

Eco-Friendly Analytical quality by design approach stability-indicating HPTLC technique for the quantitative evaluation of fluoxetine

Durgadevi PERUMAL ¹ , Manikandan KRISHNAN ^{1*} , Lakshmi K.S ¹ 

¹Department of Pharmaceutical Analysis, SRM College of Pharmacy, SRM Institute of Science and Technology, Kattankulathur – 603203, Tamil Nadu, India.

*Corresponding Author. E-mail: gurumani12@gmail.com (M.K.); Tel. +9444708710.

Received: 10 May 2022 / Revised: 06 August 2022 / Accepted: 08 August 2022

ABSTRACT: A design of experiments (DoE)-based quality by design (QbD) method was used to develop a stability-indexing assay method in high performance thin layer chromatography (HPTLC) for the kinetic study of fluoxetine. This research proposes to use an environmentally friendly HPTLC technique based on Analytical Quality by Design (AQbD) to determine environmental friendliness. According to the literature, there is currently no published article on this method. A stationary phase consisting of TLC plates (Merck) pre-coated with silica gel 60F254 on aluminum sheets was used. A mobile phase of acetone: water with 1% orthophosphoric acid (8.5:1.5 v/v) was used. Central Composite Design (CCD) provides the opportunity to optimize the HPTLC separation and determine the parameters that have the greatest influence and those that have interactions. 20 optimized experimental runs were performed, including organic solvent content (A), saturation time (B), and driving distance (C). These 3 factors were evaluated for robustness, showing an insignificant effect on retention. The R_f value was 0.72±0.07, and the calibration curves were linear in the range of 0.3-5 mcg/point with regression coefficients r² of 0.9987 for fluoxetine HCl. In the intraday and interday precision study, the percent RSD was 0.26 and 0.28, respectively. Fluoxetine was found to be highly susceptible to alkaline and acid hydrolysis compared to oxidation. All system suitability parameters were validated and were within the range specified in ICH guidelines. The novel method was also evaluated using four different approaches, including other evaluation methods: NEMI, GAPI, AGMS, AGREE, and it was found to be environmentally friendly.

KEYWORDS: Design of the experiment; HPTLC; Green analytical chemistry; Stability-Indicating Assay method; Validation.

1. INTRODUCTION

Fluoxetine hydrochloride, N-methyl-3-phenyl-3-(2, 2, 2- trifluoro-p-tolyloxy) propylamine hydrochloride C₁₇H₁₈F₃NO, HCl, MW=345.8. The structure of Fluoxetine is depicted in Figure 1 and it is an antidepressant drug that is pharmacologically and structurally distinct from tricyclic drugs. It's been a while that Presynaptic neurons are preferentially inhibited in their serotonin reuptake. Fluoxetine hydrochloride is also used to treat various diseases other than depression[1]. Benefits in obsessive-compulsive disorders have been demonstrated; Neuropathic pain and fibrositis, anxiety attacks, and nervous bulimia are all pain syndromes. Information about Medications 93 (American Hospital Formulary Service)[2]. Stability and stress testing (forced degradation studies) are essential components of medication development strategy[3]. The investigations aid in understanding the mechanism of drug decomposition, which aids in getting information on physical and chemical elements that contribute to drug instability[4]. ICH guideline Q1B describes the standard settings for photo stability testing[5].

Densitometric separation was achieved Linomat V Sample applicator (Camag, Switzerland). Hamilton microliter syringe (Bonaduz, Switzerland). Precoated aluminium plate with silica gel 60 F₂₅₄ (10cm×10cm with 250mm thickness; E. Merck, Darmstadt, Germany). TLC scanner III (Camag, Switzerland). WinCATS software Ver 4.4.1 (Camag, Switzerland), Camag Twin trough chamber (Camag, Switzerland) in this study.

How to cite this article: Perumal D, Krishnan M, Lakshmi KS. Eco-Friendly Analytical quality by design approach stability-indicating HPTLC technique for the quantitative evaluation of fluoxetine. J Res Pharm. 2023; 27(1): 274-289.

DOE is a tool for the optimization of composition parameters. It is utilized for the evaluation of principal effects along with their interactions. CCD is a part of RSM which shows quadratic response surfaces without a three level factorial design. The critical factors along with the experimental levels which are under investigation for the optimization is on the univariate preliminary studies of the chromatographic method development. Central Composite Design (CCD) has the flexibility and can be applied for the optimization of High-Performance Thin Layer Chromatography (HPTLC) separation and to view the factors which mainly effect as well that which shows interactions. 15 experimental runs and 5 center points were used for performing a three factorial experimental design. Variables which were included are: Organic solvent content (A), Saturation time (B), travelling distance (C) and 15 optimized trial experimental runs. These 3 factors were studied for its robustness which showed insignificant effect on the retention.

Green computing is the development of synthetic processes and products the use of hazardous compounds is reduced or eliminated. Environment friendly chemistry refers to every aspect of the chemical life cycle, including its production, use, and disposal. Green chemicals either break down into harmless byproducts are collected and reused. Plants and animals are affected by toxic substances in the environment. Reduced global climate change, ozone layer depletion, and smog generation. Less chemical pollution of ecosystems. Green Analytic Chemistry (GAC) is a concept that focuses on creating analytical procedures that are both environmentally and analyst-friendly. The GAC approach has many advantages, including reducing the use of harmful chemicals/reagents, the use of energy-efficient equipment, and the generation of less waste.

The analytical methods used to estimate Fluoxetine are the subject of comprehensive research. As a single and multi-component, UV Spectrophotometry[6-8], High Performance Liquid Chromatography[9-14], High Performance Thin Layer Chromatography[15-21], the approach is inefficient since this described HPTLC technique only uses one variable at a time. It produces misleading findings that should be avoided. This needs a systematic and quantitative approach to improving reaction parameters to obtain critical and accurate results with fewer experiments. One of the most frequent and beneficial chemometric optimization strategies is the experimental design, which is used to filter and optimize the impacts of selected parameters on the response by evaluating the influence of various factors on the response[22]. RSM (response surface methodology) is a mathematical and statistical tool for assessing and optimizing the development of various processes in design space[23-25]. RSM is used after factorial designs have been used to screen the experimental variables that significantly impact the response. The developed method was validated as per ICH Q2B guidelines.[26]

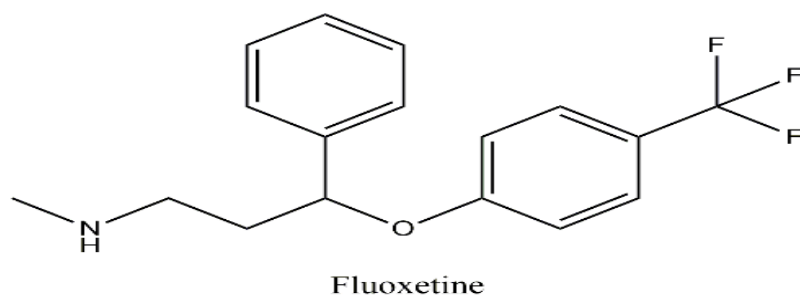


Figure 1. Structure of Fluoxetine

2. RESULTS AND DISCUSSION

Developing an analytical approach while adhering to stability-indicating principles was a new notion for a long-term method. The design of a stability-indicating assay method without applying the AQB approach, on either side, may result in poor method performance and necessitate revalidation. The combining of these ideas in the HPTLC process increases the system's stability and long-term viability. As a consequence, we've merged these two techniques to develop a useful and trustworthy approach. This is how the whole process of establishing this validation technique went.

2.1. Methods using CCD for optimization of Chromatographic condition

CCD provides versatility and may optimize separation utilizing numerous factors for HPTLC. A triple factorial experimental design was carried out using 15 experimental and 5 center points. Variables included

are Acetone content, Saturation time, traveling distance, and 15 optimized experimental runs. The responses were conducted and summarized in Table 1.

Table 1. Model of Central Composite Design.

RUN	FACTORS			RESPONSE Fluoxetine (Rf)
	A: Content of Acetone (ml)	B: Saturation time (min)	C: Developing traveled (cm)	
1	4	35	9	0.37
2	6.68	30	8	0.26
3	4	25	9	0.36
4	5	30	8	0.32
5	5	30	6.31	0.3
6	5	30	8	0.32
7	6	25	9	0.27
8	5	30	8	0.32
9	6	35	7	0.28
10	3.31	30	8	0.4
11	6	25	7	0.27
12	5	30	9.68	0.31
13	4	35	7	0.36
14	5	38.40	8	0.33
15	6	35	9	0.28
16	5	30	8	0.32
17	5	30	8	0.32
18	5	30	8	0.32
19	5	21.59	8	0.3
20	4	25	7	0.35

Model selection for the Rf Values of Fluoxetine was determined. A quadratic and linear model was selected. It was based on the PRESS value. The R² (Adjusted) value was found to be closer to 1. The model validation was performed with ANOVA, and the results are given in Table 2. Significance was found to be P < 0.05. The ratio obtained for the drugs showed an adequate signal.

The % CV < 10% the R² (Adjusted) was found to be high. This shows an effective relationship between the obtained experimental data and the models. R² values adjusted have the limit of R² ≥ 0.80, which are within the acceptable limits showing that the obtained experimental data fits with the polynomial equations. The equations with components and factors are given in Table 2.

Table 2 ANOVA table for optimized model.

Drugs	Model	Y (Equation model)	R ² (Adjusted)	P-value	% CV	Precision (Adequate)
Fluoxetine	Quadratic	0.32- 0.042A+6.62B +2.69C - 2.5AC+3.754A ² 1.549B ² - 5.085C ²	0.9957	<0.0001	0.75	83.504

The perturbation plots and 3-D plots were done to evaluate the factors and their effects (A, B &C) on retention factor (Rf) for the drugs. Figure 2, shows the perturbation plots for the predicted model. This is done to comprehend the 64 procedures under investigation. Figure 2, shows how changes in response

concerning perturbations occur from a reference value. The factors are taken to be constant at the point of reference. The steepest curvature of the steepest curve shows the sensitivity to the definite factor. Figure 2, shows that C has a more significant effect on the R_f of Fluoxetine than the other factors. Figure 3, Keeping the acetone content constant, there has been a variation in the R_f value of Fluoxetine with the function of B and C. R_f of Fluoxetine has an inverse correlation with the traveling distance. Analysis was conducted on the model's response plots and perturbation plots, exposing that A and B affect responses more than C.

The developed conditions for the separated drug were estimated with the help of Derringer's desirability function. The maximum desirability function obtained for the response surface is presented in Figure 4. The agreement between the predicted response and the experimental response was predicted and given in Table 3.

Table 3. The experimental and predicted value.

Condition	Acetone content ml	Saturation time min	Distance traveled cm	Fluoxetine R _f
1	4	25	9	
			Predicted	0.36
			Experimental	0.35
			Predicted Error %	1

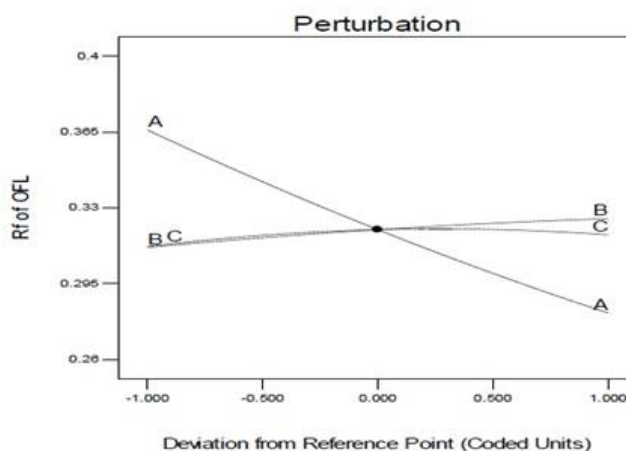


Figure 2. Perturbation graph showing the effect of all factors against R_f value of Fluoxetine.

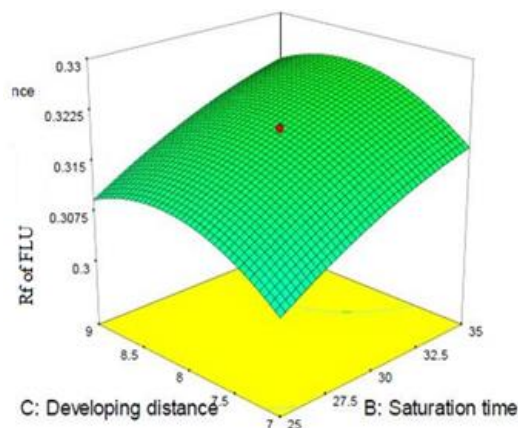


Figure 3. 3D plots of R_f of Fluoxetine against B and C.

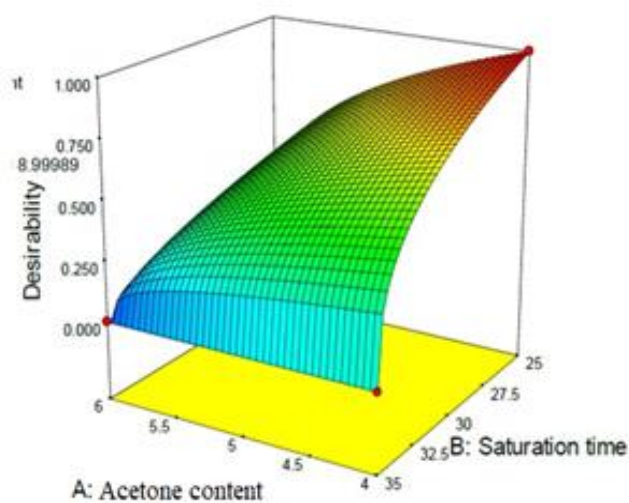


Figure 4. 3D plots for Derringer's desirability function

The % predicted error showcased a desirability value ($D = 1$) at the optimum conditions, which furnished a set of coordinates. These coordinates obtained were a measure for selecting optimum experimental conditions for analysis of Fluoxetine. The HPTLC analysis was finalized to contain Acetone: Water (5: 5 v/v). The HPTLC densitogram had an R_f value of 0.34 in the case of Fluoxetine.

2.2 Validation

The linearity of the system was tested by injecting different dosages of sample solutions. (0.5-3 mcg/spot, Figure 5). A standard solution (2 mcg/spot) was spotted six times to check the percent for system precision. Six samples were made and analyzed. Spotted in duplicate six times for RSD (relative standard deviation) and procedure accuracy. Fluoxetine was measured on one day and three different days to determine the method's Precision and accuracy inside a day (based on intra) and between days (inter-assay). Intra-assay and inter-assay were computed, and the findings are given in Table 4, respectively.

Table 4. Intra-assay and Inter-assay Precision data of proposed HPTLC method (Method Ruggedness)

Intra-assay			
	Mean (% W/W)	S.D	% RSD
Assay 1	98.61	0.21	0.23
Assay 2	99.31	0.29	0.27
Intra assay	99.01	0.26	0.26
Inter-assay			

Assay 1	98.59	0.22	0.28
Assay 2	100.63	0.26	0.31
Inter-assay	100.17	0.24	0.28

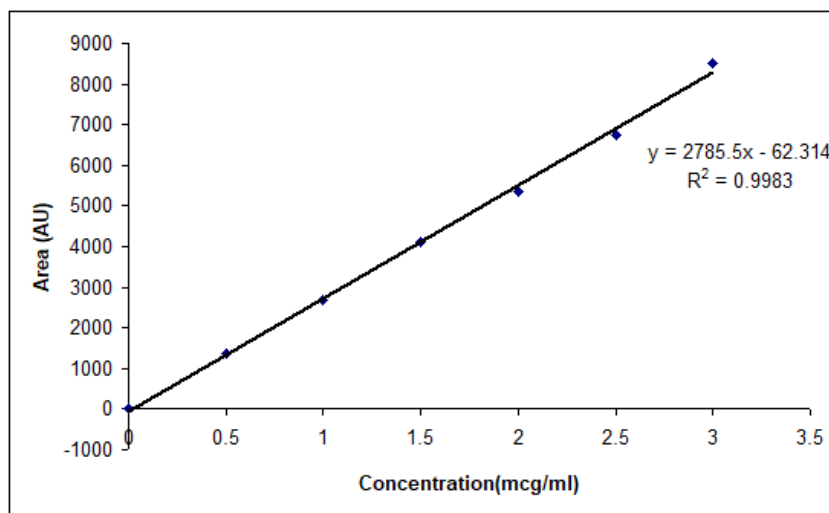


Figure 5. Linearity Curve of Fluoxetine

2.2.1 Linearity

The calibration curve for Fluoxetine exhibited remarkable linearity in the region of 0.5-3 mcg/spot, having a coefficient of correlation (r) of 0.9987 Figure 6. The regression equation is written on the calibration curve. $y = 2785.5x - 62.314$. The results are depicted in Table 5.

2.2.2 Precision

The method precision findings (percent RSD = 1.014) are determined to be within the ICH requirements (percent RSD 2 percent of method accuracy) (percent RSD 2 percent of method accuracy). The results are depicted in Table 5.

2.2.3 Inter-assay and Intra assay correlations

The approach was assessed intra-and interday, and the excellent mean assay results and low threshold variation and percentage RSD (percent RSD 2%) within a day and day-to-day oscillations for Fluoxetine demonstrated that the proposed method is exact Tables 4.

2.2.4 LOD and LOQ

Average maximum the concentration at which substance may be reliably identified LOD and LOQ values are found toward being 0.020 and 0.050 mcg/spot, accordingly. The results are depicted in Table 5.

Table 5. Analytical validation parameters.

Parameters	Fluoxetine
Linearity range (mcg/spot)	0.5-3
Correlation coefficient (r^2)	0.9987
Slope	2785.5

Intercepts	62.314
LOD (ng/spot)	0.020
LOQ (ng/spot)	0.050
Intra/Inter-day Precision	
% RSD	0.26
% RSD	0.28
Accuracy	
50%	101.46
100%	101.48
150%	101.54
% RSD	1.014
Assay results for marketed formulations	
Marketed Tablet	Fluoxet
Label claim	200
Mean± SD (n=3)	199.2 ±0.25
Recovery %	99.92
RSD %	0.27

2.3 Studies on Forced Degradation

According to literature reviews, a targeted decomposition of 20–80% is indicated for proving stability and revealing the nature of the test procedure, even though intermediate product which are degraded must not to be interfere with any phase of drug analytics. Even though these settings utilized in present research with forced degradation result in 20–80 percentage point degradation, fluoxetine degradation took a long time to achieve.

During the investigation, it was determined that when Fluoxetine was treated with a base Alkaline (0.1M NaOH), at 40°C for 12 hours, Acid (0.1M HCl), at 40°C for 48 hours, Oxidation (30% H₂O₂) at 25°C for 48 hours, Thermal at 105°C for 48 hours, Photochemical kept at direct sunlight for 48 hours. The degree of fluoxetine degradation was revealed in Table 6, under various stress situations. The Densitograms of forcibly deteriorated samples is presented. Furthermore, it is crucial to emphasize that the Densitograms show that deterioration peaks may be seen under the stress conditions used. As a result it shows that the drug Fluoxetine was stable in Acid conditions and photochemical degradation, liable in alkaline and oxidation. Under Thermal degradation circumstances, the medication is unstable slightly more degraded up to 12.62%. The findings of stability tests and the chromatograms of all degradations were portrayed in Figure 7(A- E), and resulted in Table 6.

Table 6: Fluoxetine Forced Degradation Studies Results

Stress Level / Duration	Drug Decomposed Percentage	Drug Recovered Percentage	Rf
Degradation of alkaline (0.1N NaOH, 40°C, 12h)	11.07	88.93	0.73
Acid hydrolysis (0.1N HCl, 40°C, 48h)	08.9	91.10	0.73
Degradation by oxidation (30% H ₂ O ₂ , 25°C, 48 h)	10.06	89.94	0.73
Degradation due to heat (105°C, 48h)	12.62	87.38	0.74
Photochemical degradation (48h, sunlight)	6.72	93.28	0.73

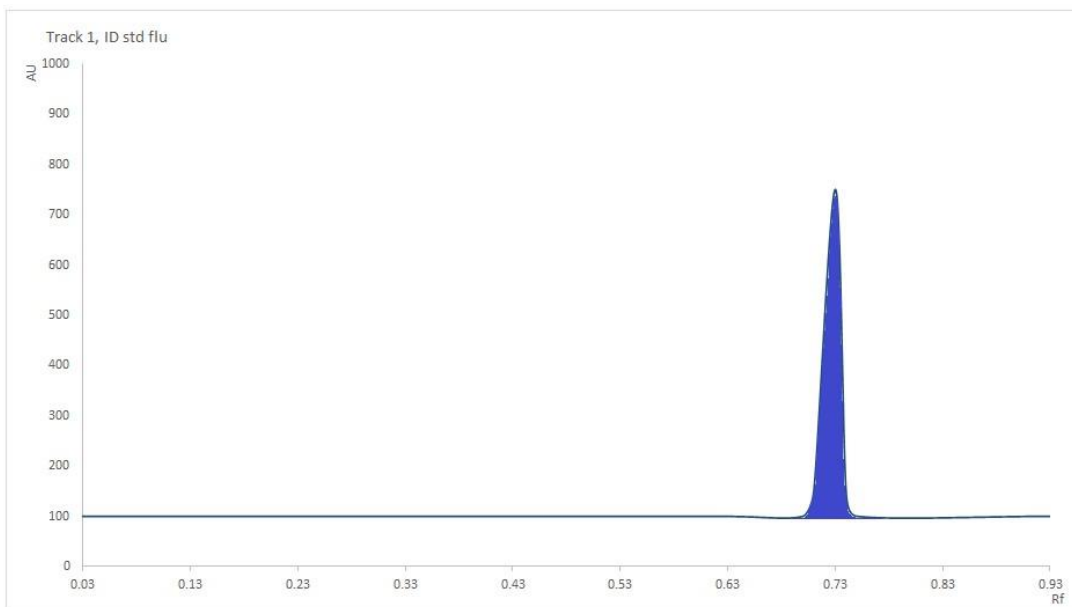
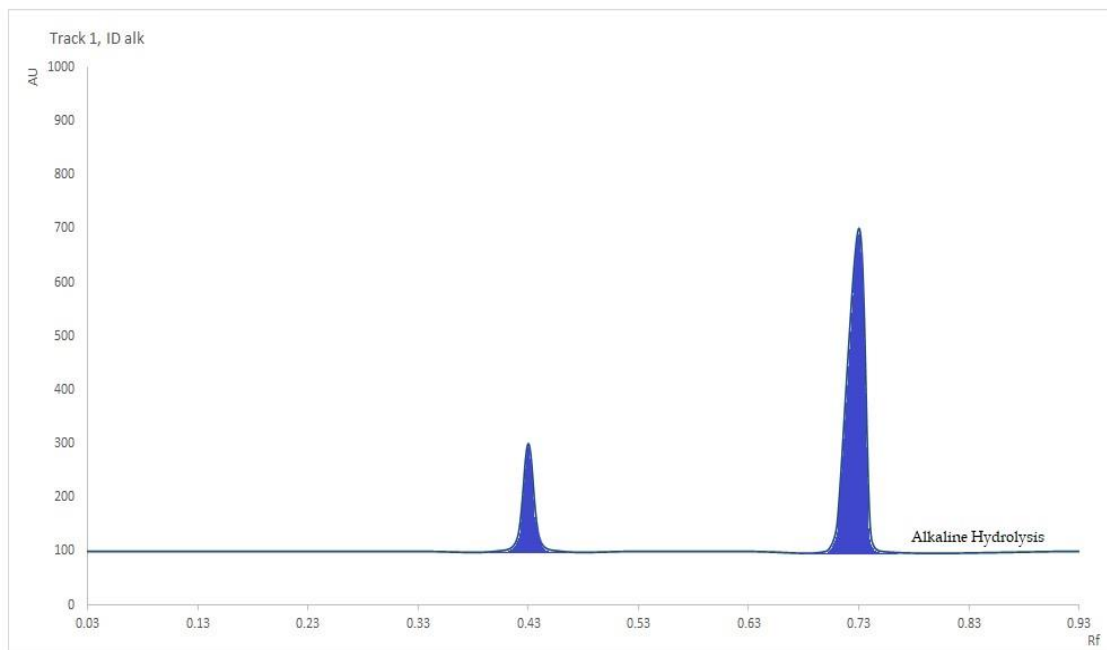
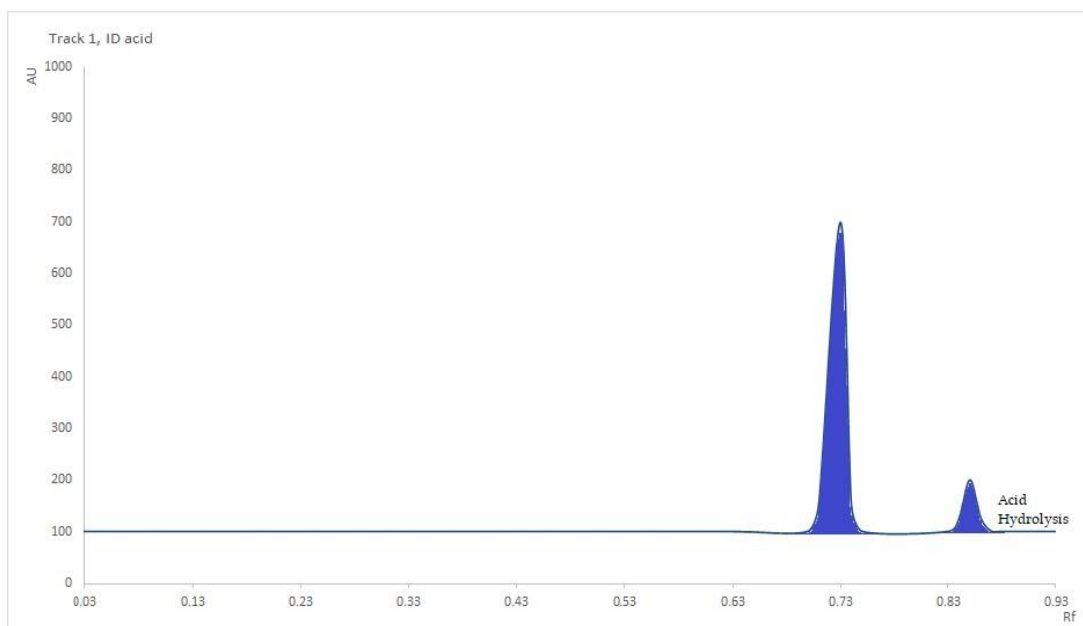


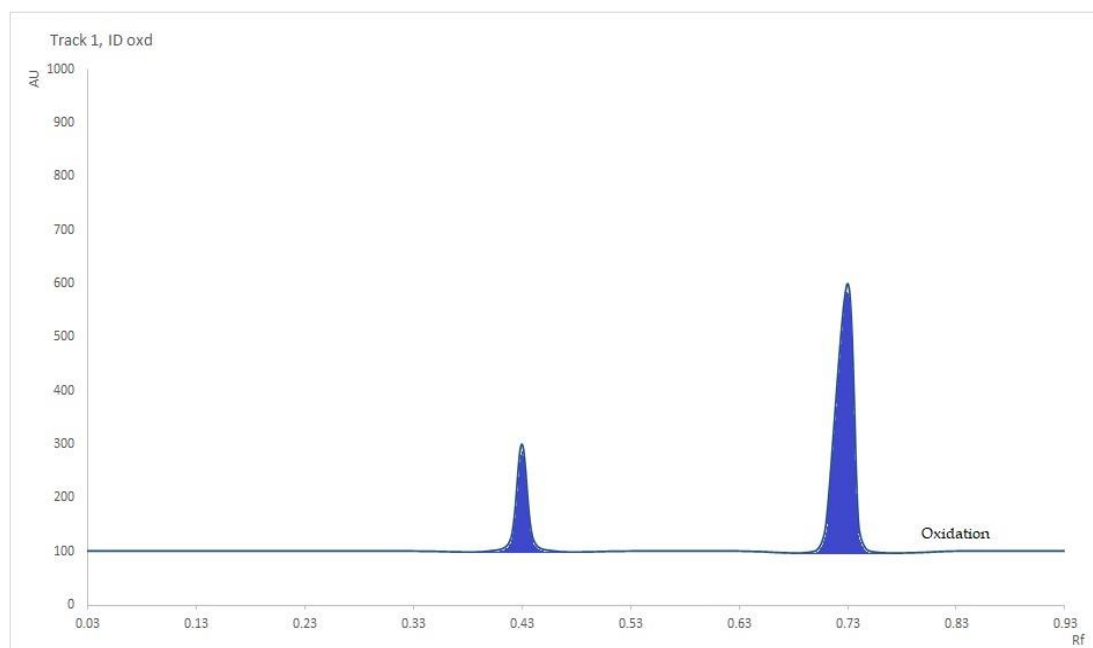
Figure 6. Standard Chromatogram for Fluoxetine



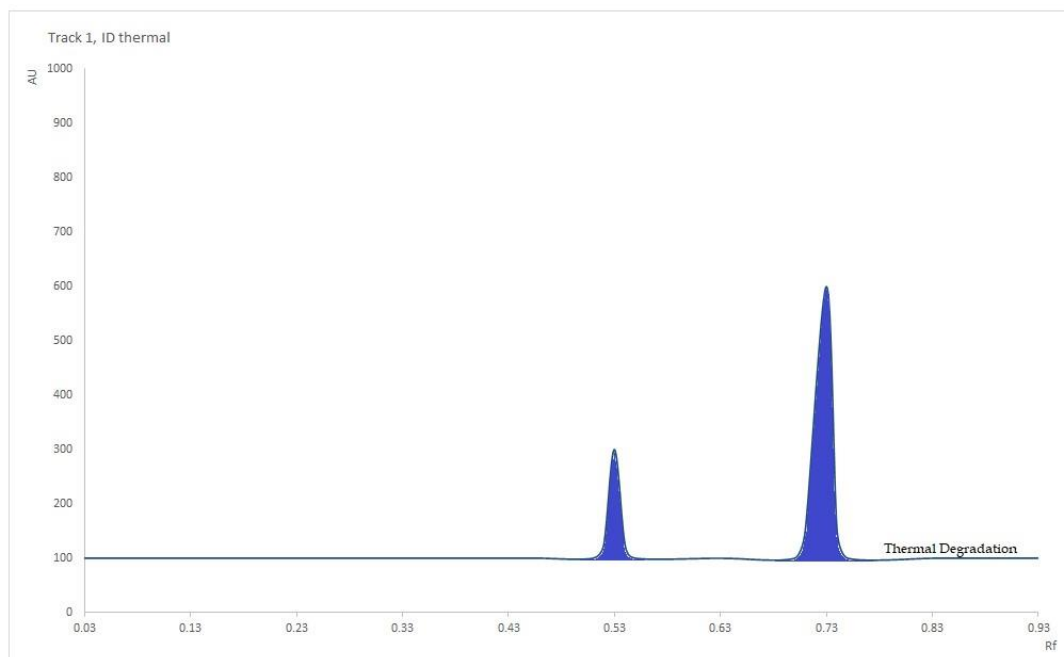
(A) Degradation of Alkaline



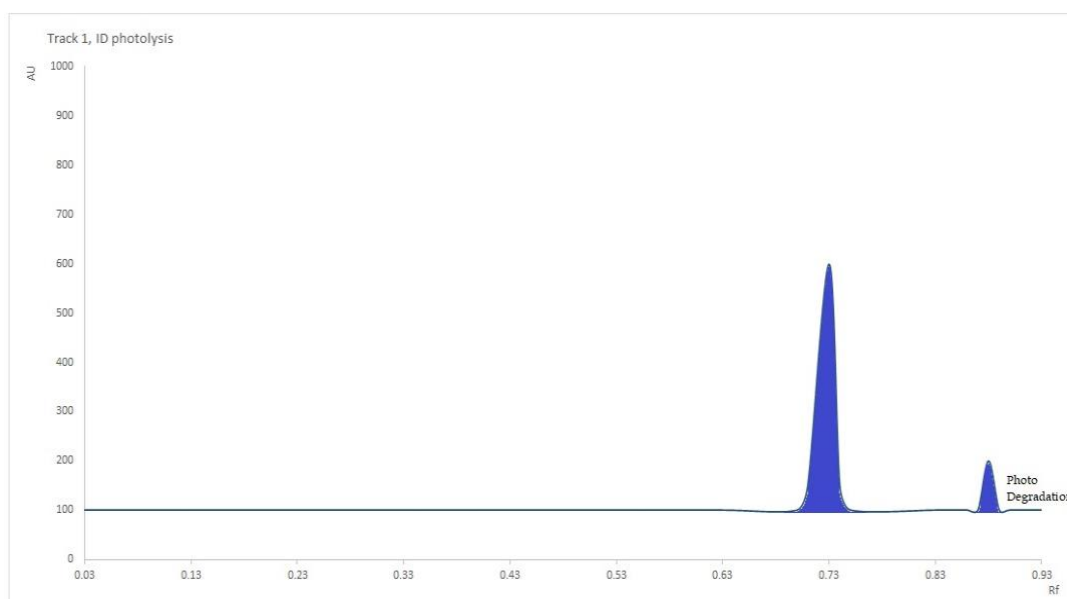
(B) Acid Hydrolysis



(C) Degradation by Oxidation



(D) Degradation due to heat



(E) Photochemical degradation

Figure 7(A-E) Densitogram for different stressed degradations for Fluoxetine.

2.4. Analytical solution for specificity and stability

The uniqueness demonstrated that the presence of a degrading sample unspoiled the pinnacle. It is important to note that Fluoxetine stayed steady in sufficient concentration at 250°C for up to 24 hours and 48 hours when designed to protect from light (kept in the dark). The linear, correctness, inter-as well and intra-day assay, LOD, LOQ, specific, and stability of the devised HPTLC testing for fluoxetine analysis were proven in analytical solution results.

2.5. Assay of marketed formulation

The commercially available dosage version contains 200 mg Fluoxetine. Fluoxetine has a percentage test of 99.92 percent. This demonstrates that this method can assess quality during tablet dose formulation testing. The results are depicted in Table 6.

2.6 Greenness assessment for the developed method

2.6.1 Green assessment for the developed method

For the examination of two medicines, the approach combines tri-combinations. In creating methods, each of these three aspects is equally important. A designed solution cannot merely claim to be environmentally beneficial without being evaluated using appropriate assessment methods in this competition. The method's greenness was analyzed using four evaluation tools: NEMI (National Environmental Methods Index), GAPI (Green Analytical Procedure Index), AGMS (Analytical Method Greenness Score), and AGREE (Analytical Greenness Metric). Every instrument seems to have its number of capabilities, disadvantages, and assessment methods. The results of each assessment tool may result in a different impact on which strategy is the most environmentally friendly and which evaluating technique to adopt. Even though numerous tools were employed in this technique evaluation process, all of the outcomes were shown in a single eco-friendly greenway. The following is how the approach was evaluated:

2.6.2 NEMI

NEMI is a well-known qualitative evaluation technique for assessing green chemistry. At first, it was the only tool available for evaluating GAC approaches. Despite developing new assessment tools for the GAC, NEMI has advantages in looking at the green analytical method. NEMI is shown as a four-quadrant circular symbol with color matching (green and colorless). Quadrant one must deal with the EPA's TRI (Toxic Regulatory Inventory) list of Persisting Bioaccumulative Toxic (PBT) chemicals[27]. In contrast, quadrant two must deal with the Toxic Compounds Regulation Inventory (TRI) list of PBT chemicals. This quadrant is tinted green since the substances used in this method are not listed in PBT. Hazardous compounds, which are controlled mainly through Environmental Protection Agency (EPA) under the RCRA, occupy the second quadrant (Resource Conservation and Recovery Act)[28].

Several substances on the RCRA list were also found in this method; thus, the second quadrant is labelled green. In order to qualify as green, the pH of the analytical solutions in the third quadrant must be below a specific range, and the mobile phase ethanol and phosphate buffer in a 60:40% v/v pH must also be below a specific range; hence, the third quadrant is a green zone. The fourth quadrant focuses on waste, which should be less than 50 g or mL altogether. The third quadrant was given a green tint since the loss in this manner is small due to the recycling process. The primary NEMI picture of a method is shown in Figure.8.

2.6.3 GAPI

GAPI is a slightly modified version of NEMI with 11 classifications and a color-coded approach signify hazard, tolerance, and environmental friendliness using red, yellow, and green. In his paper, the method is [29,30] made assessment using GAPI easier by producing free software. The method specifics which need to be evaluated must be entered into the program, which contains 11 straightforward stages to obtain the outcome. Figure.8 depicts the obtained result, demonstrating the method's viability and future potential.

2.6.4 AMGS

An alternative method of evaluation for safety, health, and the environment is the environmental assessment (EAT) and SHE (Safety, Health, and Environmental Assessment) are included with AMGS. There were three categories in the AMGS: equipment, solvent energy, and solvent environmental health and safety

[31]. The method's overall score is calculated by adding these three scores together, and that should be as minimal as possible to consider making the method as green as possible. The final result procured for the proposed approach was 1242.90 Figure.8, which showed a positive effect of the developed model on the environment after feeding the data required into the calculator made available by only AES Green Computing University for the green evaluation[32].

2.6.5 AGREE with metrics

The newest green assessment tool, AGREE with metrics[33,34] encompasses all twelve green analytical concepts. The total outcome, which was represented as 1, the individual principles score obtained from the individual's rights have been the emphasis. The method's greenness is indicated by a rating closer to one. Figure.8 displays the overall outcome after inputting the procedure specifics into the program. The method's impact on the environment has been described as "extremely benign" and "long-term sustainable." The primary purpose was to identify the method's sustainability, although five evaluation tools employed diverse methodologies or processes to analyze the method's greenness. Regardless of their tactics, all of the methodologies indicated that this technique is environmentally benign and flexible to future green assessment without any problems[35].

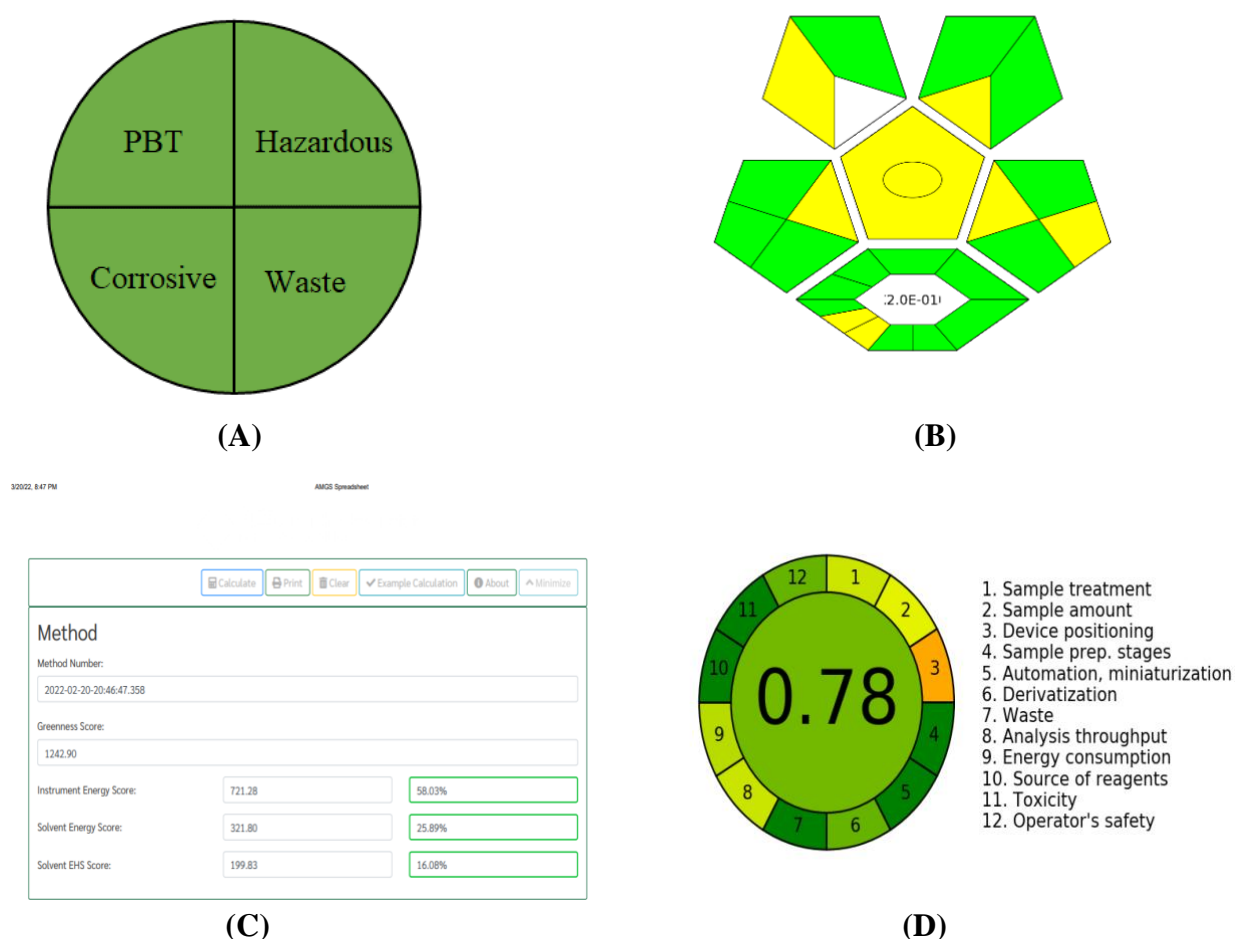


Figure 8. (a) NEMI, (b) GAPI, (c) AMGS, and (d) AGREE with metric Results of the suggested green assessment method.

3. CONCLUSION

The estimation of fluoxetine can be performed by the proposed HPTLC method. The method has the following advantages: fewer mobile phases are needed, the analysis is faster, and the cost of each study is reduced. Analysis of multiple samples can be performed simultaneously. Different wavelengths can be used for multiple plate scanning. Statistical analysis showed the repeatability of the method and can be selected to study multiple drugs. These proposed methods can be used to elucidate the degradation kinetics in biological samples under stress conditions. DOE is a method to reduce the number of parameters in a composition. It is used to study significant effects as well as interactions between them. CCD is a function of RSM that allows obtaining quadratic response surfaces without using a three-step factorial design. Factorial design is used for separation of drugs using chromatographic technique. Finally, the most environmentally friendly approach used four green assessment methods: NEMI, GAPI, AMGS (1242.90), and AGREE with metrics (0.78). This technique will also help research and testing departments in commercial and industrial laboratories to adopt and evaluate the different combinations in bulk and dosage forms for tablets. AQBd-based approaches for analyzing chemicals in green solvents could be adopted and improved by the scientific community based on the results of this study.

4. MATERIALS AND METHODS

4.1 Reagents and Materials

- Distilled water
- Acetone
- Fluoxetine, Torrent Laboratories in Gujarat, provided the reference standard as a gift sample.

4.2 Instrumentation

The experiment was carried out with the quantitative HPTLC analysis On a Camag Linomat V automated sample applicator with a TLC Scanner III. Data collecting and processing software were included with the HPTLC system.

4.3 Densitometric Conditions

Different densitometric settings were used to establish an accurate HPTLC technique for fluoxetine analysis that is linear, specific, and has adequate stability. Among the several mobile phases tested, the acetone: water with 1% orthophosphoric acid (8.5:1.5 v/v) mobile phase was determined to be suitable for the analysis of fluoxetine.

4.4 Method development

4.4.1 Standard solution preparation

Fluoxetine 10 mg was carefully weighed and transported to a 10 ml volumetric flask and diluted, where it was dissolved in acetone, and the volume was made up with acetone (1000 mcg/ml). The standard chromatogram of Fluoxetine were shown in the Figure 6.

4.4.2 Analytical wavelength selection

Bands were examined across the 400-200 nm range after chromatographic development and 270 nm was selected to estimate the drug.

4.4.3 Software aided method optimization

DOE is a method for determining the best composition parameters. It is used to investigate critical impacts as well as their interactions. CCD is a feature of RSM that allows you to create quadratic response surfaces without using a three-stage factorial design. The univariate early investigations of the chromatographic technique development are significant aspects, along with the experimental levels under research for optimization. Twenty trials and 5 center points were explored with three components (A, B & C) for Fluoxetine.

1. Acetone content in ml (A),
2. Saturation time in min (B),

3. Developing distance in cm (C)
4. Retardation factor (Rf)

The central composite design study applies a response surface approach that uses a limited number of trials to the chromatography to optimize, verify and evaluate Fluoxetine. The different factors in the experiment specified that HPTLC techniques were applied to optimize different response factors. As a result, the primary goal of this project is to create a stability-indicating assay method in HPTLC for Fluoxetine using the analytical quality by design space approach. It would be viable to apply central composite design with Response Surface Method to filter and optimize the experiment's variables and use HPTLC techniques to estimate the quantity of Fluoxetine in pharmaceuticals by utilizing the suggested method.[26]

4.5. Forced Degradation studies

Fluoxetine was hydrolyzed in 0.1M sodium hydroxide, 0.1M hydrochloric acid, and (30%) hydrogen peroxide. The thermal degradation of Fluoxetine was also studied at 80 and 105 degrees Celsius. Photo degradation was also studied in two different forms: dry powder and solution. Acetone was used to dissolve 50 mg of fluoxetine powder that had been carefully weighed. Each flask was given 5.0 ml of each base and acid, and they were mixed gently for 12 and 48 hours, accordingly. A carefully weighed amount of sample was also kept in varied concentrations of H₂O₂ solution for the experiment. The samples were collected at regular intervals, allowed to cool to room temperature, and then processed. PH 7 was achieved by neutralizing the acid and base samples. As such, the thermal and light degradation samples and the hydrogen peroxide samples were employed. All of the samples were diluted further with acetone to a 100 mcg/ml concentration. Blank received the same treatment.

Acknowledgements: The authors are thankful to the Chancellor, SRM Institute of Science and Technology, and the management of SRM College of Pharmacy, SRM Institute of Science and Technology, Kattankulathur, for allowing carrying out the research work in the university facility. We also thank Torrent laboratories, Gujarat, for providing the gift sample of Fluoxetine.

Author contributions: Concept - P.; Design - P., K.; Supervision - K.S.; Resources - P., K.; Materials - P.; Data Collection and/or Processing - P., K.; Analysis and/or Interpretation - P., K., K.S.; Literature Search - P., K.; Writing - P.; Critical Reviews - P., K., K.S.

Conflict of interest statement: The authors declared no conflict of interest" in the manuscript.

REFERENCES

- [1] M.Y. Guo, M. Etminan, R.M. Procyshyn, D.D. Kim, A. Samii, A. Kezouh, B.C. Carleton, Association of Antidepressant Use with Drug-Related Extrapiramidal Symptoms: A Pharmacoepidemiological Study, *Journal of Clinical Psychopharmacology*. 38 (2018) 349-356. [\[CrossRef\]](#)
- [2] APA, *The Treatment of Depression Across Three Age Cohorts*, (2019). [\[CrossRef\]](#)
- [3] B. Matthews, *Regulatory Aspects of Stability Testing in Europe*, 25 (2000) 579-618. [\[CrossRef\]](#)
- [4] V.R. Sinha, Monika, A. Trehan, M. Kumar, S. Singh, J.R. Bhinge, *Stress studies on acyclovir*, *Journal of Chromatographic Science*. 45 (2007) 319-324. [\[CrossRef\]](#)
- [5] ICH, *ICH Q1B, Stability Testing : Photostability Testing of New Drug Substances and Drug Products*. (1996).
- [6] K.K. Pradhan, Mukhopadhyay et al 1995.pdf, 5 (2014) 3418-3424. [\[CrossRef\]](#)
- [7] R.S. Kumar, P. Gayathri, N. Duganath, C. Kiran, C. Sridhar, K. Jayaveera, Simultaneous estimation of fluoxetine HCl and olanzapine in bulk Drug and pharmaceutical formulation by using UV-Visible spectroscopy method, *International Journal of Pharmaceutical Sciences and Drug Research*. 3 (2011) 52-55.
- [8] A. Kumar, S.K. Jain, Development and Validation of UV-Spectroscopy Based Stability Indicating Method for the Determination of Fluoxetine Hydrochloride, *Analytical Chemistry Letters*. 6 (2016) 894-902. [\[CrossRef\]](#)
- [9] A. Pathak, S.J. Rajput, Development of a stability-indicating HPLC method for simultaneous determination of olanzapine and fluoxetine in combined dosage forms, *Journal of Chromatographic Science*. 47 (2009) 605-611. [\[CrossRef\]](#)
- [10] M.M. Eswarudu, M. Anitha, N. Gayathri, T. Chaithanya, a Validated Rp-Hplc Method for the Simultaneous Estimation of Fluoxetine Hydrochloride and Olanzapine in Pharmaceutical Dosage Form, *International Research*

Journal of Pharmacy. 3 (2012) 310–313.

- [11] O.F. Fluoxetine, L. Chromatography, B.Y. High-, Laboratory Medicine, University of Connecticut School of Medicine, 499 (1990) 601–608.
- [12] G. Swapna, S.A. Rahaman, A.P. Rani, Development and validation of stability indicating analytical method for simultaneous estimation of cilnidipine, chlorthalidone and telmisartan in bulk and tablet dosage form, *Indian Drugs*. 57 (2020) 51–55. [CrossRef]
- [13] N. Yilmaz, Y. Özkan, S.A. Özkan, I. Biryol, H.Y. Aboul-Enein, High performance liquid chromatographic assay and drug dissolution studies of fluoxetine hydrochloride in capsule formulations, *Journal of Liquid Chromatography and Related Technologies*. 23 (2000) 1699–1710. [CrossRef]
- [14] A.A. Wassel, Development and Validation of (HPLC) Method for Simultaneous Determination of Atomoxetine HCl & Fluoxetine HCl in their Pharmaceutical Dosage Forms, *Biomedical Journal of Scientific & Technical Research*. 34 (2021) 26943–26950. [CrossRef]
- [15] C. Shah, B. Suhagia, N. Shah, D. Patel, N. Patel, Stability-indicating simultaneous HPTLC method for olanzapine and fluoxetine in combined tablet dosage form, *Indian Journal of Pharmaceutical Sciences*. 70 (2008) 251–255. [CrossRef]
- [16] M. Jagadeeswaran, S. Mahibalan, N. Gopal, Estimation of fluoxetine in capsule dosage form by HPTLC method, *International Journal of Pharmacy and Pharmaceutical Sciences*. (2009) 71–73.
- [17] Z. Zaheer, O. Shaikh, S. Thorat, R. Ahmed, Development and Validation of HPTLC Method of Fluoxetine Hydrochloride in Bulk and Pharmaceutical Formulation, 2 (2010) 44–48.
- [18] D. Shankar Maruti, S. Kumar Banerjee, Development and validation of HPTLC method for estimation of acyclovir in formulations, *International Journal of Research in Pharmaceutical Sciences*. 4 (2013) 310–315.
- [19] A. Maalanka, U. Hubicka, J. Krzek, M. Walczak, G. Izvorski, Determination of fluoxetine in the presence of photodegradation products appearing during UVA irradiation in a solid phase by chromatographic-densitometric method, kinetics and identification of photoproducts, *Acta Chromatographica*. 25 (2013) 465–481. [CrossRef]
- [20] A.B. Thomas, A. Naphade, S.S. Karanjikhele, Determination of alprazolam and fluoxetine HCL from spiked rat plasma using HPTLC with UV detection, *International Journal of Pharmacy and Pharmaceutical Sciences*. 8 (2016) 147–151.
- [21] S. Mennickent, R. Fierro, M. Vega, M. De Diego, C.G. Godoy, Quantitative determination of fluoxetine in human serum by high performance thin layer chromatography, *Journal of Separation Science*. 33 (2010) 2206–2210. [CrossRef]
- [22] H.F. Books, D. References, M. Windows, Design – Expert, (1996) 10–12.
- [23] S.L.C. Ferreira, R.E. Bruns, H.S. Ferreira, G.D. Matos, J.M. David, G.C. Brand, E.G.P. Silva, P.S. Reis, A.S. Souza, W.N.L. Santos, Box-Behnken design : An alternative for the optimization of analytical methods, 597 (2007) 179–186. [CrossRef]
- [24] M. Almeida, R. Erthal, E. Padua, L. Silveira, L. Am, Talanta Response surface methodology (RSM) as a tool for optimization in analytical chemistry, 76 (2008) 965–977. [CrossRef]
- [25] A. Gundala, K. Prasad, B. Koganti, Application of quality by design approach in RP-HPLC method development for simultaneous estimation of saxagliptin and dapagliflozin in tablet dosage form, (2015) 1–10.
- [26] International Conference, O.N. Harmonisation, O.F. Technical, R. For, R. Of, P. For, A. Of, requirements for registration of pharmaceuticals for human use harmonised tripartite guideline validation of analytical procedures : Parent Guideline : Text on Validation of Analytical Procedures, 1994 (2005).
- [27] C.M. Thompson, L.C. Haws, M.A. Harris, N.M. Gatto, D.M. Proctor, Application of the U.S. EPA mode of action framework for purposes of guiding future research: A case study involving the oral carcinogenicity of hexavalent chromium, *Toxicological Sciences*. 119 (2011) 20–40. [CrossRef]
- [28] EPA, Managing Your Hazardous Waste, *Marine Pollution Bulletin*. 11 (2001) 31.

- [29] B. Systems, S. Occupancies, H. Materials, Fire Code, 1 (2021) 2021–2022.
- [30] J. Plotka-Wasyłka, A new tool for the evaluation of the analytical procedure: Green Analytical Procedure Index, *Talanta*. 181 (2018) 204–209. [[CrossRef](#)]
- [31] H.M. Mohamed, N.T. Lamie, Analytical eco-scale for assessing the greenness of a developed RP-HPLC method used for simultaneous analysis of combined antihypertensive medications, *Journal of AOAC International*. 99 (2016) 1260–1265. [[CrossRef](#)]
- [32] L.J. Diorazio, P. Richardson, H.F. Sneddon, A. Moores, C. Briddell, I. Martinez, Making Sustainability Assessment Accessible: Tools Developed by the ACS Green Chemistry Institute Pharmaceutical Roundtable, *ACS Sustainable Chemistry and Engineering*. 9 (2021) 16862–16864. [[CrossRef](#)]
- [33] F. Pena-Pereira, W. Wojnowski, M. Tobiszewski, AGREE - Analytical GREENness Metric Approach and Software, *Analytical Chemistry*. 92 (2020) 10076–10082. [[CrossRef](#)]
- [34] K.S. Kokilambigai, K.S. Lakshmi, Utilization of green analytical chemistry principles for the simultaneous estimation of paracetamol, aceclofenac and thiocolchicoside by UV spectrophotometry, *Green Chemistry Letters and Reviews*. 14 (2021) 97–105. [[CrossRef](#)]
- [35] H.K. Chanduluru, A. Sugumaran, Eco-friendly estimation of isosorbide dinitrate and hydralazine hydrochloride using Green Analytical Quality by Design-based UPLC Method, *RSC Advances*. 11 (2021) 27820–27831. [[CrossRef](#)]