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HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY (HPTLC) METHOD DEVELOPMENT AND VALIDATION FOR DETERMINATION OF FINERENONE IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

The World Health Organization (WHO) reports that 10% of the medications in Low and Middle Income Countries (LMICs) are of low quality, which presents a serious risk to public health. The development and application of quick, affordable, and effective analytical techniques is one way to get around this issue. A new non-steroidal mineralocorticoid receptor antagonist called Finerenone has demonstrated encouraging reno protective and cardiac effects in Chronic Kidney Disease (CKD). A new, simple and quick HPTLC technique was developed and verified for quantitative estimation of Finerenone using densitometry detection at 235 nm. Finerenone was chromatographed on silica gel 60 F_{254} precoated TLC plates using chloroform, ethyl acetate, methanol, and 1% aqueous ammonia were combined in the mobile phase at the following ratios: 5:3.5:1.5:0.5 (v/v/v/v). The linear regression analysis data for calibration plots showed good linear relationship with r^2= 0.999 in the concentration range 400-1400 ng/band. The method was found to give compact band for the drug (Rf is 0.47 ± 0.01). The range of recoveries was 98% to 102%. For Finerenone, the limits of detection and quantitation were 65.6 and 198.7 ng/spot, respectively. In compliance with ICH requirements, the developed approach was validated. Statistical analysis of the data showed that the method is precise,

accurate, reproducible and selective for the analysis of Finerenone. The method was successfully used for the estimation of Finerenone as a bulk drug and in commercially available formulation.

Keywords: Finerenone, chronic kidney diseases, analytical method development, high performance thin layer chromatography (HPTLC), Validation

1. INTRODUCTION

A nonsteroidal mineralocorticoid receptor antagonist, Finerenone (FIN) ($C_{21}H_{22}N_4O_3$) (4S)-4-(4-cyano-2-methoxyphenyl)-5-ethoxy-2,8dimethyl-1,4-dihydro-1,6naphthyridine-3 carboxamide) is a drug used to lower the risk of cardiovascular death, non-fatal heart attacks, kidney failure, deterioration in kidney function, and hospitalization for heart failure in adults with type 2 diabetes-related chronic renal disease. It is ingested orally (by mouth). The kidneys, heart, and blood arteries contain a protein called the mineralocorticoid receptor (MR), which is blocked as part of the mechanism of action. In the kidney, heart, and blood arteries, where the MR is expressed, FIN also inhibits hypertrophy processes and sodium retention **[1, 2]**. FIN's nonsteroidal composition and bulky binding mechanism give it a high potency and selectivity for the MR. FIN does not produce sex hormone-related side effects, such as gynecomastia, because it has no discernible affinity for androgen, progesterone, estrogen, or glucocorticoids receptors. When it binds to the MR, a particular receptor ligand complex is produced, which prevents transcriptional coactivators involved in the production of mediators that promote inflammation and fibrosis from being recruited. FIN has linear pharmacokinetics and a half-life of two to three hours at doses up to twenty milligrams **[3, 4]**.

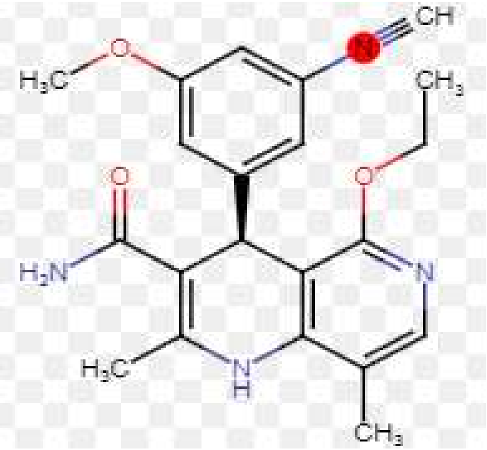

Figure 1: Chemical structure of Finerenone

Literature survey revealed that HPLC and Stability indicating HPLC method were reported for estimation of FIN **[5, 6]**. But HPTLC method has not been reported for estimation of FIN till date. High-performance thin-layer chromatography (HPTLC) is an automated variation of thin-layer chromatography (TLC) with smaller sorbent particles and pores, shorter analysis times, and more efficient development chambers that use less mobile phase. Sample application in HPTLC is automated, and a UV/Visible/Fluorescence scanner—a sophisticated kind of densitometer—scans the whole chromatogram both qualitatively and quantitatively. Its benefits, which include improved separation efficiency and detection limits, lower analysis costs, shorter analysis times, no need for solvent pre-treatment (such as filtration and degassing), lower mobile phase consumption per sample, and the elimination of interference from prior analyses because each analysis uses a fresh stationery phase and mobile phase, have made it increasingly important in the pharmaceutical industry. Additionally, the technique permits the determination of up to 18 sample sites to be applied in a single run. It is vitally crucial to establish a quick, easy, affordable, accurate, and exact analysis method like HPTLC in order to control the quality of Finerenone **[7]**. Thus, the development and validation of an HPTLC approach for FIN estimation in tablet dosage was deemed interesting.

2. EXPERIMENTALS

2.1 Reagents and Chemicals:

Pure sample of FIN was kindly supplied as gift sample by Zydus Pharma Ltd. HPLC grade methanol and analytical reagent grade Toluene, ethyl acetate, ammonia, distilled water were used. Ethyl acetate, methanol ammonia and toluene was used in preparing the mobile phase. Tablet formulations containing Finerenone 20 mg purchased from local pharmacy in Vadodara.

2.2 Instrumentation and chromatographic condition:

Camag HPTLC apparatus consisting of Linomat V Sample applicator (Camag, Muttenz, Switzerland), 100 μL syringe (Hamilton-Bonaduz Schweiz, Camag, Switzerland), TLC Scanner IV (Camag, Muttenz, Swtzerland), VisionCATS version 1.4.8 software (Camag, Muttenz, Switzerland) were used in the study Chromatography was performed on Merck silica gel 60 F254 precoated TLC plates (20 cm x 20 cm with 200 mm thickness), Saturation pad (Camag, Muttenz, Switzerland) was used for saturating development chambers. Samples were applied as bands under a stream of nitrogen using the 100 microliter syringe (Hamilton-Bonaduz Schweiz, Camag, Swtzerland).

Ascending development to a distance of 7 cm was performed in a 30 min presaturated 20 x 20 cm twin trough TLC developing chamber (Camag). Developed plates were dried using hair drier. Densitometry scanning and quantitative evaluation were performed using the TLC scanner and VisionCATS software, respectively.

2.3 Preparation of standard solution:

A standard solution of FIN was prepared by dissolving a quantity of working standard equivalent to 100 mg of Finerenone in 100 ml of methanol in 100 ml volumetric flask make up with methanol. From this take 10 ml of solution and diluted upto mark with methanol (100 μg/ml).

2.4 HPTLC Method and Chromatographic Conditions

2.4.1 Sample Application

On precoated TLC plates, the standard and formulation samples of FIN were identified as thin bands measuring 6 mm in length, 15 mm from the left and bottom margins, and 10.4 mm apart from one another. Samples were applied at a constant 150 nL/s under a continuous nitrogen gas drying stream.

2.4.2 Mobile Phase and Migration

The mobile phase used to develop the plates was Chloroform: ethyl acetate:methanol: 1% aqueous ammonia (5.0 + 3.5 + 1.5 + 0.5 v/v/v). The development process was linear ascending in a 10 cm × 10 cm twin trough glass container that was mobile phase equilibrated. At 25 ± 2 °C, the ideal chamber saturation time for the mobile phase was 20 minutes. For every development, ten milliliters of the mobile phase (5 mL in the trough holding the plate and 5 mL in another trough) were used, and it took 10 min for the mobile phase to migrate 70 mm. Following development, the TLC plates underwent thorough drying.

2.4.3Densitometric Evaluation and Quantification Method:

VisionCATS planar chromatography was used to control the Camag TLC scanner III in absorbance mode during densitometric scanning. The deuterium lamp was the radiation source that was used. The spots were examined at 235 nm in wavelength. The analysis employed slit size of 5 mm for length and 0.45 mm for width, with a scanning rate of 20 mm/s. These are chosen in accordance with the CAMAG TLC Scanner IV manual's recommendations. It covers between 70 and 90 percent of the application band length, or 6 mm in this instance. A 20 nm bandwidth was used for the monochromator. The intensity of diffusely reflected light was used to calculate the concentrations of the substance chromatographed, and peak areas were compared to concentrations utilizing linear regression equation.

2.5 Method validation

The International Conference on Harmonization (ICH) criteria Q2 (R1) for specificity, sensitivity, accuracy, precision, repeatability, and robustness were followed in the validation of the developed HPTLC method **[8]**.

2.5.1 Specificity

By examining sample solutions containing FIN from commercialized tablets in relation to interferences from formulation constituents, the specificity of the suggested approach was established. The confirmation of the FIN spot in the sample was achieved by comparing the retardation factor (Rf) values of the spot with that the standard.

2.5.2 Sensitivity

The method's sensitivity was assessed in relation to the limits of quantification (LOQ) and detection (LOD). Six scans of the blank spot (methanol) were used to identify noise. To ascertain LOD and LOQ, a range of drug solution concentrations (400-1400 ng/spot) were put to a plate and examined. Three times the noise level was used to determine LOD, and ten times the noise level was used to calculate LOQ. By dilution of the known concentrations of FIN until the average responses were roughly 3–10 times the standard deviation (SD) of the responses for six repeat measurements, LOD and LOQ were experimentally validated.

2.5.3 Linearity

In order to assess the method's linearity, calibration curves at six concentration levels were built. Throughout the concentration range of 400-1400 ng/band, calibration curves were drawn. Aliquots of FINs standard working solution (4, 6, 8, 10, 12, 14 µL/band) were applied to the plate. Using the Vision CATS program, peak area versus concentrations (n = 6) was plotted to create the calibration curves.

2.5.4 Precision

Both intra- and inter-day precisions were used to assess precision. Bands were applied to the HPTLC plate using a working standard solution containing 100 µg/mL FIN for the precision investigation. By examining a solution of FIN (400, 800,1400 ng/band) at three levels covering the low, middle, and high concentrations of the calibration curve three times on the same day (n = three), intra-day precision was ascertained. The analysis of a solution of FIN (400, 800,1400 ng/band) at three levels covering low, medium, and high concentrations over a period of three days (n = 3) was used to evaluate the inter-day precision. The mean and relative standard deviation (% RSD) values were computed using the acquired peak regions. In order to assess the injection's repeatability, the peak area measurement's repeatability was assessed by evaluating the FIN sample (800 ng/band) six times without moving the plate's location.

2.5.5 Accuracy

To confirm the accuracy of the proposed method, recovery experiments were carried out by the standard addition technique. It was carried out by adding known amounts of drug (reference standard) to samples of Finerenone tablet corresponding to three concentration levels (80, 100, and 120% of the working concentration) along with the excipients and to the working standard.

2.5.6 Robustness

Modest adjustments were made to the mobile phase composition, solvent migration distance and chamber saturation duration, and the impact on the outcomes was assessed. The method's robustness was assessed at 800 ng/band of FIN concentration level. The peak areas' mean and percentage RSD values were computed.

2.6 Examination of the formulation:

2.6.1 Preparation of sample solution:

Finerenone tablets was weighed and triturated. Powder equivalent to 20 mg Finerenone was weighed accurately and transferred to 10 ml volumetric flask and volume was made up to mark with methanol. This solution was used as 1º stock solution (2000 µg/ml of Finerenone). From this take 5 ml aliquot and diluted upto 100 ml with methanol (100 µg/ml**)**.

3. RESULTS AND DISCUSSION

The mobile phase was chosen based on polarity in order to develop the HPTLC method for the determination of FIN. It is desired to have a mobile phase that yields a compact and dense band with a suitable Rf value for FIN. In the early stages of developing the approach, several mobile phases were tested. Initially, Chloroform, methanol, toluene, glacial acetic acid and ethyl acetate were attempted as the mobile phase. The following combinations were tried as mobile phases: Ethyl acetate: methanol (6.5:3.5, v/v), Ethyl acetate: methanol: glacial acetic acid (6.5: 3.0:0.5, v/v/v), Ethyl acetate :methanol: ammonia (5.0:4.5:0.5 v/v/v),), methanol-chloroform-glacial acetic acid (5:4:1, v/v/v), and ethyl acetate-chloroform-acetic acid (3:6:1, v/v/v). Of them, chloroform: ethyl acetate: methanol: 1% aqueous ammonia (5: 3.5: 1.5: 0.5, v/v/v/v) solvent combination produced excellent FIN separation from its matrix, with an Rf value of 0.47. Additionally, it was noted that solvent migration distance and chamber saturation time are important factors in chromatographic separation, with solvent migration lengths larger than 70 mm and chamber saturation times of fewer than 15 minutes

resulted in the analyte spot diffusing. Consequently, the solvent system of Toluene: Chloroform: Ethyl acetate: Ammonia (5: 3.5: 1.5: 0.5, v/v/) proportion was employed as the mobile phase, with a solvent migration distance of 70 mm and a chamber saturation period of 20 min at 25°C. The lowest quantity of drug that could be measured under the experimental conditions used was 198.7 ng/band, while the lowest amount that could be detected was 65.6 ng/band. Specificity is the ability of an analytical method to assess unequivocally the analyte in the presence of sample matrix. FIN was separated from excipients with an Rf value of 0.47± 0.01. There was no interfering peak at Rf value of FIN so, no interference from excipients, present in commercial formulation, thereby confirming specificity of method. Linearity of an analytical method is its ability, within a given range, to obtain test results that are directly, or through a mathematical transformation, proportional to concentration of analyte. Method was found to be linear in a concentration range of 400-1400 ng/ban (n=6), with respect to peak area. The regression data as shown in Table I reveal a good linear relationship over the concentration range studied demonstrating its suitability for analysis. No significant difference was observed in the slopes of standard curves (ANOVA, P>0.05). Accuracy of an analytical method is the closeness of test results to true value. It was determined by the application of analytical procedure to recovery studies, where known amount of standard is spiked in preanalysed samples solutions. Results of accuracy studies from excipient matrix were shown in Table II; Recovery values demonstrated the accuracy of the method in the desired range. The precision of an analytical method express the degree of scatter between a series of measurement obtained from multiple sampling of the same homogeneous sample under prescribed conditions. Intraday precision refers to the use of analytical method within a laboratory over a short period of time with the same instrument whereas Interday precision involves estimation of variations in analysis when a method is used within a laboratory on different days. The results obtained are shown in table III. In all instances, %RSD values were less than 2 confirming the precision method. Intraday and interday variations were performed by analysis of three different concentrations (400, 800 and 1400 ng/spot) of the drug three times on the same day and on different days, respectively. In order to control scanner parameter, that is repeatability of measurement of peak area, one spot as analysed without changing position of plate (n=6). By spotting and analysing the same amount several times (n=6), precision of automatic spotting device was evaluated. % RSD was consistently less than 2 (Table IV), which was well below the instrumental specifications, ensuring repeatability of developed method as well as proper functioning of the HPTLC system. The low values of %RSD (Table V) obtained after introducing small deliberate changes in developed HPTLC method confirmed the robustness of the method. A single spot at Rf 0.47 was observed in the chromatogram of FIN. No interference from the excipients present in the marketed tablet formulation observed. Analysis of FIN tablets showed a drug content of 19.79± 0.38. (Table VI). The low %RSD value indicated the suitability of this method for routine analysis of FIN in various formulations.

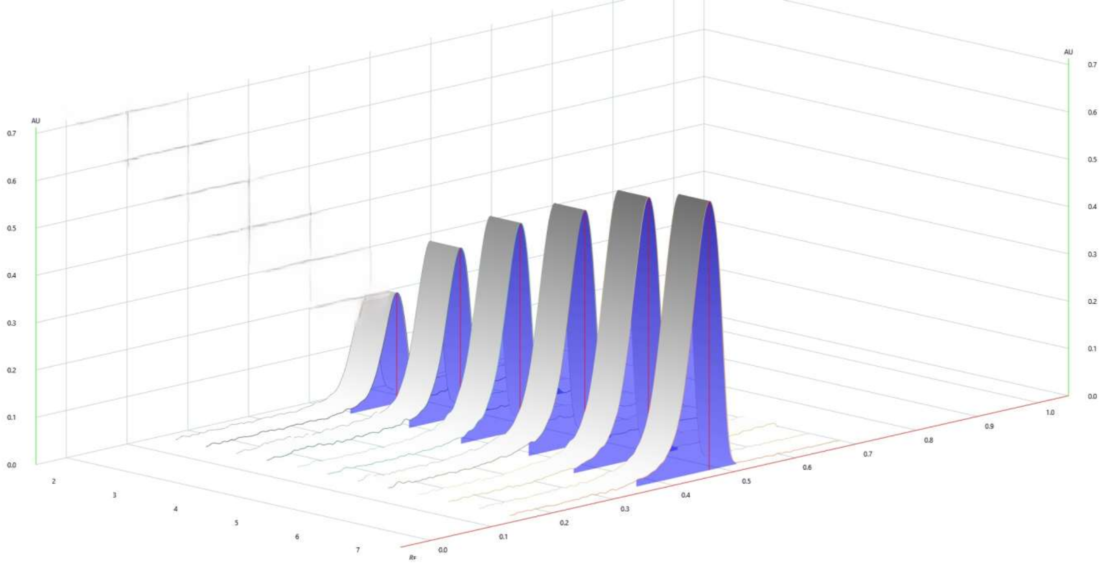

Figure 2: 3-D Chromatogram of FIN (400-1400 ng/band) in optimized chromatographic conditions

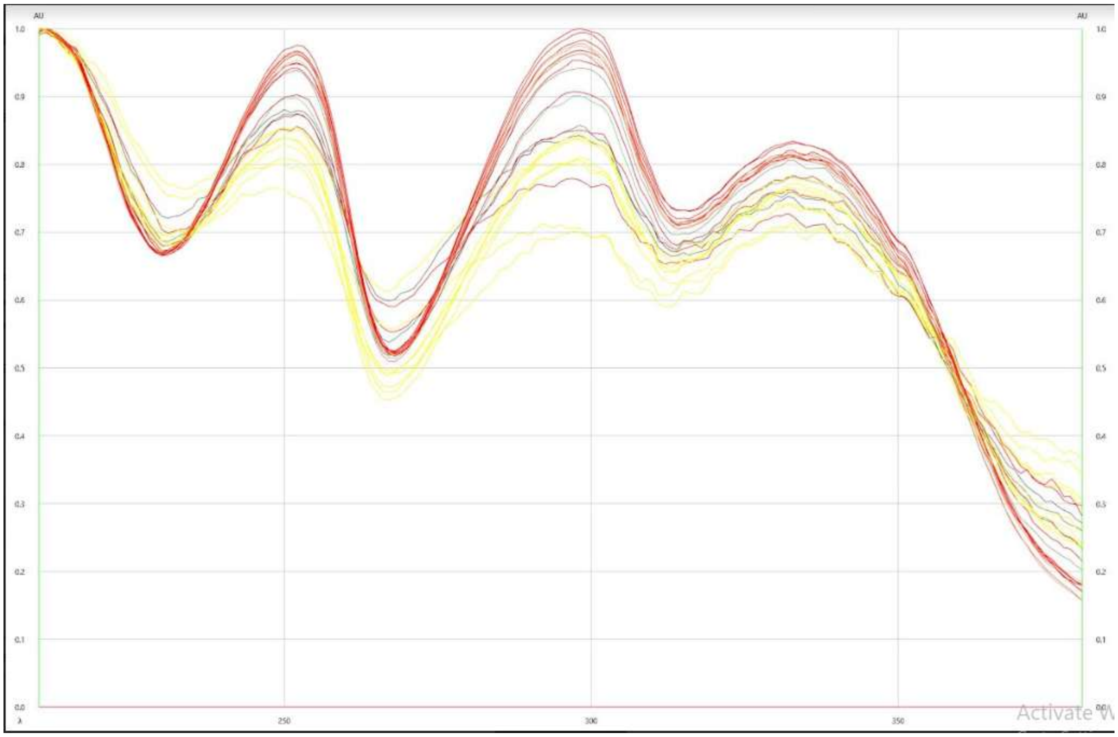

Figure 3: Overlain UV spectra of standard and sample FIN

Table I: Linear regression data for the calibration curves (n = 6).

Range (ng/band)	r2	Slope ± SD	Intercept ± SD
400-1400	0.999	1.3559 ± 0.001	373.9±1.17

Table II: Recovery studies (n = 9)

Level %	Amount of drug analysed (ng/band)	Std concentration spiked (ng/band)	Total concentration found (ng/band)	Spiked concentration recovered (ng/band)	% recovery	% Mean recovery ± SD	%RSD
50	400	200	600	601.23	100.20	100.08 ± 0.11	0.11
	400	200	600	600.47	100.07		
	400	200	600	599.89	99.98		
100	400	400	800	799.85	99.98	100.08 ± 0.09	0.09
	400	400	800	800.98	100.12		
	400	400	800	801.26	100.15		
150	400	600	1000	1001.23	100.12	100.03 ± 0.08	0.08
	400	600	1000	1000.36	100.03		
	400	600	1000	999.54	99.95		

Table III: Intra- and inter precision studies (n = 3)

Amount of drug spotted (ng/band)	Amount of drug detected (ng/band) (mean ± SD)	% RSD
Intraday (n=3)		
400	99.85 ±0.26	0.26
800	100.05± 0.08	0.08
1400	99.97 ±0.10	0.10
Interday (n=3)		
400	99.94±0.43	0.43
800	100.03±0.10	0.10
1400	99.99±0.15	0.15

Table IV: Repeatability Studies (n=6)

Amount of drug spotted (n=6) (ng/band)	Mean Peak area ± SD	%RSD
800	1533.5±29.82	1.94

Table V: Robustness study

Parameters	Amount of FIN spotted (ng/band) (n=3)	Amount of FIN detected (ng/band) (n=3) Mean ± SD	%RSD
Mobile Phase composition Chloroform:ethyl acetate:methanol: and 1% aqueous ammonia (4.5:4.0 :1.5:0.5)	800	100.08±0.09	0.09
Mobile Phase composition Chloroform:ethyl acetate:methanol: and 1% aqueous ammonia (5.5:2.5 :1.5:0.5)	800	99.97±0.18	0.18
Migration distance (68 mm)	800	99.94±0.11	0.11
Migration distance (72 mm)	800	99.95±0.23	0.23
Chamber saturation time (15 min)	800	100.04±0.10	0.10
Chamber saturation time (25 min)	800	100.98±0.30	0.30

Table VI: Contents of FIN in formulation (n=6)

Formulation	Label Claim	Amount Found (mean ± SD)	%RSD
F1	20 mg/tab	19.79±0.38	1.94

4. CONCLUSION

A new HPTLC method has been developed for the identification and quantification of FIN. Accurate, precise, economical and robust are the main features of this developed method. Method was successfully validated as per ICH guideline and statistical analysis proves that method is sensitive, specific and repeatable. It can be conveniently used for routine quality control analysis of FIN as bulk drug in marketed tablets without any interference from excipients.

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