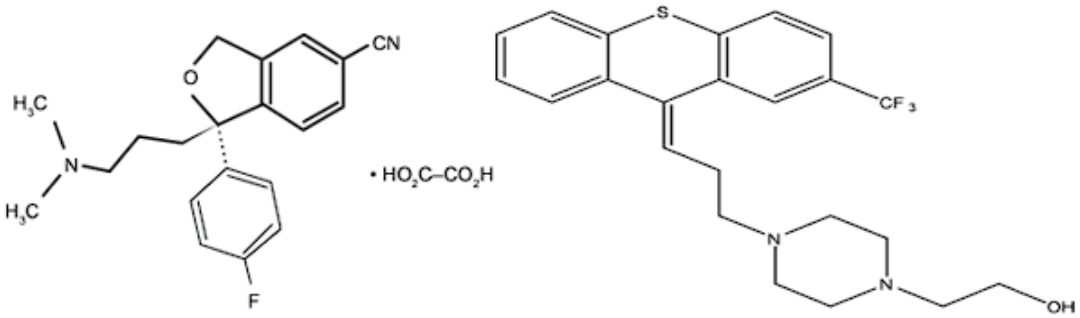
guidelines. In light of this, endeavours have been made to establish a novel RP-HPLC method for simultaneously determining ESC and FLU in tablet dosage form. This study describes Ultra High-Performance Liquid Chromatography (UHPLC), a well-established stability-indicating technique and methodology towards estimating Esc and Flu simultaneously when their degradation products are present. The proposed strategy is described in terms of simple, accurate, repeatable, and stability-indicating. It functions correctly for routinely determining ESC and FLU in combination dose forms. The validation process adhered to the ICH guidelines, ensuring the method's reliability and robustness for pharmaceutical applications. The structure of Escitalopram and Flupentixol is shown in figure 1.



Escitalopram **Flupentixol**

Figure 1. Structure of Escitalopram and Flupentixol.

Materials and Methods

Materials

Pharmaceutical grade Escitalopram and Flupentixol were generously provided as gift samples by Swapnaroop Drugs and Pharmaceuticals located in Sambhajinagar (431003), Maharashtra, India. Various chemicals and reagents were used in the study: Rankem in New Delhi provided sodium hydroxide, Fischer Scientific in Mumbai provided potassium dihydrogen phosphate, Himedia in Mumbai provided the nylon sixty-six filter membrane of (0.45µ), and Mumbai-based Loba Chemie Pvt. Ltd. supplied H_2O_2 For the analysis of the marketed formulation, REXIPRA FX 10 tablets including 0.5mg for Flupentixol and 10 mg for Escitalopram were acquired from a local pharmacy and used as the sample for the study.

Instrumentation

The HPLC system utilized in this study was an AGILENT (1100) equipped with a UV detector and a 20 µl fixed loop for injections. Applying LC Solution software, a chromatographic analysis was conducted on 4.6 mm in the centre and 100 mm in length in a column. Additionally, a variety of analytical tools were used in the experimental procedures, including a hot air oven (Biotech) and a pH indicator (Frontline FS 4, Mumbai, India).

Method Development

Various mobile phases were experimented with, incorporating combinations of different proportions of methanol, water, acetonitrile, and buffers. Ultimately, A 0.1% trifluroacetic acid (TFAA) and triethylamine (TEA) adjusted to pH 4.2 in a 65:35% v/v ratio was selected as the mobile phase. This selected mobile phase demonstrated excellent peak parameters obtained with an acceptable resolution for both escitalopram and flupentixol. System Suitability Studies were carried out, and Table 1 presents the calculated values for resolution, number of theoretical plates, and peak asymmetry from the standard solutions. These results affirm the system's suitability for the simultaneous analysis of these drugs.

Table 1. System Suitability Studies

Parameter	Method
Column	id 4.6 x100 mm length
Particle size	2.5µm
Stationary phase	$C1_8$(AGILENT)
Mobile Phase	methanol: 0.1 % Tri fluoro acetic acid
Detection Wavelength	235 nm
Flow rate	0.7 ml/min
Temperature	33
Size of sample	20 µl

Preparation of standard solutions

1. Working standard 10 mg of escitalopram weighed and transferred to 100 ml volumetric flasks and diluted with mobile phase to 100 ml gives a final 100 µg/ml concentration used as standard stock solution.
2. Working standard 0.5 mg of flupentixol weighed and transferred to 100 ml volumetric flask and diluted with mobile phase to 100 ml, giving a final

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concentration of 5 µg/ml used as standard stock solution.

Table 2. Results of assay experiment.

Drug	Labeled claim (mg)	Amount found (n=6)	%RSD	%Assay
Flupentixol	4	4.03	1.45	100.87
Escitalopram	80	78.56	1.42	98.20

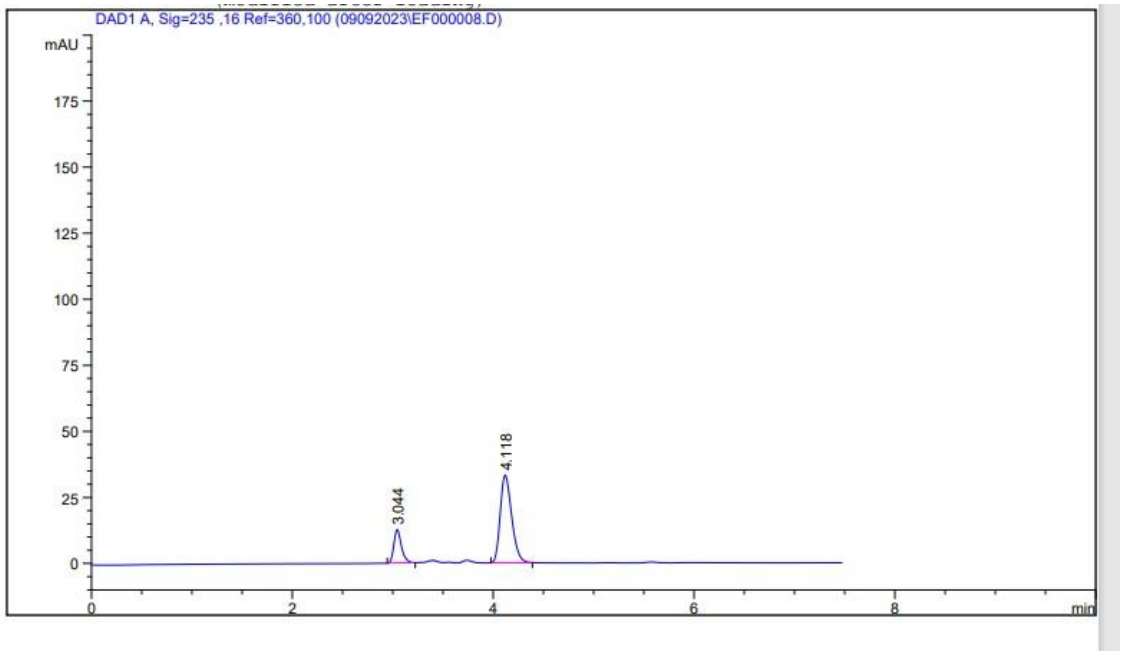

Figure 2. Chromatogram of Flupentixol (3.044 min) and Escitalopram 4.118 min, respectively.

Preparation of Sample Solutions

The method is effectively working for the analysis of a commercial sample. Twenty (20) tablets were assessed and their average weight was determined to be 18 gms. An equivalent weight of 10 mg is 18 mg. Next the tablet was ground into a uniform size, and 18 milligrams drug was dissolved in 100 millilitres of methanol to give final concentration of 50 µgm/ml Flupenthixol and 1000 µgm/ml Esitalopram. This solution underwent 15 minutes of sonication and 5 minutes of cyclomixing to ensure the extraction of the drug into the solution. The stock concentration A Millipore syringe filter (0.42µ) was then used to filter the resulting solution. Following the specified protocol, the cleared solution was subsequently injected into the HPLC system twice. It proposed method exhibited specificity, with no observable interference resulting from typical excipients in tablets such as lactose. Further 0.8 ml solution was pipetted out from the stock solution and diluted to 10 ml, which gives final concentration of 4 µgm/ml of Flupenthixol and 80 µgm/ml Escitalopram. The assay was calculated and the results are shown in table 2. The analysis results indicate a close alignment between the quantities of drugs found and the labelled claims of the formulations. This alignment attests to the analytical method's precision and consistency employed, confirming its suitability for evaluating the composition of the studied formulations. Chromatogram of Flupentixol and Escitalopram is shown in figure 2.

Method Development

In adherence with the ICH guidelines, the validation process of the method encompassed assessing key parameters, including limits on quantitation, detection, linearity, accuracy, precision, and resilience. These evaluations collectively ensure the method's suitability for its planned application, confirming its ability to generate precise, accurate, and reliable results across a specified range and under varying experimental conditions.

Result and Discussion

Linearity and Range

By serially diluting the stock solutions to create a concentration range from 20 µg/ml to 100 µg/ml for escitalopram and from 1 µg/ml to 5 µg/ml for flupentixol. Peak area was plotted against concentration to create calibration curves. The formulation's linearity was tested at five concentration levels. Escitalopram regression line equation was $y = 26.073x - 73.821$ ($R^2 = 0.9999$) and for Flupenthixol, $y = 143.83x + 30.146$ ($R^2 = 0.9996$). These

results establish a strong correlation between peak area and drug concentration across the specific concentration range. The Calibration Curve of Escitalopram and Flupentixol is shown in figure 3.

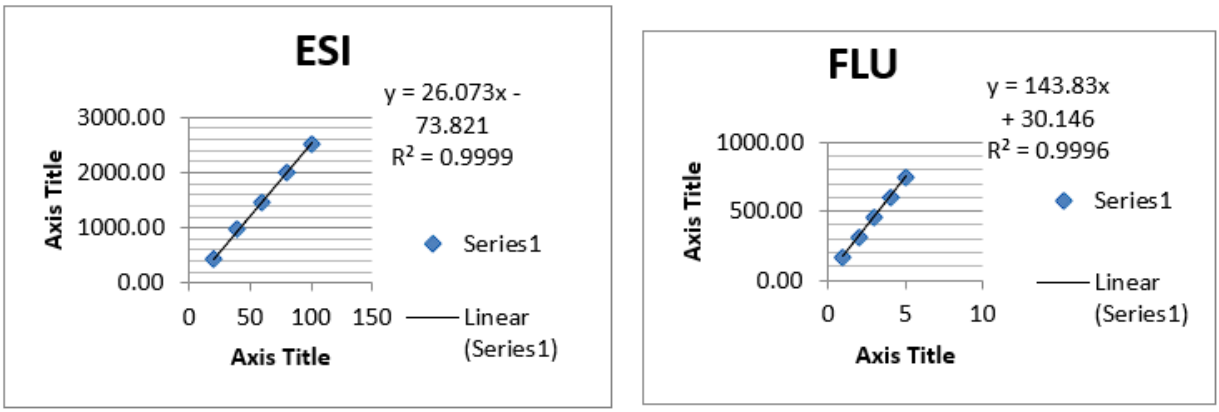

Figure 3. Calibration Curve of Escitalopram and Flupentixol.

Table 3. Recovery Studies of flupentixol (FLU) and escitalopram (ESI)

% Recovery	% Mean Recovery		% R.S.D.	
	FLU	ESI	FLU	ESI
80	100.68	99.68	0.49	1.5
100	101.54	101.44	0.32	0.20
120	102.01	99.14	0.67	0.61

Table 4. Robustness study for Escitalopram and Flupentixol.

Studied Parameter	Flow Rate		Mobile Phase		Wavelength	
	0.6ml	0.8ml	(1% OPA: MEOH) 64:36	(1% OPA: MEOH) 66:34	234 nm	236 nm
% Assay Escitalopram	100.56	99.20	100.64	101.36	101.20	101.28
% Assay Flupentixol	100.03	100.18	100.18	100.51	100.52	100.26

Accuracy and Precision

Recovery tests at three different levels (80%, 100%, and 120%) were used to assess the correctness of the approach; Table 2 provides specifics on the computed recovered percentages. The recovery results confirm the method's correctness, which is within the range of 100 ± 2%. Studies on both intra-day and inter-day variance showed precision. Investigations conducted within a single day involved three consecutive injections of sample solutions. The percentage to evaluate precision, Relative Standard Deviation (%RSD), was computed. Inter-day variation tests, including three repeated standard and sample solutions injections over successive days, yielded %RSD values (Table 3).

The gathered data, which has a percentage RSD of less than 1.5%, shows the accuracy of the developed UHPLC process.

Limit of Detection and Limit of Quantification

The limits of detection (LOD) and quantification (LOQ) were also established. To calculate LOD, the formula LOD = (3.3 x standard deviation / Slope of the calibration curve) represents the lowest analyte concentration, generating a detectable reaction. Escitalopram and Flupentixol had LOD values of 0.018 g/ml and 0.22g/ml, respectively. The formula for LOQ, which represents the lowest accurately measurable concentration, is LOQ = (10 x standard deviation / Slope of the calibration curve). The determined LOQ values for Escitalopram and Flupentixol were 0.057 g/ml and 0.67 g/ml, respectively.

Robustness

Robustness was evaluated through deliberate, minor adjustments in the experimental procedures. Specifically, changes in wavelength, Mobile phase, and flow rate were intentionally introduced, and the subsequent effects were closely monitored. The method exhibited robustness, demonstrating its resilience to variations in wavelength, Mobile phase, and flow rate. The results are shown in Table 4.

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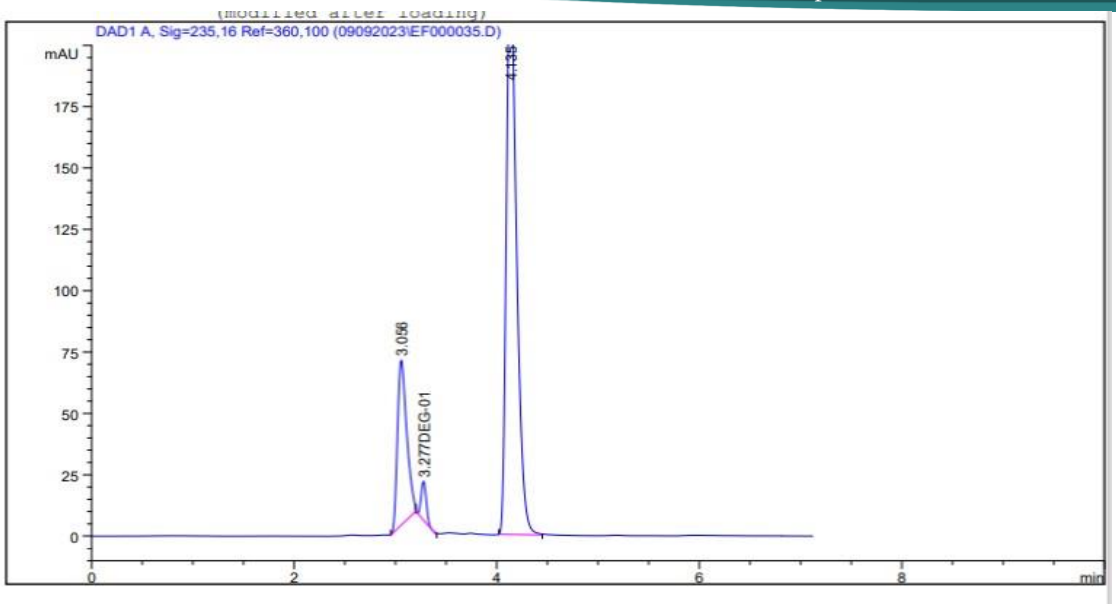

Figure 4(a). Force Degradation Study by Acid Hydrolysis.

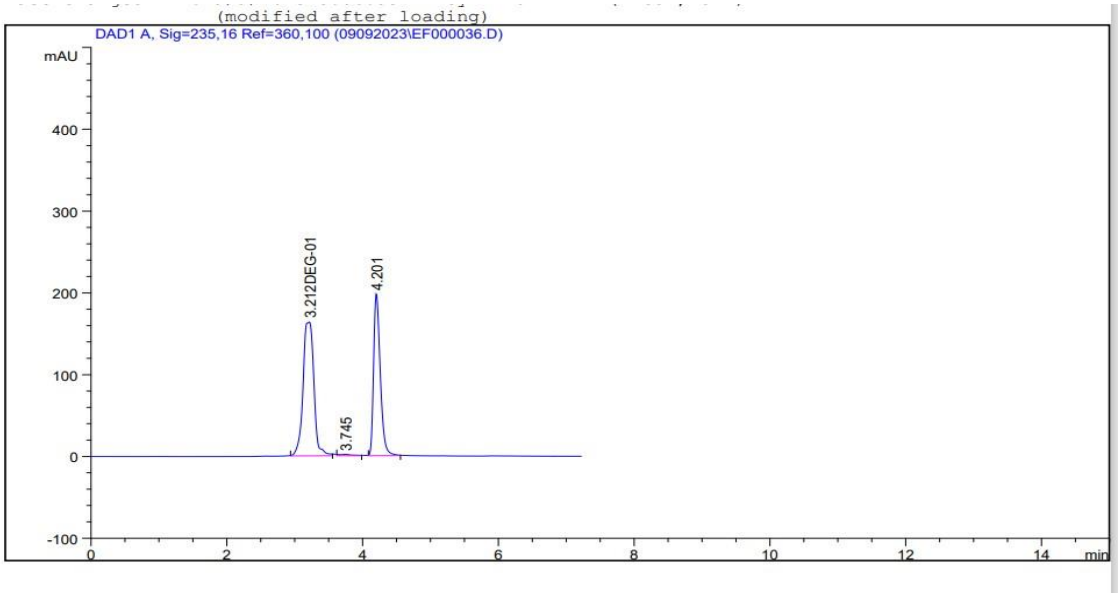

Figure 4(b). Force Degradation Study by Alkali Hydrolysis.

Forced Degradation Studies

Studies on the induced degradation of both medications were carried out in a variety of environments, such as sunlight, oxidation, dry heat, and hydrolysis. Twenty tablets in all were weighed and pulverized and their average weight was determined to be 18 mg. Next, the tablet was ground into a uniform size, and 18 milligrammes of that size were dissolved in 100 millilitres of methanol. This solution underwent 15 minutes of sonication and 5 minutes of cyclomixing to ensure the extraction of the drug into the solution. During filtration, 10 millilitres of the solution were mixed with the mobile phase in a second 100 ml volumetric flask to obtain a 100 µg/ml concentration. To attain the ultimate concentrations of 3 µg/ml for Flupentixol and 60 µg/ml for Escitalopram, the sample solution was further diluted. This stock solution sample, which contained 100 µg/ml, was used for the research on forced degradation. These studies aim to simulate and assess the stability and degradation patterns of the drugs under different stress conditions. 0.6 ml of the sample base solution of Escitalopram and Flupentixol were obtained in separate round-bottom flasks to start the forced degradation process in alkaline conditions. Then, 5 ml of 0.1 N NaOH was added, and the mobile phase was injected to fill the remaining amount. The combination was left to rest at room temperature for an hour. 5.0 ml of 0.1 N HCl and 0.6 ml of the sample stock solution were brought into contact for forced degradation in an acidic environment.

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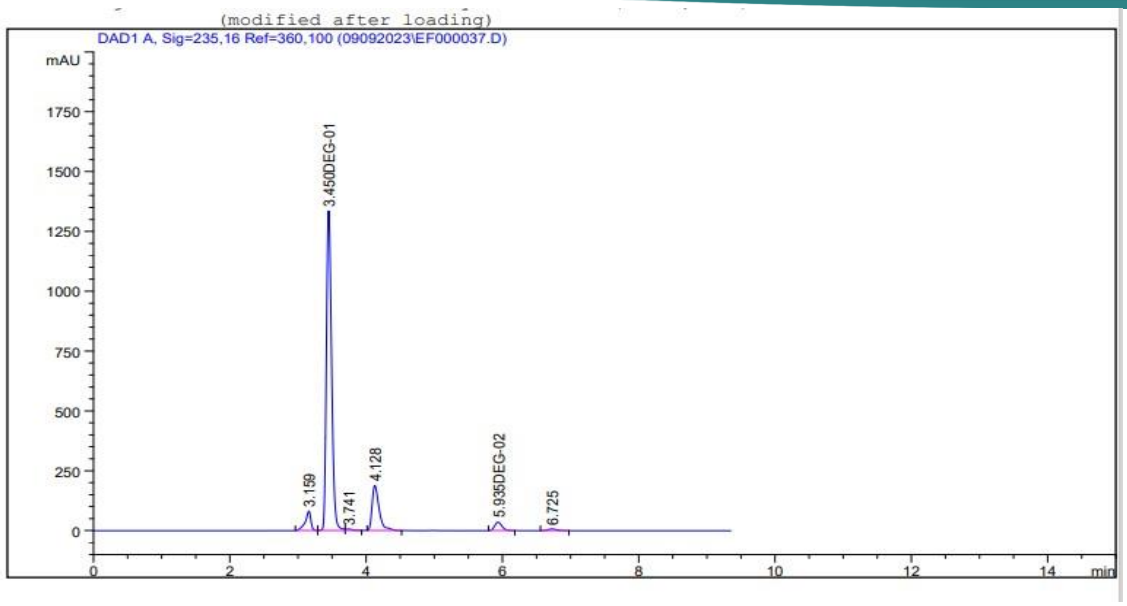

Figure 4(c). Force Degradation study by hydrogen peroxide.

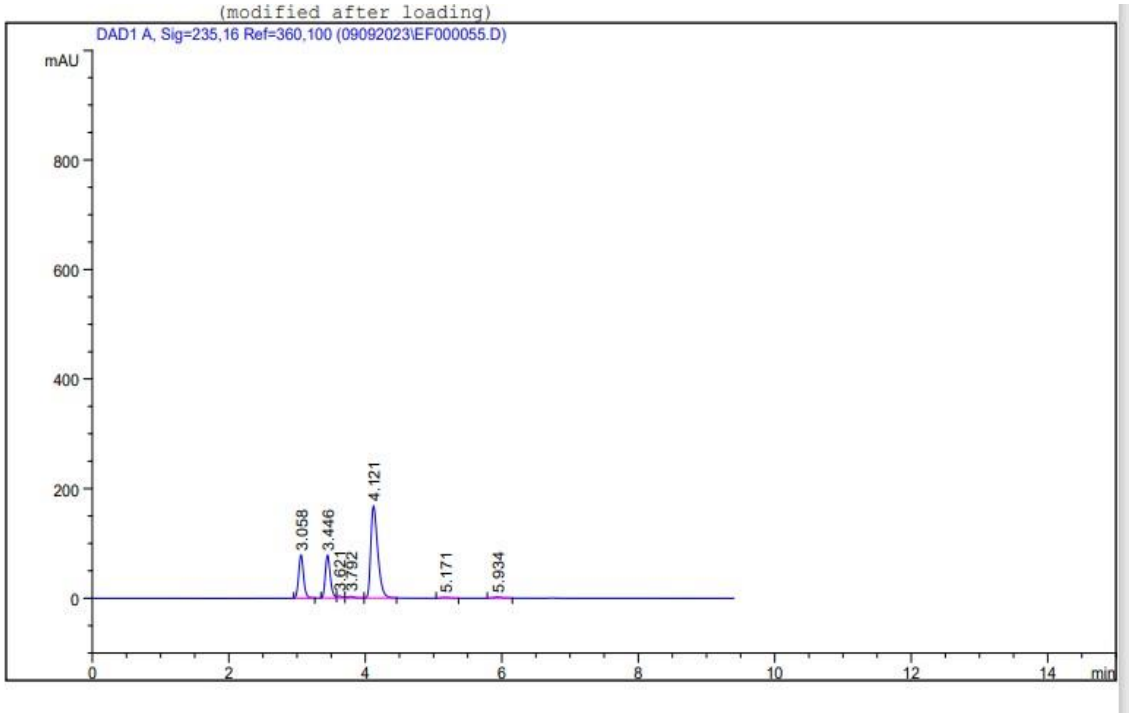

Figure 4(d). Force Degradation Sun heat.

After the mobile phase was included to adjust the final amount, the mixture was let to stand at room temperature for a maximum of two hours. Five millilitres of 3% (w/v) hydrogen peroxide were mixed with 0.6 millilitres of the sample stock solution in the flask to initiate the hydrogen peroxide degradation process. This combination could only be used at room temperature for an hour at most. In order to assess photo-stability, 50 mg of the active pharmaceutical ingredients (API) for Escitalopram and Flupentixol were subjected to direct sunlight for a whole day. All of the degraded sample solutions were diluted with the mobile phase in order to reach their final concentrations of 10 µg/ml for Escitalopram and 5 µg/ml for Flupentixol in UHPLC analysis. To evaluate the stability and drug degradation patterns, these investigations of drug degradation imitate various stressful environments. Force degradation study is shown in figure 4 and the Summary of degradation studies for Escitalopram and Flupentixol in their tablet dosage form is shown in table 5.

Discussion

The recommended method was simple, with linearity demonstrated at concentration ranges of 1 to 5 g/ml for Flupentixol and 20 to 100 g/ml for Escitalopram. Accuracy and precision were confirmed through recovery studies, revealing % RSD values not exceeding 1.5% for Esc and Flu, the LOD and LOQ values are 0.223 µg/ml

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Table 5. Summary of Degradation studies for Escitalopram and Flupentixol in their tablet dosage form.

Degradation condition	Time (h)	% Degradation	
		Escitalopram	Flupentixol
Acid (0.1N HCL) at room temperature	2h	8.15	6.05
Alkali, (0.1N NaOH) at room temperature	1h	100.00	8.62
Oxidation, (3% H_2O_2) at room temperature	1h	7.10	8.44
Photolytic Direct sunlight	24h	14.65	19.00

and 0.189µg/ml, respectively, and 0.676 µg/ml and 0.057 µg/ml, accordingly, the method also showed sensitivity. The method's sensitivity and specificity are validated by these results taken together. A discernible decrease in peak areas in the degradation research indicated drug degradation without the emergence of novel degradation peaks. To calculate the percentage of deterioration, the areas of the peaks for both treatments under non-degradation conditions and the areas of the deteriorated peaks under each degradation condition were compared. The following protocols were applied: 3% v/v H_2O_2, 0.1 N NaOH for one hour, photo-degradation for thirty minutes, and forced deterioration under 0.1 N HCl for two hours. Using the created HPLC method, it was discovered that under the stated conditions, the percent degradation for both Escitalopram and Flupentixol in their tablet dosage form was between 10 and 20 percent. For the simultaneous assessment of Escitalopram and Flupentixol stability-indicating, the HPLC technique was created in this work and verified in accordance with ICH recommendations. Statistical analysis verified the procedure's accuracy, precision, and repeatability. The created method's simplicity, sensitivity, and selectivity for Escitalopram and Flupentixol analysis in combination, free from excipient interference, were its defining features. Crucially, the approach precisely quantifies both medications when all degradants produced by forced degradation experiments are present. The combined dosage form's assay results, obtained through the suggested strategy, revealed 99.14±0.61% of Esc and 101.54±0.32% of Flu. The results obtained demonstrate the technique's suitability for researching Escitalopram and Flupentixol stability across a range of forced degradation scenarios, including as oxidation, basic, acid, and photolytic degradation. It is significant that this investigation did not involve the identification of degradation products.

Conclusion

In the present study, a precise, simple, accurate, sensitive and cost-effective stability-indicating assay method for simultaneous estimation of Escitalopram and Flupentixol in mixed dose forms by UHPLC was established. The results obtained by analysing the forced degraded samples depict no other co-eluting interference peaks due to variable stress components with the main peaks. The method was seen to be specific for the determination of Escitalopram and Flupentixol amongst various degradants. The method can also be successfully used for assay and dissolution studies for tablet formulation.

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Conflict of Interest

The authors declare no conflict of interest.

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