Article

Development and Validation of a Stability-Indicating Greener HPTLC Method for the Estimation of Flufenamic Acid

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Abstract: The literature on ecofriendly/greener high-performance thin-layer chromatographic (HPTLC) methods for quantifying flufenamic acid (FFA) is scant. In order to develop and validate a stability-indicating greener HPTLC densitometry assay for FFA determination in marketed products, this research was conducted. The ecofriendly eluent system was composed of ethanol–water (70:30 *v*/*v*). FFA was measured at 290 nm of wavelength. The greenness scale of suggested analytical assay was derived using "Analytical GREENness (AGREE)" methodology. The suggested stability-indicating HPTLC assay was linear for FFA determination in 25–1400 ng/band range with a determination coefficient of 0.9974. The suggested analytical assay for FFA analysis was simple, rapid, accurate, precise, robust, selective, stability-indicating, and greener. The AGREE scale for the developed stability-indicating HPTLC assay was derived to be 0.77 utilizing AGREE methodology, indicating an outstanding greenness characteristic of the suggested densitometry technique. The ecofriendly HPTLC technique was able to detect FFA degradation product under forced degradation studies, indicating its stability-indication characteristics and selectivity. The amount of FFA in marketed tablets brand A and B was determined to be 101.28 and 99.17%, respectively, indicating the suitability of the suggested analytical technique in the assay of FFA in marketed products. These results indicated that FFA in marketed products may be routinely measured using the stability-indicating greener HPTLC technique.

Keywords: AGREE; flufenamic acid; greener HPTLC; stability-indicating; validation

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1. Introduction

Flufenamic acid (FFA) is used to relieve pain and inflammation brought on by several types of disorders [1,2]. Its chemical structure is shown in Figure 1. It is administered both topically and orally [2,3]. It belongs to the anthranilics class of non-steroidal anti-inflammatory medicines (NSAIDs) [2]. It is a potent NSAID, which is a chemical compound with high permeability and weak acidity [3]. It has been reported practically insoluble in water, soluble in ethylene glycol, propylene glycol, and polyethylene glycol-400, freely soluble in Carbitol, methanol, ethanol, isopropanol, 1-butanol, and 2-butanol, and very soluble in dimethyl sulfoxide [4,5]. Its practical insolubility is the main hurdle of its formulation development especially in case of liquid formulations design [3]. FFA is available in several commercial products. As a result, both qualitative and quantitative methods must be used to estimate FFA in a variety of marketed dosage forms.

Figure 1. Chemical structure of flufenamic acid (FFA).

An exhaustive literature survey demonstrated the wide range of analytical techniques for FFA determination in marketed products and biological fluids. For the determination of FFA in marketed products and pharmaceuticals, some flow injection analytical approaches have been developed and validated [6,7]. A flow injection analysis approach has also been developed to determine FFA in urine and plasma samples [7]. Various "high-performance liquid chromatography (HPLC)" methodologies were designed and evaluated to determine FFA in combination with other NSAIDs in pharmaceuticals, plasma, serum, and urine samples [8–11]. Some "liquid-chromatography mass-spectrometry (LC-MS)" approaches were also developed to determine FFA in combination with other NSAIDs in pharmaceuticals and river water samples [12,13]. Some photochemically-induced fluorescence detection of FFA in pharmaceuticals and urine has also been carried out [14,15]. An adsorptive voltammetry with in situ surfactant modified carbon paste electrode has also been used to determine FFA in human serum samples [16]. Capillary electrophoresis and capillary isotachophoretic techniques have also been developed to determine FFA in combination with other NSAIDs in dosage forms and pharmaceuticals [17,18]. Capillary electrophoresis has also been used to determine FFA in serum and urine samples [17]. A gas chromatography approach was also used to determine FFA in rat plasma and uterus [19]. Some other techniques, such as amperometry [20], electrochemical [21], and luminescence [22] techniques have also been used to determine FFA in dosage forms and pharmaceuticals.

There are numerous analytical techniques for FFA measurement in the literature. However, neither a regular HPTLC method nor an ecofriendly/greener HPTLC method have been described for FFA detection. In addition, no greenness profile of literature analytical techniques was determined. Numerous approaches to forecast the greenness characteristics of the analytical techniques were provided in the published literature [23–27]. To forecast greenness characteristics, only the "analytical greenness (AGREE)" technology takes into account all twelve components of green analytical chemistry (GAC) [25]. In order to anticipate the greenness profile of the proposed densitometry assay, the AGREE approach was applied [25].

Based on all of these presumptions, this research's goal was to design and evaluate a stability-indicating, greener/ecofriendly reverse-phase HPTLC densitometry assay for FFA quantification in marketed formulations. The proposed stability-indicating greener HPTLC approach for FFA detection was proven efficient using the International Council for Harmonization (ICH)-Q2-R1 requirements [28].

2. Materials and Methods

2.1. Materials

The standard FFA was obtained from Sigma Aldrich (St. Louis, MO, USA). The chromatography grade ethanol and methanol were provided by E-Merck (Darmstadt, Germany). The chromatography-grade water (deionized water) was collected from the Milli-Q® (Milli-Q, Lyon, France) unit. The marketed tablet brand A and B of FFA (each containing 200 mg of FFA) were obtained from New Delhi, India. All additional reagents were of the analytical grade.

2.2. Equipment and Measurement Procedures

The HPTLC CAMAG TLC system (CAMAG, Muttenz, Switzerland) was used to analyze FFA in marketed tablet brand A and B. To apply the samples as a 6 mm bands, a CAMAG Automatic TLC Sampler 4 (ATS4) Sample Applicator (CAMAG, Geneva, Switzerland) was utilized. TLC plates used for the FFA separation were RP-60F254S plates (E-Merck, Darmstadt, Germany). The CAMAG microliter syringe (Hamilton, Bonaduz, Switzerland) was linked to the sample applicator. Throughout the course of the examination, the application rate for FFA separation remained constant at 150 nL/s. The TLC plates were set up with a distance of 80 mm in a CAMAG automated developing chamber 2 (ADC2) (CAMAG, Muttenz, Switzerland). The ecofriendly eluent system was made of the binary combination of ethanol–water (70:30 v/v). The greener eluent system's vapors were used to completely saturate the chamber for 30 min at 22 °C. At a wavelength of 290 nm, FFA was measured. The slit size was fixed to 4×0.45 mm^2 and the scan speed was set to 20 mm/s. For each examination, three or six replicates were used. The WinCAT's (version 1.4.3.6336, CAMAG, Muttenz, Switzerland) program was utilized for analyzing the data.

2.3. Calibration Curve for FFA

The needed amount of FFA was dissolved in the prescribed amount of the greener eluent system to produce the FFA stock solution with the final concentration of 100 µg/mL in triplicates (n = 3). From the stock solution, serial dilutions were prepared to obtain FFA concentrations in the 25–1400 ng/band in triplicates (n = 3). Around 200 µL of each FFA solution was spotted onto reverse-phase TLC plates and densitometry response was noted. The FFA calibration plot was produced by graphical representation of observed densitometry response vs. FFA concentrations in six replicates (n = 6). Three distinct quality control (QC) concentrations were also freshly produced for the evaluation of various parameters for validation studies.

2.4. Sample Processing for the Assay of FFA in Marketed Tablets

The average weight of ten commercially available tablet brands A and B—each containing 200 mg of FFA—was computed. The average weight of brands A and B was 450 and 455 mg, respectively. Based on the average weight, the ten tablets to determine FFA content in commercially available tablets were sufficient. Ten tablets from each brand were broken down and turned into a fine powder. Ten milliliters of methanol were combined with a portion of the powder (450 mg for brand A and 455 mg for brand B) from each brand. Then, 1 mL of this solution for each brand of tablet was mixed with 50 mL of the greener eluent system. To get rid of any undissolved components, the prepared solutions of the marketed tablets were filtered via Whatman filter paper (No. 41) and sonicated at 25 °C and the frequency of 26 kHz for 15 min. Using the stability-indicating greener HPTLC technique, the obtained solutions were used to analyze FFA in marketed tablets.

2.5. Validation Parameters

Using the ICH-Q2-R1 procedures, the stability-indicating greener HPTLC technique for FFA measurement in solution was validated for various parameters [28]. To determine the specificity of the stability-indicating ecofriendly densitometry technique for FFA deter-

mination, the retardation factor (R_f) values and UV spectra of FFA in tablet brands A and B were compared to that of standard FFA.

To assess the system's suitability for the stability-indicating ecofriendly densitometry procedure for FFA measurement, the assessment of R_f, asymmetry factor (As), and theoretical plates number/meter (N/m) were used. The appropriate equations, which are reported in the literature, were used to obtain the "R_f, As, and N/m" values [27].

FFA linearity was investigated by graphing measured peak area versus FFA concentrations. The linearity of the proposed analytical technique for FFA analysis was derived in the 25–1400 ng/band range in six replicates (n = 6).

The sensitivity of the stability-indicating greener densitometry assay for FFA was estimated as "limit of detection (LOD) and limit of quantification (LOQ)" utilizing a standard deviation approach. For the suggested analytical assay, the blank sample was analyzed in six replicates (n = 6) and standard deviation was determined. FFA "LOD and LOQ" values were then computed utilizing their reported equations in six replicates (n = 6) [29].

The intra-day and inter-day precision of the stability-indicating greener HPTLC assay was evaluated for FFA. The intra-day variation for FFA was derived by analyzing freshly produced FFA solutions at three distinct QC solutions, namely low QC (LQC = 50 ng/band), middle QC (MQC = 400 ng/band), and high QC (HQC = 1400 ng/band) on the same day in six replicates (n = 6). The FFA inter-day variation was derived by evaluating freshly produced FFA solutions at same QC levels on three distinct days in six replicates (n = 6). Both precisions were measured as relative standard deviation in percent (%RSD).

The intra-day and inter-day accuracy of the stability-indicating greener HPTLC assay for FFA estimation was evaluated using the standard addition/spiking method in terms of % recovery [29]. The pre-analyzed solution of FFA (50 ng/band) was spiked with 0, 350, and 1350 ng/band extra solution of FFA in order to obtain the solutions at LQC (50 ng/band), MQC (400 ng/band), and HQC (1400 ng/band). Six replicates (n = 6) were analyzed on the same day to determine intra-day accuracy at three different QC solutions. On three distinct days, six replicates (n = 6) of FFA's LQC, MQC, and HQC solutions were employed to measure inter-day accuracy. The % recovery for intra-day and inter-day accuracy was calculated at each QC level.

By intentionally modifying the mixture of the greener eluent system, the FFA robustness was measured for the stability-indicating ecofriendly densitometry assay. The ecofriendly eluent system ethanol–water (70:30, v/v) for FFA was modified to ethanol–water (72:28, v/v) and ethanol–water (68:32, v/v) for the ecofriendly densitometry assay, and variations in FFA chromatographic response and R_f values were determined.

2.6. Force Degradation/Selectivity Studies

To examine the selectivity/stability-indicating capabilities of the ecofriendly HPTLC assay, force degradation studies under a various stress conditions, including acidic (HCl), alkali (NaOH), oxidative (H_2O_2), and thermal stress conditions, were performed [27,30]. These studies were performed under mild conditions according to ICH recommendations [28]. The MQC level (400 ng/band) of FFA was freshly produced into the greener eluent system for acid and alkali-induced degradation. By mixing 4 mL of 1M HCl or 4 mL of 1M NaOH into an aliquot (1 mL) of MQC solution, acid and alkali hydrolysis were evaluated. Both mixtures were diluted with greener eluent system to obtain a total volume of 10 mL. For the detection of FFA in the presence of its acid- and alkali-degradation products, respectively, these mixtures were refluxed for 48 h at 60 °C, and examined using the greener HPTLC assay [27].

The MQC level (400 ng/band) of FFA was freshly produced and introduced into the greener eluent system for oxidative degradation studies. This MQC solution (1 mL) was oxidatively degraded by adding 2 mL of 30% H_2O_2. The mixture was diluted with greener eluent system to obtain a total volume of 10 mL. For the detection of FFA in the presence of its oxidative-degradation products, this mixture was refluxed for 48 h at 60 °C, and examined using the greener HPTLC assay [27].

An aliquot of MQC (400 ng/band) level (1 mL) was subjected to a hot air oven for 48 h at 60 °C for thermal degradation studies. The mixture was diluted with greener eluent system to obtain a total volume of 10 mL. The humidity of a hot air oven was not monitored. It was then analyzed using the greener HPTLC assay for the determination of FFA in the presence of its thermal-degradation products [27].

2.7. Application of the Greener HPTLC Assay in the Determination of FFA in Marketed Tablets

For the stability-indicating greener HPTLC assay, prepared samples of commercially available tablets were put to TLC plates. Chromatographic responses were obtained using the same experimental techniques employed for the measurement of standard FFA in triplicates (n = 3). The % assay of FFA in packaged tablets was calculated for the stability-indicating HPTLC test using the FFA calibration curve.

2.8. Greenness Assessment

Using the AGREE technique, the greenness features for the suggested stability-indicating analytical assay for FFA measurement were evaluated [25]. Using AGREE: The Analytical Greenness Calculator (version 0.5, Gdansk University of Technology, Gdansk, Poland, 2020), the AGREE scale (0.0–1.0) for the stability-indicating densitometry assay was established.

3. Results and Discussion

3.1. Analytical Method Development

To provide an acceptable band for FFA separation, a number of ethanol and water concentrations were investigated as the ecofriendly eluent systems, including ethanol–water (30:70, *v*/*v*), ethanol–water (40:60, *v*/*v*), ethanol–water (50:50, *v*/*v*), ethanol–water (60:40, *v*/*v*), ethanol–water (70:30, *v*/*v*), ethanol–water (80:20, *v*/*v*), and ethanol–water (90:10, *v*/*v*). All greener eluent systems were established using the chamber saturation conditions. A representative TLC chromoplate for the standard FFA, marketed formulations, and degradation samples is presented in Figure 2.

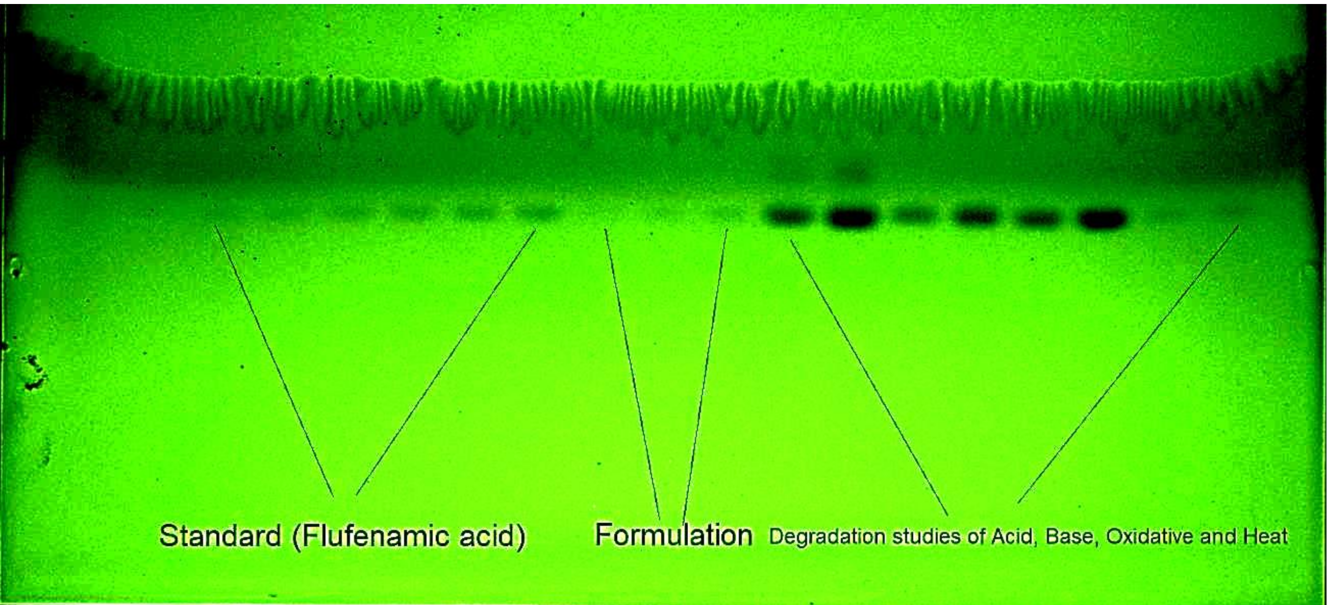

Figure 2. A representative thin-layer chromatographic (TLC) chromo-plate of standard FFA, marketed products, and forced degradation studies samples obtained using ecofriendly ethanol–water (70:30 *v*/*v*) eluent system for the ecofriendly high-performance-TLC (HPTLC) assay.

According to the findings, the ecofriendly eluent systems, such as ethanol–water (30:70, v/v), ethanol–water (40:60, v/v), ethanol–water (50:50, v/v), ethanol–water (60:40, v/v), ethanol–water (80:20, v/v), and ethanol–water (90:10, v/v), caused unfavorable FFA chromatographic peaks with undesirable As values (As >1.20) when examined. However, upon closer inspection, it was discovered that the ecofriendly ethanol–water eluent system (70:30, v/v) generated a well-resolved and uninterrupted FFA chromatographic peak at R_f = 0.71 ± 0.01 (Figure 3) when examined. Additionally, it was determined that FFA had a suitable As value of 1.06 for FFA separation. As a consequence, the final ecofriendly eluent system for the stability-indicating ecofriendly densitometry assay of FFA analysis was chosen to be ethanol–water (70:30, v/v). The greatest chromatographic response for FFA was detected at 290 nm wavelength when the spectral bands for FFA were measured under densitometric mode. Thus, 290 nm was utilized for all of the FFA examinations.

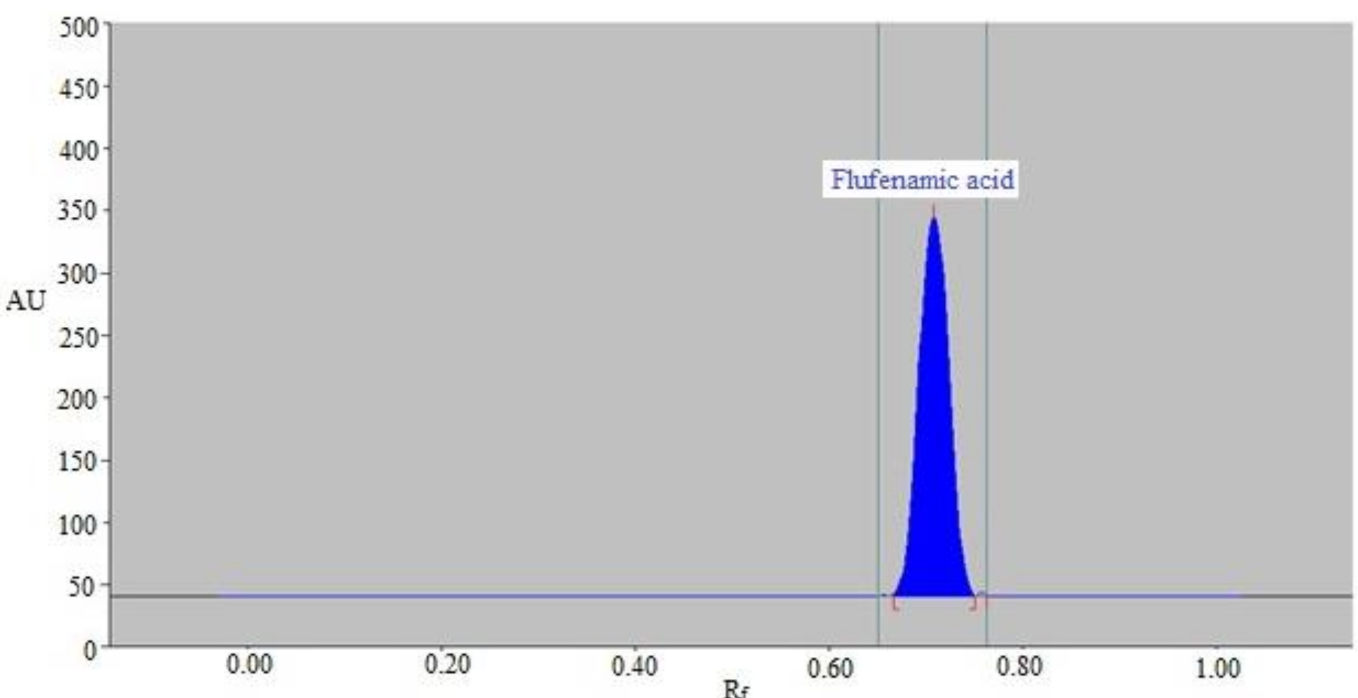

Figure 3. Representative chromatograms of standard FFA for the ecofriendly HPTLC assay.

3.2. Analytical Method Validation

In order to obtain numerous validation parameters for FFA estimation in solutions, the ICH-Q2-R1 guidelines were used [28]. The specificity of the suggested analytical technique for determining FFA levels could be assessed by contrasting the R_f data and UV spectrum of the FFA in formulations A and B with those of standard FFA. The combined UV-absorption spectra of standard FFA and FFA in formulation A and B are illustrated in Figure 4 for comparison. At 290 nm, the peak response of formulations A and B and standard FFA was observed. The standard, formulations A and B, all had similar UV spectrum, R_f data, and detection wavelengths, demonstrating the specificity of the suggested analytical procedure for determining FFA.

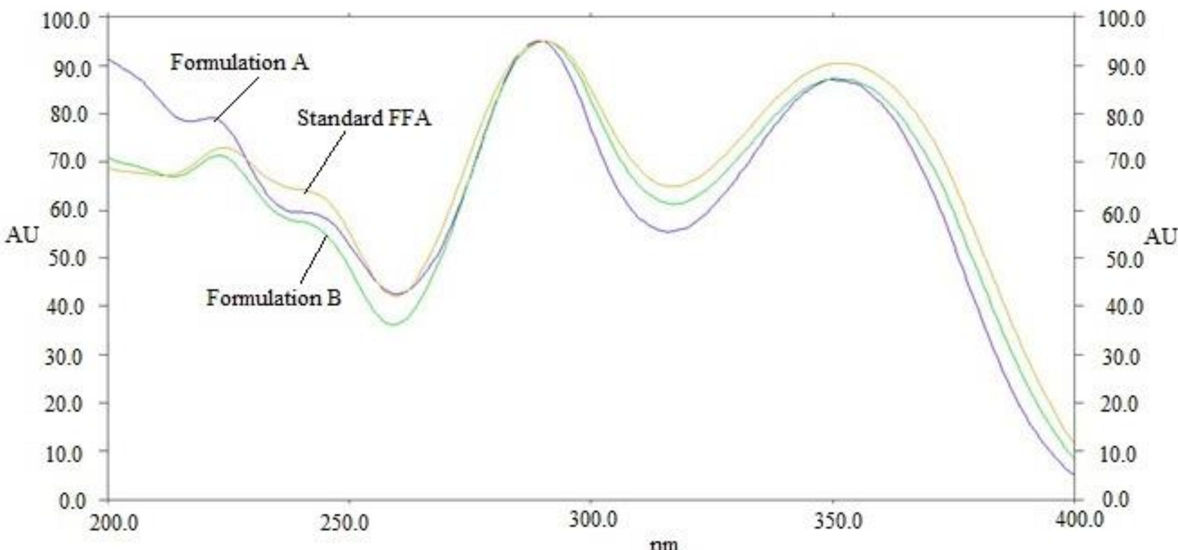

Figure 4. UV-absorption spectra of standard FFA and marketed products, superimposed.

The system appropriateness parameters for the suggested analytical assay are included in Table 1. The suggested densitometry technique's R_f, As, and N/m for FFA measurement were found to be 0.71, 1.06, and 4987, respectively, which were reliable for FFA analysis.

Table 1. System appropriate parameters of FFA for the ecofriendly HPTLC assay (mean ± SD; n = 3).

Parameters	Greener HPTLC
R_f	0.71 ± 0.01
As	1.06 ± 0.02
N/m	4987 ± 4.67

R_f: retardation factor; As: asymmetry factor; N/m: theoretical plates number/meter.

Table 2 shows the data from the FFA calibration curve's linear regression analysis for the stability-indicating greener analytical assay. The suggested analytical assay's FFA calibration plot was observed linear in the concentration range of 25–1400 ng/band. FFA's determination coefficient (R^2) and regression coefficient (R) for the suggested analytical assay were found to be 0.9974 and 0.9986, respectively. These results pointed to a strong correlation between the measured peak response and the FFA concentrations. The linearity of the stability-indicating greener analytical assay for FFA estimation over a wider range of concentrations was revealed by these results.

Table 2. Findings for the linearity of flufenamic acid (FFA) for the greener high-performance thin-layer chromatography (HPTLC) assay (mean ± SD; n = 6).

Parameters	Value
Linearity range (ng/band)	25–1400
Regression equation	y = 14.447x + 511.64
R^2	0.9974
R	0.9986
SE of slope	0.28
SE of intercept	1.97
95% CI of slope	13.19–15.69
95% CI of intercept	503.13–520.14
LOD ± SD (ng/band)	8.51 ± 0.04
LOQ ± SD (ng/band)	25.53 ± 0.12

R^2: determination coefficient; R: regression coefficient; y: peak area; x: concentration (ng/band); SE: standard error; CI: confidence interval; LOD: limit of detection; LOQ: limit of quantification.

To measure the sensitivity of the proposed analytical technology of FFA estimation, the LOD and LOQ were calculated. Table 2 summarizes the resulting values of LOD and LOQ of FFA for the proposed densitometry assay. As per the findings, FFA's LOD and LOQ were calculated to be 8.51 ± 0.04 and 25.53 ± 0.12 ng/band, respectively. The obtained findings revealed that the proposed densitometry assay was sensitive enough for FFA estimation.

The specificity results of the proposed method showed that there were no significant changes in the R_f value and absorption spectra of standard FFA and marketed products (Figure 4), so it was not necessary to determine the precision and accuracy on synthetic mixtures of the product components (active substance and ingredients). As a result, the precision and accuracy of the proposed method were determined in solutions instead of tablets or synthetic mixtures [29]. The precision data for FFA estimation are expressed as % RSD, and the intra-day/inter-day precision of the proposed analytical assay was examined. The outcomes of both precisions for the proposed analytical assay of FFA estimation are illustrated in Table 3. The proposed analytical technology was shown to have a 0.81–0.90% RSD of FFA for intra-day precision. The proposed analytical technology was shown to have an RSD of FFA for inter-day variation of 0.84-0.95%. These results demonstrated the precision of the proposed analytical procedure to estimate FFA.

Table 3. Determination of FFA intra-day and inter-day precision for the ecofriendly HPTLC assay (mean ± SD; n = 6).

Conc. (ng/band)	Intra-Day Precision			Inter-Day Precision		
	Conc. (ng/band) ± SD	SE	RSD (%)	Conc. (ng/band) ± SD	SE	(%) RSD
50	49.54 ± 0.45	0.18	0.90	49.42 ± 0.47	0.19	0.95
400	405.21 ± 3.51	1.43	0.86	409.22 ± 3.61	1.47	0.88
1400	1415.51 ± 11.56	4.72	0.81	1381.12 ± 11.68	4.76	0.84

The suggested analytical assay of FFA measurement's intra-day and inter-day accuracy was calculated as % recovery. The % recovery data for the suggested densitometry assay is summarized in Table 4. The intra-day % recoveries of FFA at three distinct QC concentrations were found to be 99.10–100.87% using the proposed analytical assay. FFA inter-day % recoveries at three distinct QC levels were estimated to be 98.36–99.80% for the proposed analytical assay. The obtained findings revealed that the suggested densitometry technology was accurate for the estimation of FFA.

Table 4. Intra-day and inter-day accuracy data of FFA for the ecofriendly HPTLC assay (mean ± SD; n = 6).

Conc. (ng/band)	Conc. Found (ng/band) ± SD	Recovery (%)	RSD (%)
	Intra-day accuracy		
50	50.31 ± 0.56	100.62	1.13
400	403.51 ± 3.76	100.87	0.93
1400	1387.41 ± 10.12	99.10	0.72
	Inter-day accuracy		
50	49.61 ± 0.61	99.22	1.22
400	393.45 ± 3.84	98.36	0.97
1400	1383.21 ± 10.38	98.80	0.75

The robustness of the proposed densitometry technology for FFA measurement was evaluated by deliberately modifying the composition of the greener eluent system. The resulting data of robustness evaluation for the proposed densitometry technology are illustrated in Table 5. FFA % RSD for the proposed analytical technology was found to be 0.98–1.01%. FFA R_f values were found to be 0.70–0.72 for the proposed densitometry technology. These results demonstrated that the suggested analytical technology for FFA estimation was robust.

Table 5. Findings of FFA robustness for the ecofriendly HPTLC assay (mean ± SD; n = 6).

Conc. (ng/band)	Greener Eluent System (Ethanol–Water)			Results		
	Original	Used	Level	Conc. (ng/band) ± SD	RSD (%)	R_f
400	70:30	72:28	+2.0	391.23 ± 3.87	0.98	0.70
		70:30	0.0	397.84 ± 3.97	0.99	0.71
		68:32	-2.0	408.51 ± 4.15	1.01	0.72

3.3. Selectivity/Force Degradation Studies

The selectivity/degradation of the suggested densitometry assay was examined under various stress conditions. The findings of the proposed analytical methodology are illustrated in Figure 5 and Table 6. The FFA peak was easily separated from other peaks of degradation products in the chromatographic peaks from circumstances of degradation (Figure 5). Under the acid-degradation scenario, FFA remained at 87.35%, while 12.65% was decomposed (Table 6 and Figure 5A). As a consequence, FFA was found to be stable enough to withstand acid deterioration. Peak 2 in Figure 5A, which represents the acid-induced degradation peak, was separated by R_f value of 0.79. Under acid-degradation scenario, FFA's R_f value was slightly changed (R_f = 0.70). Because there was 100% of FFA left after alkali, oxidative, and thermal degradation, no FFA was decomposed under these circumstances (Table 6 and Figure 5B–D). As a result, FFA was found to be highly stable under alkali, oxidative, and thermal degradation conditions. Under alkali-degradation conditions, FFA's R_f value was also slightly changed (R_f = 0.70). However, under oxidative and thermal degradation conditions, FFA's R_f value was unchanged (R_f = 0.71 under both conditions). Using the greener HPTLC assay, the highest FFA decomposition under acid-degradation scenario was recorded. All of these results revealed that FFA can be detected using the greener HPTLC technology in the presence of its degradation products. These findings and observations indicated that the greener HPTLC assay had a selectivity and stability-indicating property.

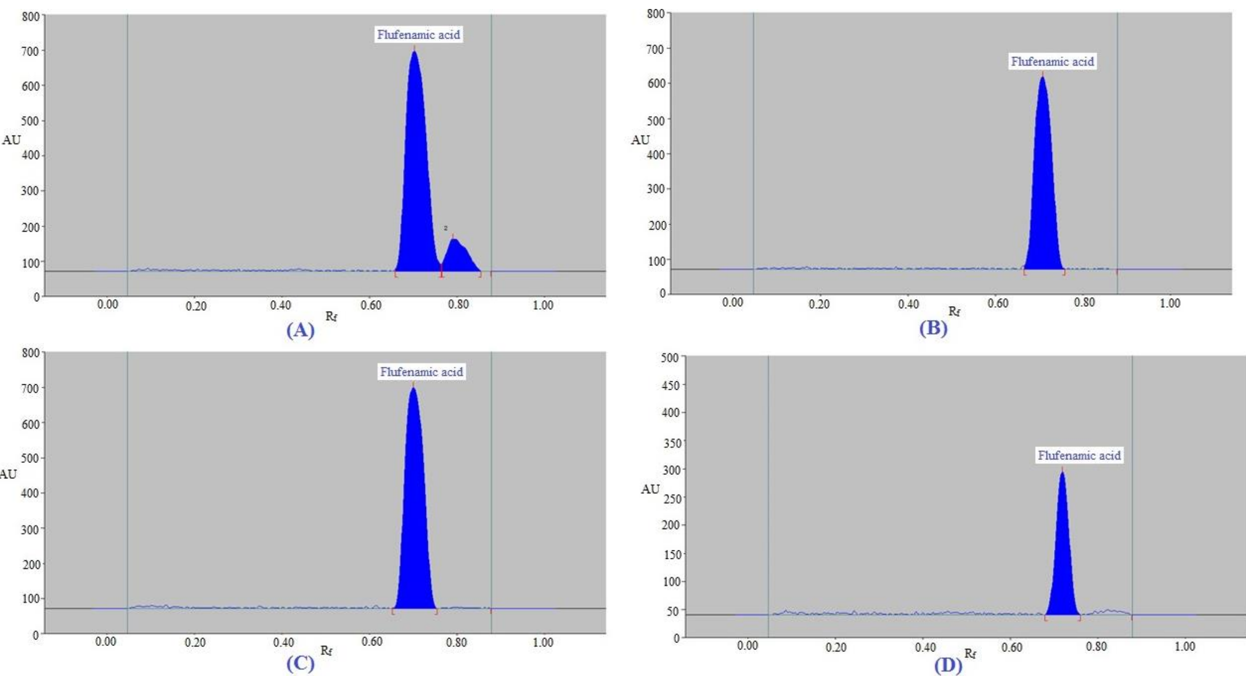

Figure 5. Representative densitometry chromatograms of FFA derived under (**A**) acid-induced degradation, (**B**) base-induced degradation, (**C**) oxidative degradation, and (**D**) thermal degradation of FFA.

Table 6. Results of forced-degradation studies of FFA under various stress conditions for the greener HPTLC assay (mean ± SD; n = 3).

Stress Condition	Number of Degradation Products (R_f)	FFA R_f	FFA Remained (ng/band)	FFA Recovered (%)
1M HCl	1 (0.79)	0.70	349.40	87.35 ± 1.41
1M NaOH	0	0.70	400.00	100.00 ± 0.00
30% H_2O_2	0	0.71	400.00	100.00 ± 0.00
Thermal	0	0.71	400.00	100 ± 0.00

3.4. Application of Greener HPTLC Assay in the Determination of FFA in Marketed Products

For the determination of FFA in marketed products, the stability-indicating greener HPTLC assay was used as an alternative strategy to regular analytical methods. By contrasting the TLC spot at R_f = 0.71 ± 0.01 for FFA with standard FFA utilizing the ecofriendly analytical technology, the chromatogram of FFA from the formulation A and B was confirmed. FFA in the formulation A and B had the same chromatographic peak as standard FFA when employing the ecofriendly densitometry assay. Additionally, no new formulation excipient peaks were found in formulations A or B, indicating that there was no interaction between FFA and the excipients. Utilizing the ecofriendly HPTLC assay, FFA content was determined by the FFA calibration plot. Using the greener HPTLC assay, the % content of FFA in formulation A and B was found to be 101.28 and 99.17%, respectively. The obtained outcomes indicated the suitability of the stability-indicating ecofriendly densitometry assay for FFA pharmaceutical assay.

3.5. Greenness Assessment

Various qualitative and quantitative technologies are used to derive the greenness of analytical assays [23–27]. However, only AGREE considers all twelve GAC criteria to derive the greenness profile [25]. As a consequence, the AGREE technology was applied to gauge the analytical strategy's greenness characteristics. A representative diagram for the AGREE scale of the proposed analytical assay is shown in Figure 6. For the suggested HPTLC assay, the AGREE scale was derived to be 0.77, pointing that the suggested analytical assay for FFA estimation had an outstanding greenness profile.

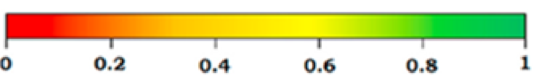

Figure 6. The representative pictogram for AGREE scale for the proposed HPTLC strategy derived using AGREE calculator.

4. Conclusions

There are no regular or greener HPTLC assays available for FFA determination. As a result, this work was carried out to develop and validate a simple, rapid, sensitive, and stability-indicating ecofriendly HPTLC assay for FFA measurement in marketed products. The suggested analytical assay of FFA measurement is simple, accurate, precise, rapid, robust, selective, greener, and stability-indicating. AGREE results indicated an outstanding greenness profile of the suggested analytical assay. The maximum degradation of FFA was recorded under acid degradation conditions and it was found to be stable under alkali, oxidative, and thermal degradation conditions. The recommended analytical assay's selectivity and stability-indicating properties were demonstrated by the greener HPTLC assay's ability to detect FFA even in the presence of its breakdown products. These results demonstrated that the stability-indicating greener HPTLC assay can be used to determine FFA in marketed products.

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