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Development and Validation of HPTLC SIAM for Furosemide and Spironolactone

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"Diuretics," like spirolactone and furosemide, help the kidneys eliminate excess water. It also reduces fluid-induced hypertension and maintains blood potassium levels. Both Furosemide (FRU) and Spironolactone (SPL) can be measured at the same time with the help of HPTLC chromatographic method that have been shown to be very selective and accurate. There are a number of causes of edema, and FRU can help with all of them, including hepatic cirrhosis, chronic congestive heart failure, and excessive blood pressure. Heart failure and ascites caused by hepatic diseases are commonly treated with spironolactone due to its properties as an aldosterone antagonist and potassium-sparing diuretic. HPTLC methods were developed in this research to determine FRU and SPL simultaneously without using the solvents generally needed in chromatographic procedures. The proposed HPTLC approach stood out as an analytical method for quality control laboratories due to its speed, low cost, and ability to concurrently determine the target chemicals with a small number of solvents. The selectivity, accuracy, and reproducibility of the procedures for the simultaneous determination of the pure and mixed drug forms studied were further confirmed by statistical analysis.

Keywords: Spironolactone, Furosemide, HPTLC, HPLC, and Validation.

In order to lessen the amount of fluid that is retained in the body, loop diuretics such as Furosemide (FRU) are taken. 4-Chloro-2-[(FRUan-2-ylmethyl) amino] The IUPAC designation for this compound is 5-sulfamoylbenzoic acid. It is acknowledged as a legitimate medication in a variety of pharmacopoeias. There are a variety of generic names for FRU, including Furosemide, Aisemide, Beronald, Desdimin, and Lasilix, amongst others. Conditions such as hypertension, chronic congestive heart failure, and edema caused by hepatic cirrhosis are all able to benefit from the application of FRU[1-2].

Both spirolactone and furosemide are examples of the class of medications known as "diuretics," which are commonly used to assist the kidneys in excreting excess water from the body. In addition, it prevents hypertension, which is defined as high blood pressure that is brought on by the retention of fluid, and it maintains a healthy potassium balance in the blood[3, 4].

Heart failure and ascites due to hepatic diseases are two of the most common indications for the use of spironolactone (SPL; 17-hydroxy-7-mercapto-3-oxo-17-pregen-4-ene-21-carboxylic acid—lactone acetone). It is a diuretic that spares potassium and acts as an antagonist of aldosterone.

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It is imperative that both medications be taken at the same time in order to mitigate the negative effects of hypokalemia brought on by FRU. A variety of different analytical methods for identifying the presence of both drugs were discovered as a result of the search for relevant literature[5-7]. Calculations of FRU were made using a variety of analytical methods, such as spectrophotometry, thin-layer chromatography, spectrofluorimetry, and high-performance liquid chromatography. Furosemide is the first loop diuretic ever developed; hence it is the one that sets the standard. In addition to these side effects, FRU may also cause hyponatremia, hypokalemia, hyperuricaemia, paresthesis, cloudy vision, and orthostatic hypotension[8-10].

Several methods for the determination of Furosemide in bulk, in pharmaceutical samples, and in biological samples[11, 12] have been published as a result of this study. In addition to or instead of other medications, these methods can be utilized. In this study, HPTLC strategies were created for the concomitant measurement of FRU and SPL. These methods did not require the use of the solvents that are customarily necessary for chromatographic operations. The development and validation of HPTLC SIAM for the diuretic medications furosemide and spironolactone were the aims of this work.

MATERIALS AND METHODS

The Camag HPTLC System was used in this study. For this procedure, you will need a UV-Visible Double beam spectrophotometer, a Hamilton syringe (100 ul), a Camag TLC Scanner 3, Win CATS software V- 1.4.2, and a Linomat - 5 sample applicator (Jasco Model V-730 with a single Monochromator). All of these chemicals and reagents can be found in a product called SPIROMIDE, made by RPG Life Sciences Ltd. According to the product label, each film-coated tablet contains 20 milligrams of furosemide and 50 milligrams of spironolactone.

Method Development

Chromatographic conditions and mobile phase selection

Chromatographic separation studies employed standard solutions of FRU (400 ng/band) and SPL (1000 ng/band). Studies were conducted before hand to determine the ideal solvent concentration and plate temperature for HPTLC analysis. Chloroform, methanol, and glacial acetic acid (7.5:2:0.5 v/v/v) proved to be the mobile phase that provided the best resolution and peak characteristics overall. By adjusting the chromatographic parameters (such as the chamber saturation time, run length, distance between tracks, and detection wavelength), we were able to achieve constant Rf values and a symmetrical peak shape for the drug.

The samples were applied to a precoated silica gel aluminum plate 60 F254 with a thickness of 250mm (E. MERCK, Darmstadt, Germany) using a CAMAG Linomat 5 sample applicator and a 100 µL sample syringe (Hamilton, Bonaduz, Switzerland). The width of the bands was 6 mm, and there was an 8 mm gap between each band (Switzerland). The width of the slit was 0.45 mm, and the scanning rate was 20 mm/sec. The mobile phase was used for linear ascending development in a 10 x 10 cm twin trough glass chamber (CAMAG, Muttenz, Switzerland). It took fifteen minutes to completely saturate the compartment with mobile phase. The chromatogram had a development period of about 30 minutes and a run length of 8 cm. An air blast from a hair dryer was used to dry the TLC plates. All developments were scanned for density using a CAMAG thin layer chromatography scanner, with the wavelength set to 234 nm and the software WINCATS 1.4.2 under control. As the radiation source, we opted for deuterium lamps because of their continuous UV spectrum from 200 to 400 nm.

Making a standard stock solution

A 1000 µg/ml standard stock solution of each medication was prepared by dissolving 10 mg into 10 ml of methanol. Working standard solutions of FRU and SPL, both in methanol at concentrations of 100 µg/ml, were prepared from their respective standard stock solutions.

Selection of Detection Wavelength

The spectra were collected by scanning stock solution dilutions in methanol from 200 to 400 nm. High absorbance at 234 nm was measured for both medications (Fig. 1).

Tablet Formulation Analysis Sample Preparation

We weighed and powdered ten SPIROMIDE (RPG Life Sciences Ltd) tablets, each of which contained 20 mg of FRU and 50 mg of SPL. A volume of methanol was added to a

volumetric flask holding powder corresponding to 10 mg of FRU and 25 mg of SPL, and the volume was adjusted to 10 ml (1000 µg/ml of FRU and 2500 µg/ml of SPL). The ultimate concentrations of FRU and SPL were determined by filtering the solution and diluting it further with mobile phase.

Drug system compatibility parameters and chromatogram

Once we got the chromatographic conditions just right, we loaded up a TLC plate with 400 ng/band of FRU and 1000 ng/band of SPL and measured the retention factor as,

FRU = 0.29 ± 0.03

SPL = 0.69 ± 0.02

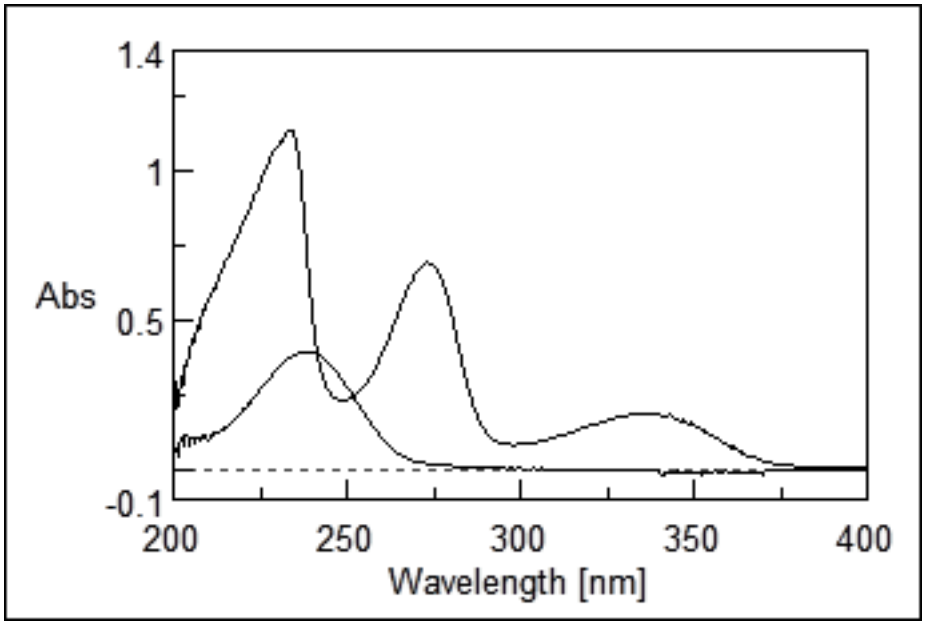

Fig. 1. FRU and SPL UV-VIS Spectra (10 µg/ml) Superimposed

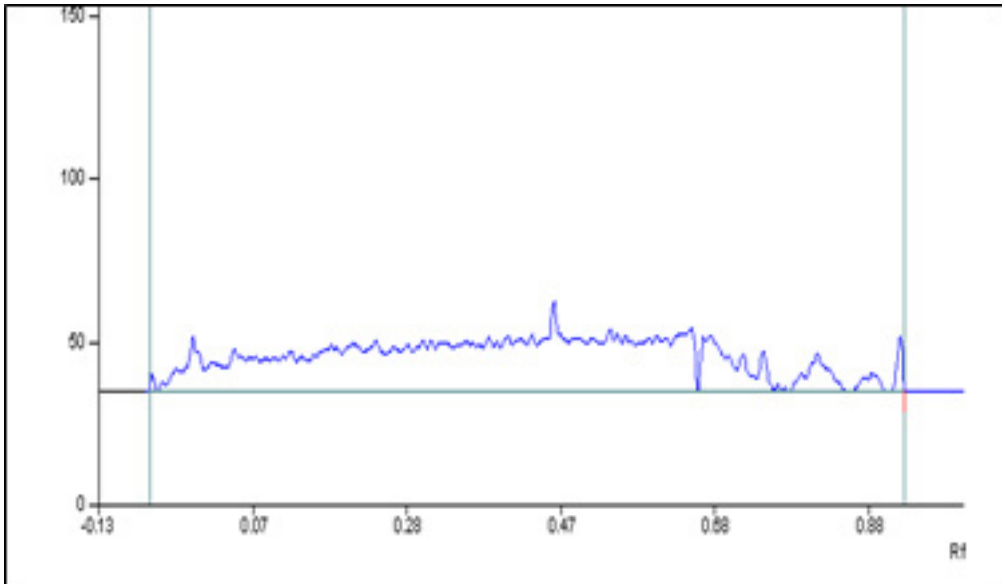

Fig. 2. Densitogram of Mobile Phase blank (Methanol)

Chromatogram of Methanol blank, FRU, SPL and Mixture are shown in Figure 2, 3, 4 and 5

Synopsis of Chosen Chromatographic Parameters

Table 2 summarizes certain chromatographic parameters.

Bulk medication stress degradation studies

The effects of numerous stress degradation processes, such as acid and base hydrolysis, oxidation, dry heat, and photolysis, were investigated. At least three replicates of each sample were made for each experiment.

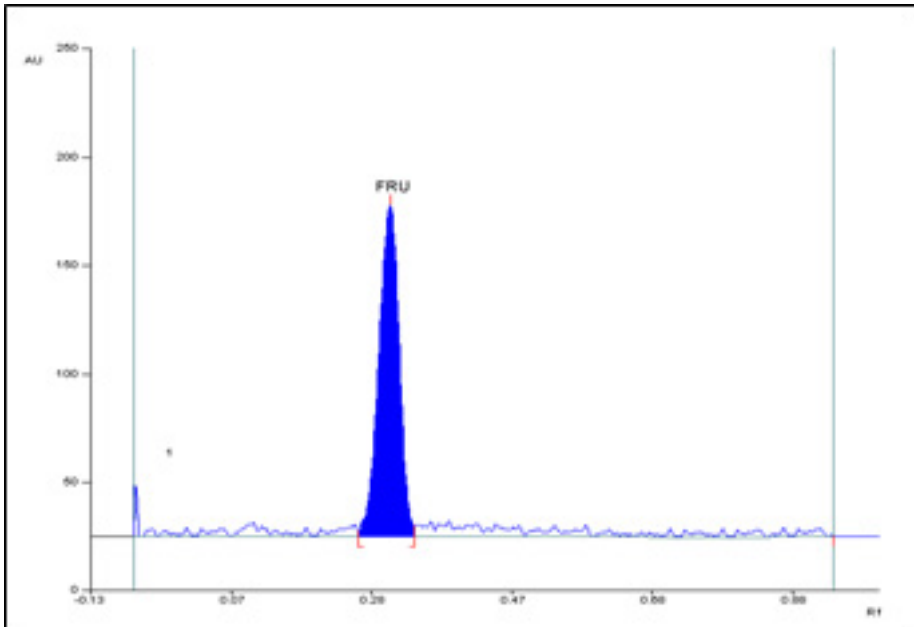

Fig. 3. FRU density plot

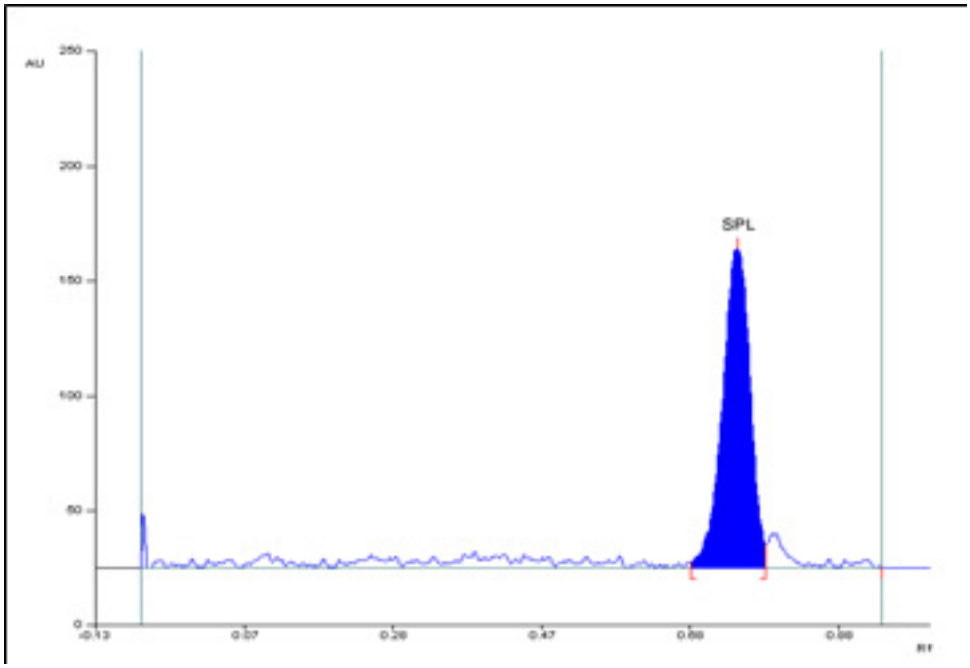

Fig. 4. Densitogram of SPL

The tension was applied to the blank in the same way that it would be applied to the medication. Substances were solidified and then degraded using dry heat and photolysis.

Alkaline hydrolysis

One milliliter of 0.1 N NaOH (methanolic) was mixed with one milliliter of methanol to create a standard working solution of FRU (100 µg/ml). For 24 hours, the solution was kept in the dark. The final concentration of the SPL solution, prepared in the same manner as the FRU solution, was similarly 250 µg/ml. After putting 4 µl of solution to a TLC plate, we found that the concentrations of FRU were 400 ng/band and SPL was 1000 ng/band. FRU only had one degradation peak (D1) after being exposed to alkali and it was located at Rf 0.17, with a recovery of 89.14%. SPL 83.52% recovery rate indicated that there was no peak of degradation.

Acidic hydrolysis

An FRU (100 µg/ml) working standard solution, 0.1 N HCl (methanolic), and 8 ml of methanol were mixed together. For 24 hours, the solution was kept in the dark. The final concentration of the SPL solution, prepared in the same manner as the FRU solution, was similarly

Table 1. Optimal System Parameter

Name	Rf Mean ± % RSD	Concentration (ng/band)	Area	Asymmetry
FRU	0.29 ± 0.03	400	3247	0.99
SPL	0.69 ± 0.02	1000	5955	0.97

Table 2. Characteristics of a chromatograph

Sr. No.	Parameter	Analytical Conditions
1	Stationary phase	TLC precoated silica gel 60 F_{254} aluminum plate
2.	Mobile phase	Chloroform: Methanol: Glacial acetic acid (7.5: 2:0.5 v/v)
3.	Detection Wavelength	234 nm
4.	Saturation time	15 mins

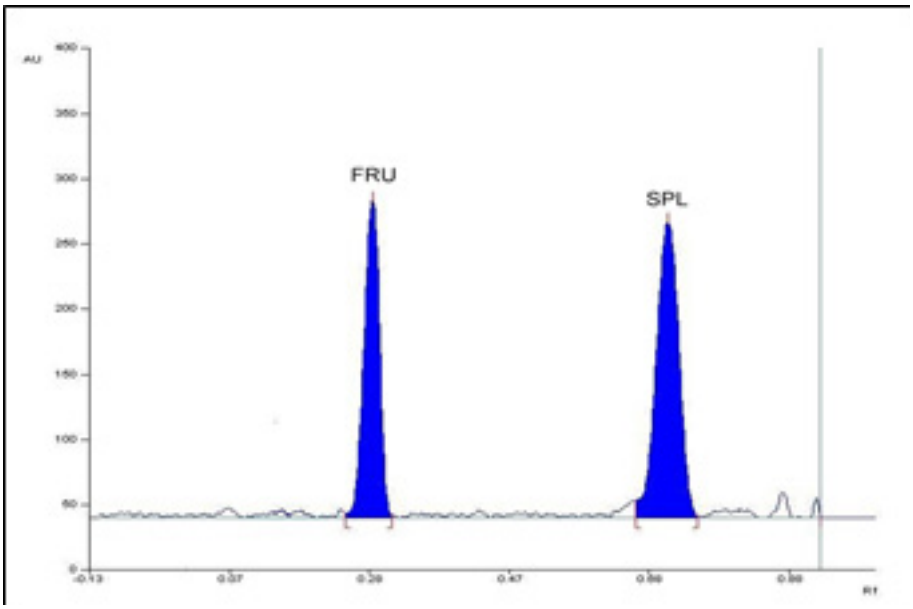

Fig. 5. Densitometric analysis of a reference mixture containing 400 ng/band FRU and 1000 ng/band SPL

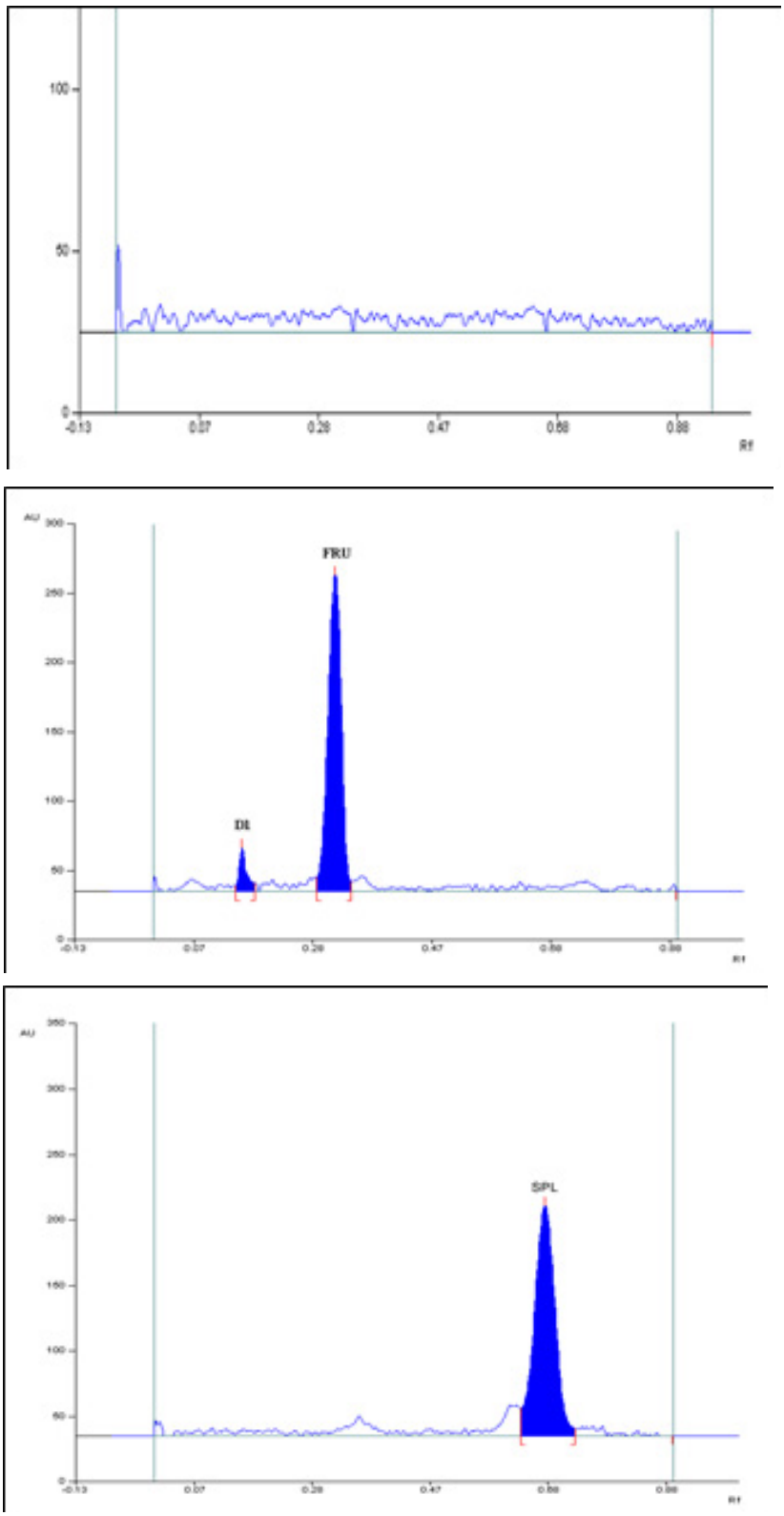

Fig. 6. Densitogram of: I- Alkali blank, II- Alkali treated FRU, III- Alkali treated SPL

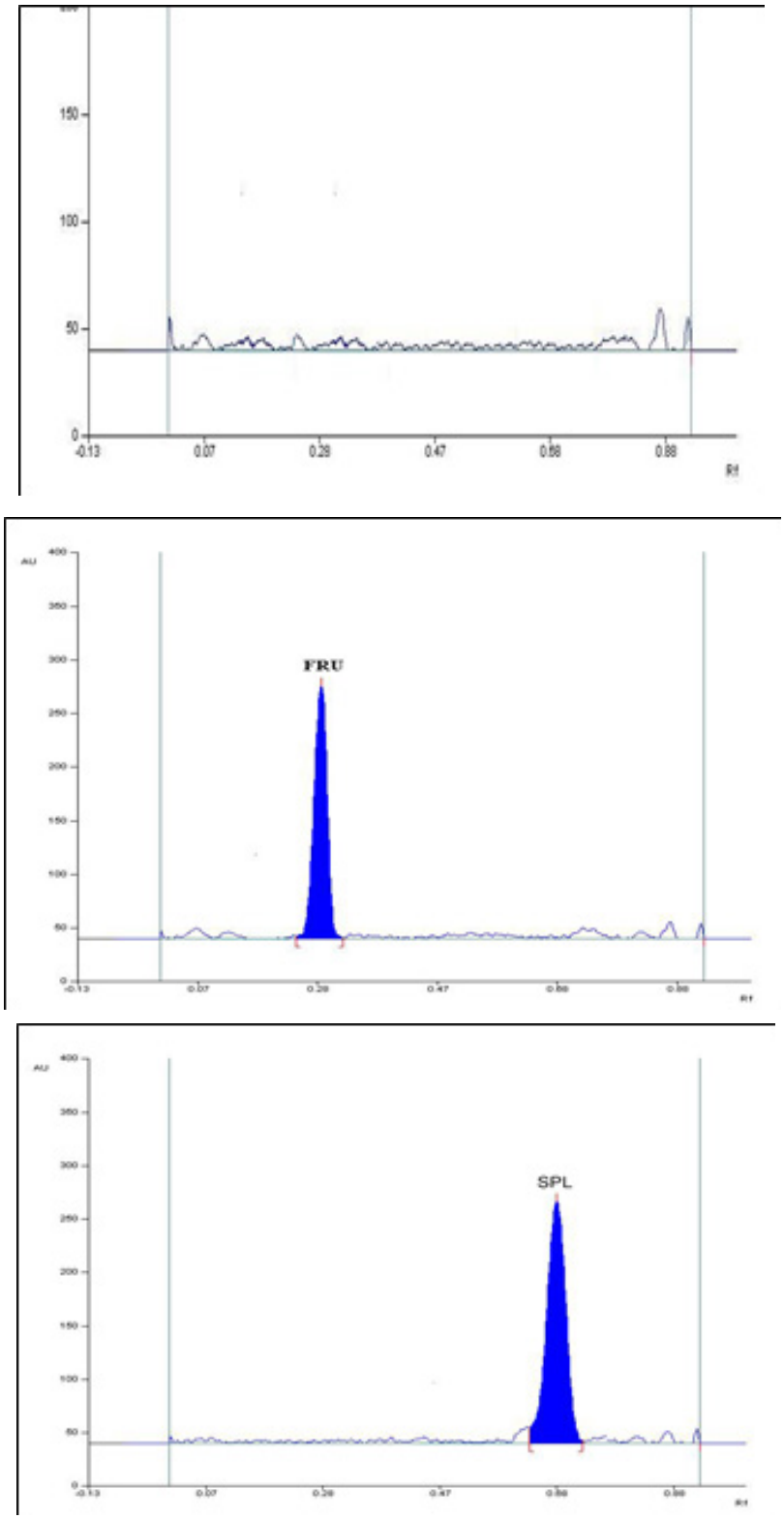

Fig. 7. I- Densitogram of Acid blank, II- Acid treated FRU, III- Acid treated SPL

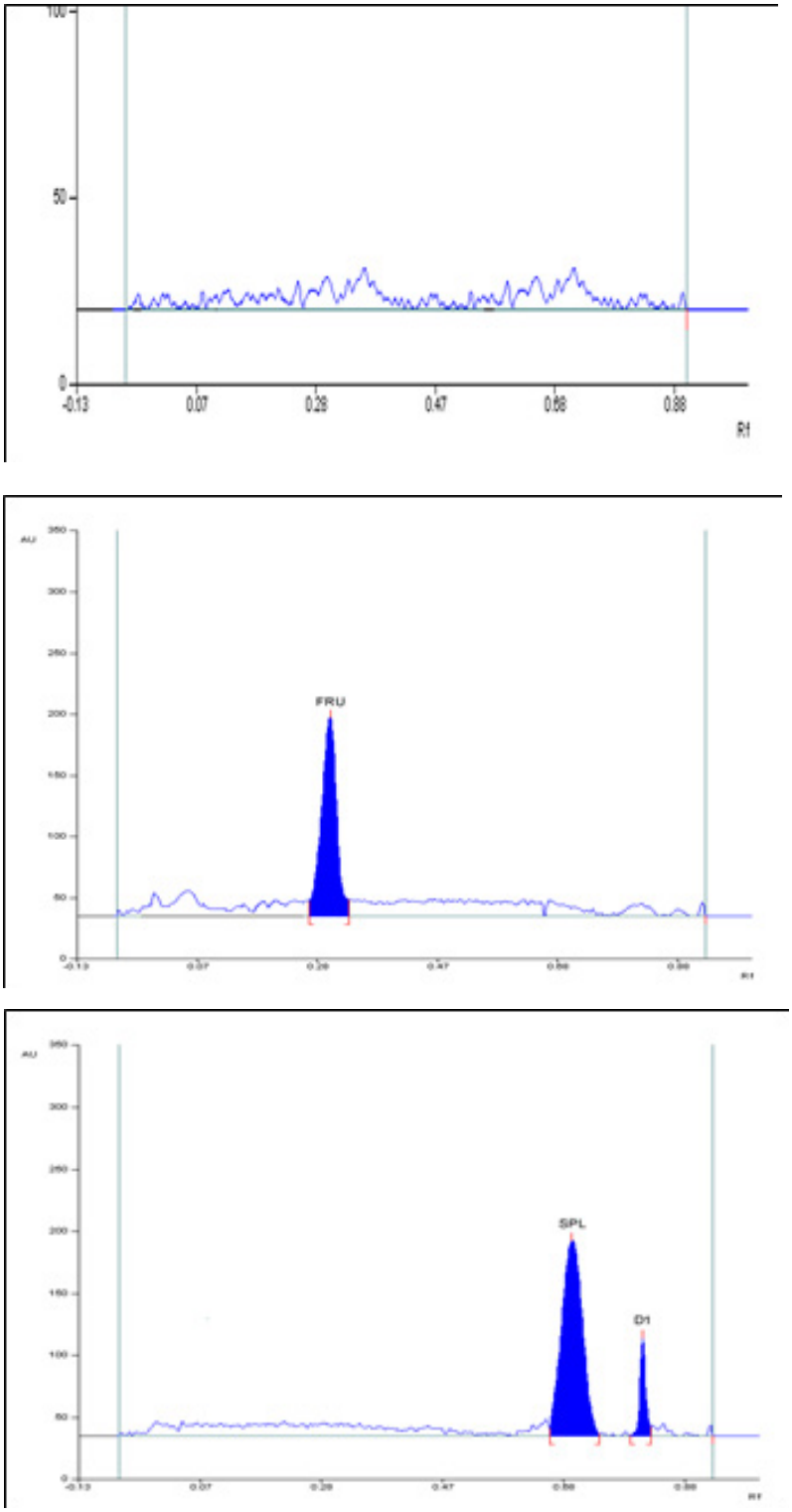

Fig. 8. I- Densitogram of: I) H_2O_2 blank, II) H_2O_2 treated FRUU, III) H_2O_2 treated SPL

250 µg/ml. After putting 4 µl of solution to a TLC plate, we found that the concentrations of FRUU were 400 ng/band and SPL was 1000 ng/band.

Recovering 96.84% of its original mass after acid hydrolysis, FRU showed no degradation peak. Nonetheless, SPL was restored to 95.66 percent without any noticeable degradation peaks.

Oxidation

A 100 µg/ml FRU working standard solution was combined with a 1 ml 30% H_2O_2 solution. This solution was stored in the dark for a full day. The final SPL solution was also 250 g/ml, and was made in the same way as the FRU solution. Two-liter quantities were used to apply to the TLC plate, resulting in concentrations of 400 ng/band for FRU and 1000 ng/band for SPL. The oxidative condition resulted in a percent recovery of 86.42% for FRU and no peaks of degradation products, and a recovery of 77.38% for SPL and one peaks of degradation products (D1) at Rf 0.81.

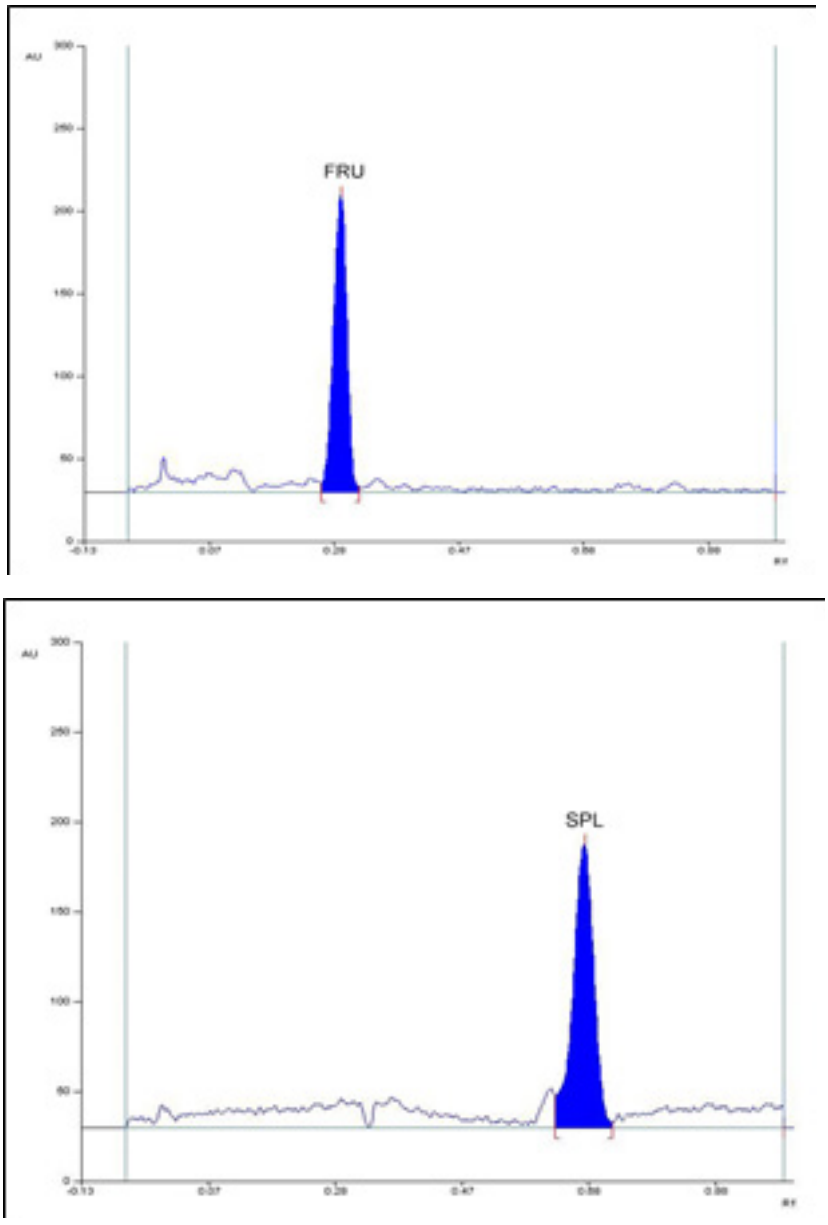

Fig. 9. Densitogram of drug after Dry heat degradation: I) FRUU, II) SPL

Deterioration in dry heat

The medication sample was heated to 100^{0}C for two hours during the dry heat test. After two hours, a sample of FRU was taken, dissolved in methanol to make a solution with a 100 µg/ml concentration, spotted in a volume of 4 µl at a concentration of 400 ng/band on a TLC plate.

The final concentration of the SPL solution, prepared in the same manner as the FRU solution, was similarly 250 µg/ml. 4 µl was used to apply to the TLC plate, resulting in concentrations of 1000 ng/band for SPL. FRU was recovered at a rate of 98.94% under dry heat degradation conditions, whereas SPL was recovered at a rate of 99.10%. No degradation products were detected under these conditions.

Photo-degradation studies

For the photolytic investigations, the medication was first exposed to UV light at a power density of 200 watt hours per square meter, and then to cool fluorescent light at a lumen intensity of 1200 Lux Hrs. Spots of 4 µl (400 ng/band) of the resultant solution were made on a TLC plate SPL solution, made in the same way as FRU solution, had final concentration of 1000 ng/band for SPL were achieved. The results of the UV and

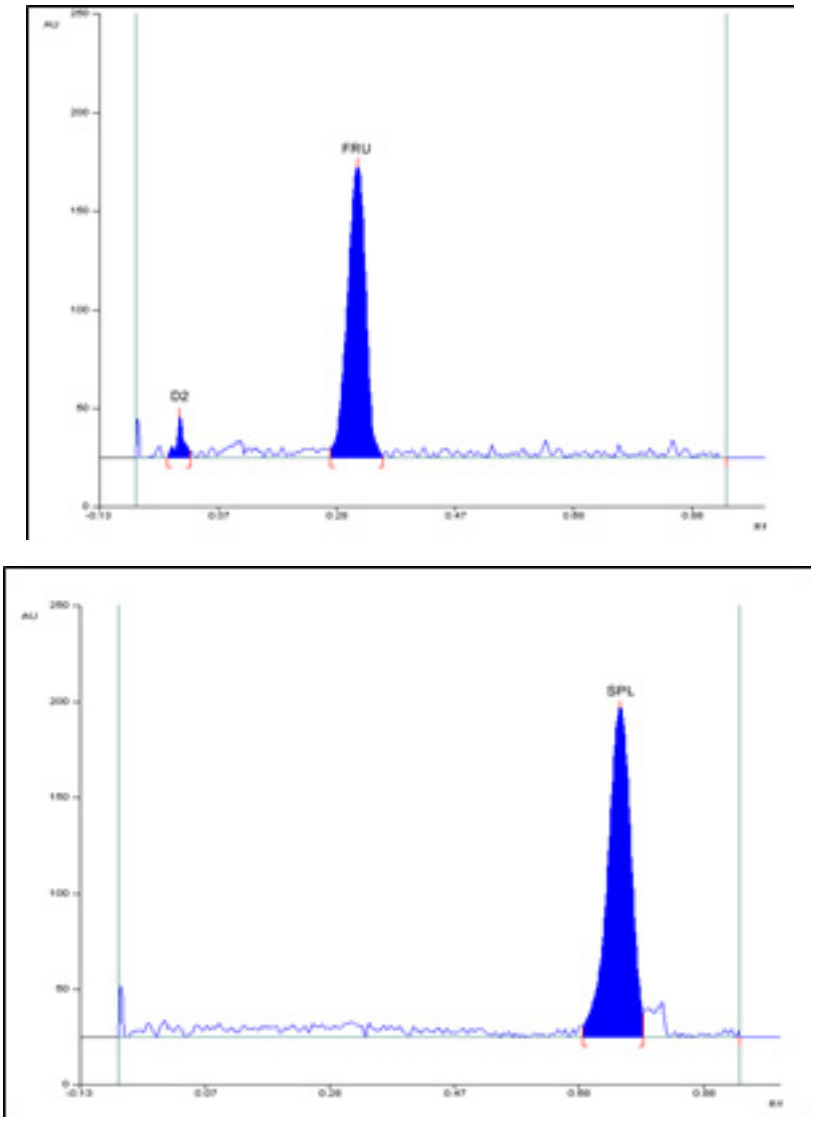

Fig. 10. Densitogram of drug after photo degradation: I) FRU, II) SPL

fluorescence photo degradation studies showed that FRU recovered 96.19 % of its original mass after being exposed to UV light, whereas SPL recovered 98.21 % of its original mass after being exposed to fluorescence light.

Analytical Method Validation

Specificity

Studies of peak purity profiling confirmed the method's sensitivity. As the peak purity values

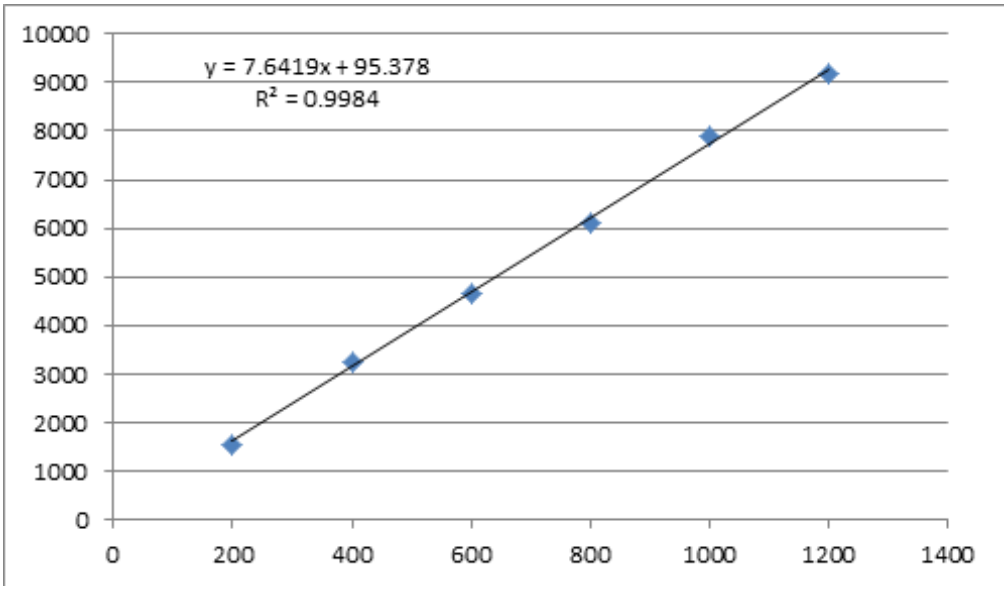

Fig. 11. Calibration curve for FRU

Table 3. Degradation of FRU and SPL under Stress: A Synopsis

Sr. No.	Deteriorating stress condition	% Recovery (FRU)	Degradation Product (Rf) (FRU)	% Recovery (SPL)	Degradation Product (Rf) (SPL)
1	Base	89.14	D1 (0.17)	83.52	—
2	Acid	96.84	—	95.66	—
3	H_2O_2 30% (kept for 24 hrs)	86.42	—	77.38	D1 (0.81)
4	Dry heat (100^0C for 2 hrs.)	98.94	—	99.10	—
5	Photostability	96.19	D2 (0.04)	98.21	—

Table 4. Analysis of FRUU Linearity

Replicates	Concentrations of FRU (ng/band)					
	200	400	600	800	1000	1200
	Peak Area					
1	1512	3287	4677	6119	7849	9192
2	1578	3247	4794	6123	7929	9264
3	1549	3262	4626	6042	7866	9132
4	1570	3271	4602	6084	7923	9120
5	1537	3226	4583	6195	7986	9133
6	1564	3266	4686	6124	7926	9165
Mean	1551.667	3259.833	4661.333	6114.500	7913.167	9167.667
Std.dev.	24.402	21.047	76.628	50.694	49.313	54.084
%RSD	1.573	0.646	1.644	0.829	0.623	0.590

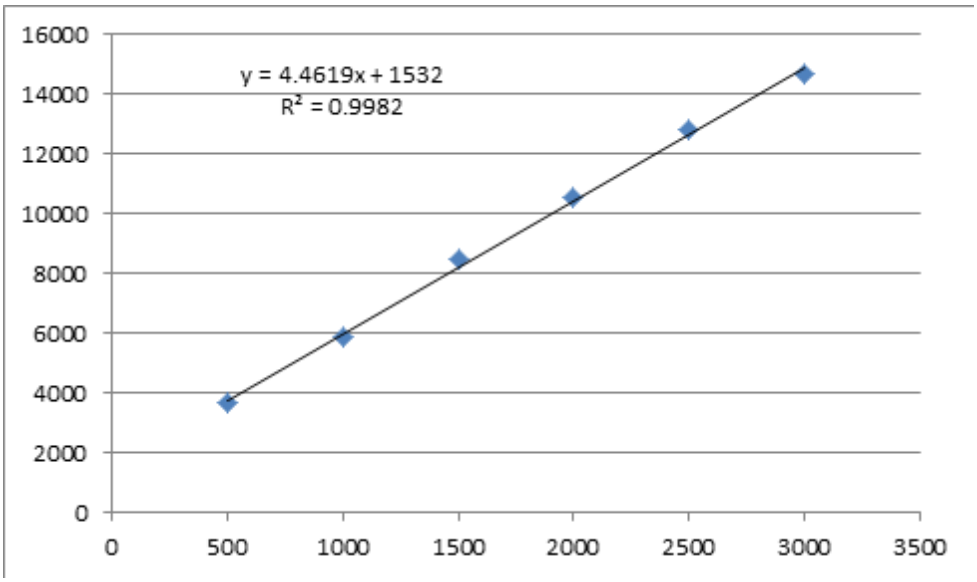

Fig. 12. Calibration curve for SPL

Table 5. SPL Linearity Analysis

Replicates	Concentrations of SPL (ng/band)					
	500	1000	1500	2000	2500	3000
	Peak Area					
1	3631	6032	8547	10614	12927	14722
2	3615	5955	8415	10523	12693	14708
3	3603	5868	8523	10565	12704	14671
4	3628	5793	8438	10490	12743	14708
5	3709	5733	8415	10541	12894	14777
6	3752	5829	8621	10398	12747	14718
Mean	3656.333	5868.333	8493.167	10521.833	12784.667	14717.333
Std.dev.	59.872	109.394	84.134	73.586	100.277	34.361
%RSD	1.637	1.864	0.991	0.699	0.784	0.233

Table 6. Intra-day precision study FRU

Concentration (ng/band)	Area	% Recovery	Avg % Recovery ± % RSD
100	1615	99.439	99.505± 0.693
	1627	100.224	
	1606	98.850	
200	3145	99.778	100.291± 0.775
	3188	101.185	
	3149	99.909	
300	4644	99.215	99.688± 0.493
	4664	99.652	
	4689	100.197	

were higher than 0.997%, it was determined that there was no contamination From other degradation products or pollutants.

Linearity

Using a normal stock standard solutions of 100 µg/ml FRU and 250 µg/ml SPL The concentration range used to establish linearity (peak area as a function of concentration) for FRU was 200–1200 ng/band, whereas for SPL it was 500–3000 ng/band. Each concentration has six identical replicates. Table 4 displays the results for FRU, while Table 5 displays the results for SPL.

Range

FRU = 200 – 1200 ng/band
SPL = 500- 3000 ng/band

Table 7. Inter-day precision of FRU

Concentration (ng/band)	Area	% Recovery	Avg % Recovery ± % RSD
100	1638	100.944	100.944± 0.454
	1631	100.486	
	1645	101.402	
200	3224	102.363	101.251± 0.971
	3167	100.498	
	3179	100.891	
300	4563	97.449	99.492± 1.846
	4726	101.004	
	4681	100.022	

Table 8. SPL for an intraday precision study

Conc. (ng/band)	Area	% Recovery	Avg % Recovery ± % RSD
1600	3771	100.381	101.083 ±0.649
	3789	101.188	
	3800	101.681	
3200	5993	100.000	100.590 ±0.614
	6017	100.538	
	6048	101.233	
4800	8310	101.293	100.765 ±0.469
	8265	100.620	
	8249	100.381	

Table 9. Inter-day SPL investigation of precision

Concentration (ng/band)	Area	% Recovery	Avg % Recovery ± % RSDa
1600	3741	99.036	99.813 ±0.764
	3759	99.843	
	3775	100.560	
3200	5945	98.924	99.694 ±0.911
	5969	99.462	
	6024	100.695	
4800	8244	100.306	99.729 ±0.538
	8199	99.634	
	8173	99.245	

Precision

Intra- and inter-day variance analyses proved the reliability of the technique. Intra-day research involved analyzing three replicates of three different concentrations on a single day, with the % RSD being determined. Three separate concentrations were examined over the course of three days for the inter-day variation investigations, and the % RSD was determined. Tables 6, 7, 8, and 9[13, 14] display the results obtained for intraday and interday variations.

Assay

The analysis of the tablet formulation was performed as described in Tablet Formulation Analysis. Six times through the procedure. Each medicine had a sample solution sprayed on it, and the resulting area was measured. A linear equation was used to figure out the concentration and the purity percentage. The outcomes are detailed in Table 10. The chromatogram in Figure 13[15, 16] is typical of the type of chromatogram used in sample analysis.

Table 10. Assay results of tablet formulation

Sr. No.	FRU Peak area	FRU Amt. recovered (ng/band)	FRU % recovery	SPL Peak area	SPL Amt. recovered (ng/band)	SPL % recovery
1	3175	403.040	100.760	5976	996.189	99.619
2	3162	401.339	100.335	6018	1005.604	100.560
3	3198	406.050	101.513	5944	989.016	98.902
4	3156	400.554	100.138	5946	989.464	98.946
5	3183	404.087	101.022	6022.5	1006.613	100.661
6	3155	400.423	100.106	5973	995.517	99.552
Mean	3171.50	402.582	100.646	5979.92	997.067	99.707
SD	17.03	2.228	0.557	33.96	7.613	0.761
% RSD	0.537	0.554	0.554	0.568	0.764	0.764

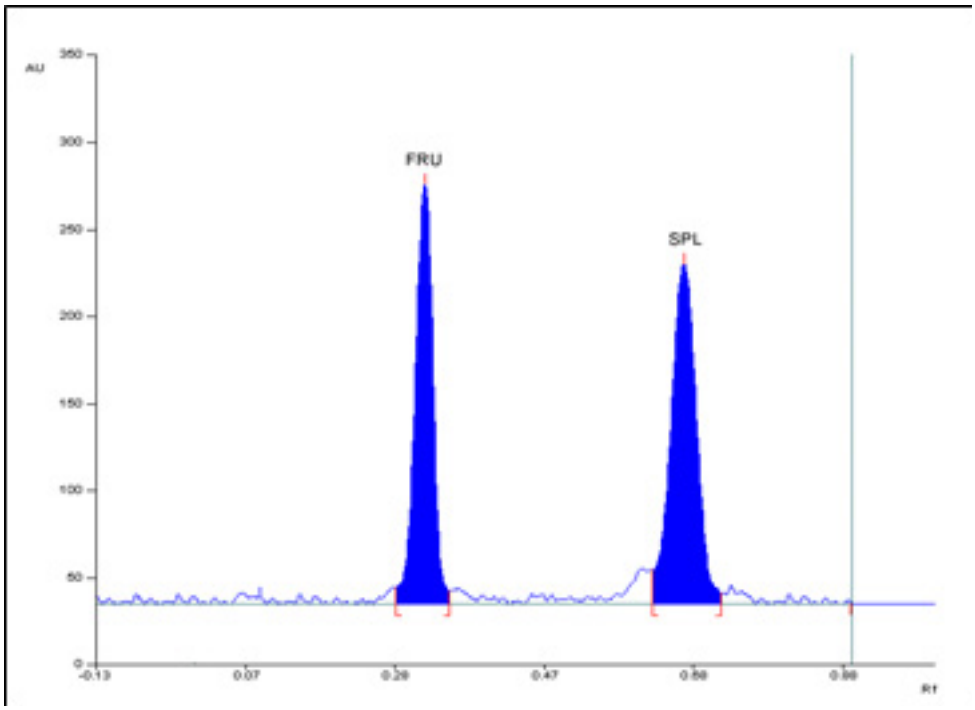

Fig. 13. Test Solution FRU (400 ng/band) and SPL (1000 ng/band) Densitogram

Accuracy

Recovery trials were performed by adding 50, 100, and 150% of a standard medication to a sample to verify the method's accuracy. Four microliters of 100 µg/ml FRU and of 250 µg/ml SPL were used as the basic sample concentrations. Three replicate applications of these solutions were performed to TLC plates to generate the densitogram. Using linearity equations for FRU and SPL, we were able to determine their respective medication concentrations. Table 11 and Table 12 illustrate the acquired results.

Limit of Detection (LOD)

The formula used to determine LOD: -

$$LOD = 3.3 * \sigma/s$$

Where,

ó = The range's minimum concentration's standard deviation

S = The gradient of the measuring system

LOD of FRU = 9.28 ng/band

LOD of SPL = 46.78 ng/band

Table 11. Recovery studies of FRU

Level	Conc. (ng/band)		Area	%	Mean %
	Sample	Std.	Recovery	Recovery ± % RSD	
50 %	400	200	4646	99.259	99.230 ± 0.704
			4676	99.913	
			4612	98.517	
100 %	400	400	6235	100.439	99.757 ± 0.621
			6161	99.228	
			6184	99.605	
150 %	400	600	7742	100.074	99.943 ± 0.652
			7776	100.519	
			7678	99.236	

Table 12. SPL recovery studies

Level	Conc. (ng/band)		Area	% Recovery	Mean % Recovery ± SD
	Sample	Std.			
50 %	1000	500	8249	100.381	100.037 ± 0.406
			8196	99.589	
			8233	100.142	
100 %	1000	1000	10471	100.191	100.585 ± 0.444
			10498	100.493	
			10550	101.070	
150 %	1000	1500	12691	100.058	99.958 ± 0.565
			12737	100.466	
			12612	99.350	

Table 13. Robustness Analysis

Drug	% RSD Found for Robustness Study								
	Wavelength (nm)			Saturation Period of a Chamber (Min)			Time form application to development (min)		
	233	234	235	14	15	16	25	30	35
FRU	0.727	0.560	0.881	1.083	0.704	0.808	1.220	1.224	1.348
SPL	0.783	1.563	0.801	0.301	0.655	0.967	1.047	0.631	0.677

Table 14. Overview of the validation study

Sr. No.	Metric for Validation	Results FRU	SPL
1.	Linearity	y = 7.641 x + 95.37 R^2 = 0.9984	y = 4.461 x + 1532 R^2 = 0.9982
2.	Range	200-1200 ng/band	500 - 3000 ng/band
3.	Assay (Mean ± % RSD)	100.646 ± 0.554	99.707 ± 0.764
4.	Precision	%RSD	%RSD
	A) Intraday precision	0.493 – 0.775 %	0.469 – 0.649 %
	B) Interday precision	0.454 – 1.846 %	0.538 – 0.911 %
5.	Accuracy	% recovery	% recovery
	50%	99.230 ± 0.704	100.037 ± 0.406
	100%	99.757 ± 0.621	100.585 ± 0.444
	150%	99.943 ± 0.652	99.958 ± 0.565
6.	LOD	9.28 ng/ band	46.78 ng/band
7.	LOQ	28.13 ng/band	141.75 ng/band
8.	Specificity	Specific	Specific
9.	Robustness	Robust	Robust

Limit of Quantification (LOQ)

In order to express the Quantitative bound, we have:

$$LOQ = 10 * \sigma/s$$

LOQ of FRU = 28.13 ng/ band
LOQ of SPL = 141.75 ng/band

Robustness

To ensure the reliability of the procedure, multiple trials were conducted using a range of parameters, including wavelength, chamber saturation time, and Time form application to development. Table 13 displays the final findings.

Overview of the validation study

Method was validated as per ICH guideline[20, 21, 22]

Table provides a summary of the validation parameters.

DISCUSSION

The findings of these research led to the development of solvent-free HPTLC methods for determining FRU and SPL. As a result of the fact that the suggested HPTLC method simultaneously determined the target compounds while utilizing minute amounts of solvents, it stood out as an analytical tool for quality control laboratories that was both quick and inexpensive. One of the primary contributors to the overall decrease in cost per analysis is the capacity of HPTLC technology to rapidly analyze a number of samples while requiring only a small volume of solvent. Following the completion of pharmacokinetic research, the established procedures will be put to use in order to successfully isolate and measure the components that were under investigation in human samples. This will be accomplished without the interference of confusing biological constituents. Statistical significance tests were used to validate the procedures that were devised for the simultaneous determination of the pure and mixed drug forms that were under consideration. These processes were verified for selectivity, accuracy, and repeatability[17, 18].

During the chromatographic separation investigations, standard solutions of FRU (200 ng/ band) and SPL (3200 ng/band) were utilized. In the past, research was conducted to determine the ideal solvent content as well as the plate temperature for HPTLC analysis. In general, the best peak characteristics and resolution were achieved by using a mobile phase that was composed of chloroform, methanol, and glacial acetic acid in the proportions of 7.5:2:0.5 volume/volume/volume. By adjusting the chromatographic parameters (such

as the chamber saturation time, run length, distance between tracks, and detection wavelength), we were able to keep the Rf values constant and produce a medicine with a symmetrical peak shape. A standard stock solution with a concentration of 1000 µg/ml was prepared by dissolving 10 mg of each medication into 10 ml of methanol. Standard stock solutions of FRU and SPL were used to generate working standard solutions of those chemicals at concentrations of 100 µg/ml FRU and 250 µg/ml SPL in methanol. These solutions were used to measure the concentration of the substances. In order to produce the spectra, stock solution dilutions in methanol were scanned between the wavelengths of 200 and 400 nm. It was discovered that both medicines possessed a high absorption when measured at 234 nm[19, 20].

Ideal conditions were used for the preparation of the TLC plates, and a volume of 4 µl was utilized for the application[21, 22].

Research was conducted on a number of different kinds of deterioration that can occur as a result of stress. These included acid and basic hydrolysis, oxidation, dry heat, and photolysis. At a minimum of three copies of each sample were used in each experiment. The strain was applied to the blank in a manner that was analogous to how one may apply medication. Solid compound was subjected to dry heat condition and then photolyzing them. During the process of validating, each and every validation parameter was utilized[23, 24, 25].

CONCLUSION

These studies paved the way for the development of solvent-Free HPTLC methods for the simultaneous determination of FRU and SPL. As an analytical strategy for quality control laboratories, the proposed HPTLC method stood out for its speed and low cost due to its simultaneous determination of the target chemicals utilizing tiny amounts of solvents. The ability of the HPTLC technology to rapidly analyze several samples with a minimal amount of solvent adds directly to the decreased cost per analysis. The established methods effectively separated and measured the examined components in human samples devoid of confounding biological components, paving the path for their application in subsequent pharmacokinetic studies. The selectivity, accuracy, and repeatability of the devised processes for the simultaneous determination of the pure and mixed drug forms tested were validated by statistical significance analyses.

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Conflict of interest

The authors declared that there is no conflict of interest.

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