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A New Validated Method for the Estimation of Fineranone in Bulk and Pharmaceutical Dosage Form by UPLC

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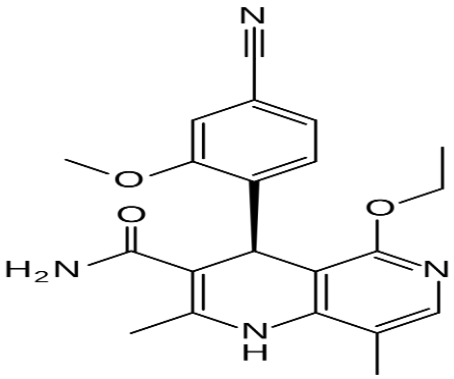
ABSTRACT

A straightforward, sensitive, focused, and accurate RP-UPLC technique is provided for the measurement of Finerenone in pharmaceutical dosage form and API. HSS C18 100 x 3.8 mm, 2.0µm was used to conduct the chromatogram. At a flow rate of 0.4 ml/min, the mobile phase comprising OPA: Methanol in the ratio 80:20 was passed through the column. Orthophosphoric acid buffer was employed in this procedure. 30°C was kept as the temperature. The chosen optimised wavelength was 227.0 nm. Finerenone retention time was discovered to be 1.426 minutes. Finerenone %RSD was determined to be 0.3. Its %RSD for method accuracy was determined to be 0.8. Its recovery was determined to be 100.10%. Finer none's regression equation yielded LOD and LOQ values of 0.38 and 1.16. Finerenone regression equation is y = 36857x + 884291. As a result of shorter retention durations and shorter run times, the method was created to be straightforward and cost-effective, and it may be used for routine Quality Control Tests in Industries.

INTRODUCTION:

Sun Pharmaceuticals developed Finerenone (Lyvelsa), In adults with chronic kidney disease associated with type II diabetes mellitus, finerenone is a non-steroidal mineralocorticoid receptor antagonist that is indicated to lower the risk of sustained decline in glomerular filtration rate, end stage kidney disease, cardiovascular death, heart attacks, and hospitalization due to heart failure. The recommended dosage of finerenone is 20 mg once daily, to be taken with food, The mineralocorticoid hormone aldosterone controls blood pressure, potassium excretion, and salt reabsorption.2. Malignant hypertension, which can lead to organ fibrosis and inflammation to agonism of the MR and elevated salt intake.2,3.



Structure finerenone

Pharmacodynamics:

With negligible affinity or activity at androgen, progesterone, estrogen, and glucocorticoid receptors, finerenone is a non-steroidal selective antagonist of the mineralocorticoid receptor (MR).[4,5]. Given that patients were given doses ranging from 1.25 mg to 80 mg during clinical trials, it has a broad therapeutic window and a moderate duration of action due to its once-daily administration.7. Patients need to be informed about the possibility of hyperkalemia.[4].

Pharmacokinetics:

Over a dosage range of 200-400 mg once a day, there are approximately dose proportionate increases in the maximum plasma concentration of finerenone (Cmax) and the area under the plasma concentration-time curve (AUC). Lumosudil has an accumulation ratio of 1.4. The median time to Cmax at steady state was 1.26-2.53 h after Fineranone 20 mg was given once or twice daily to patients with cGVHD. Fineranone Cmax and AUC dropped 2.2-fold and 2-fold, respectively, when a single dose was given together with a high-fat, high-calorie meal,

while the median time to Cmax was prolonged by 0.5 h. Fineranone had a mean bioavailability of 64% after a single dose. Following a single dose, the geometric mean volume of distribution finerenone is 184 L. 99.9% of the medication is bound. Over a dosage range of 200-400 mg once a day, there are approximately dose proportionate increases in the maximum plasma concentration of finerenone (Cmax) and the area under the plasma concentration-time curve (AUC). Lumosudil has an accumulation ratio of 1.4. The median time to Cmax at steady state was 1.26-2.53 h after Fineranone 20 mg was given once or twice daily to patients with cGVHD. Fineranone Cmax and AUC dropped 2.2-fold and 2-fold, respectively, when a single dose was given together with a high-fat, high-calorie meal, while the median time to Cmax was prolonged by 0.5 h. Fineranone had a mean bioavailability of 64% after a single dose. Following a single dose, the geometric mean volume of distribution finerenone is 184 L. 99.9% of the medication is bound.[2]

Adverse Events:

Phase II trials using Fineranone to treat cGVHD showed that it was well tolerated.[6,7]The most frequent adverse events (AEs) associated with Fineranone 20 mg once daily (incidence 20%; n = 83) were infection (53%); asthenia (46%); nausea (42%); diarrhoea (35%); dyspnea (33%); cough (30%); oedema (27%); haemorrhage (23%); musculoskeletal pain (22%); abdominal pain (22%); headache (21%) and hypertension (21%).[2]

Allogeneic hematopoietic cell transplantation (HCT), a potential cure for a variety of haematological malignancies, is a therapy option for Fineranone. The prevalence of chronic graft-versus-host disease (cGVHD), a frequent and clinically relevant complication that affects up to 50% of HCT patients, can reduce the efficacy of allogenic HCT[8]. According to United States (US) claims analysis, approximately 14,000 individuals have cGVHD at this time, and 5,000 new cases are identified every year.[9]

Treatment:

There are several methods for treating cGVHD, but many of them have limitations that reduce their overall efficacy. A considerable portion of patients fail systemic therapy or develop steroid resistance despite the fact that systemic corticosteroids are the cornerstone of first-line treatment for cGVHD. Within two years of receiving initial systemic treatment, 50–70% of patients with cGVHD require additional treatment.[10]. Despite the fact that there are numerous second-line standard-of-care options (such as mTOR inhibitors, calcineurin inhibitors,

extracorporeal photopheresis, mycophenolate mofetil, steroid combination therapy, ibrutinib, and ruxolitinib) available, these therapies are connected to increasing immunosuppression and side-effects, and there is no agreement on which secondary cGVHD therapy is the most beneficial.[9,11]. Due to a higher risk of infections, cytopenias, and secondary primary cancers, current treatment options could also put patients' chances of recovering in danger. Due to these challenges, individuals with cGVHD use a significant amount of resources in the US healthcare system, and their inability to return to the labor costs the country's economy an estimated $27 billion annually.[12].

CLINICAL TRIALS[13,14,15]:

The terms "Finerenone" and "chronic graft-versus-host disease" were used in a literature search. NCT03640481; KD025-213) ROCK star: Efficacy and safety finerenonewere examined in a phase 2 US-based, multicenter, randomized, open-label trial in patients with cGVHD who needed further treatment after receiving 2 lines of systemic medication. Patients with platelets less than 50 109/L, absolute neutrophil count (ANC) less than 1.5 109/L, aspartate aminotransferase (AST) or alanine aminotransferase (ALT) greater than 3 ULN, total bilirubin greater than 1.5 ULN, corrected QT interval using Fridericia's formula (QTcF) greater than 480 ms, eGFR less than 30 mL/min/1. Patients who met the criteria were randomly assigned to receive either Fineranone 20 mg once daily or a placebo.

MATERIALS AND METHODS

Chemicals and reagents

Finerenone pure drug (API), Fineranone tablets (Rezurock), Distilled water, Acetonitrile, Phosphate buffer, Methanol, Potassium dihydrogen ortho phosphate buffer, Ortho-phosphoric acid. All the above chemicals and solvents are from Rankem.

Instrumentation:

- Electronics Balance-Denver
- pH meter -BVK enterprises, India
- Ultrasonicator-BVK enterprises
- WATERS HPLC 2695 SYSTEM equipped with quaternary pumps, Photo Diode Array detector and Auto sampler integrated with Empower 2 Software.

UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2mm and 10mm and matched quartz cells integrated with UV win 6 Software was used for measuring absorbance finerenone solution.

Preparation of Standard stock solution:

Accurately weighed 20mg finerenone is transferred to a 50ml volumetric flask. 3/4 th of diluents were added to the flask and sonicated for 10 minutes. Flask was made up with diluents and labeled as Standard stock solution. (400µg/ml finerenone)

Preparation of Standard working solution: 1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (40µg/ml finerenone).

Preparation of Sample stock solution: 10 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to 1 tablet was transferred into a 100ml volumetric flask, 50ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters (400µg/ml finerenone)

Preparation of Sample working solution: 0.2ml of filtered sample stock solution was transferred to a 10ml volumetric flask and made up with diluent. (40µg/ml finerenone).

Chromatographic conditions:

Mobile phase	**:**	45**%** OPA: 55% Acetonitrile
Flow rate	**:**	0.4ml/min
Column	**:**	**HSS** (3.8 x 100mm, 2.6µm).
Detector wave length	**:**	227.0nm
Column temperature	**:**	30°C
Injection volume	**:**	5.0µL
Run time	**:**	3 min
Diluent	**:**	Water and Methanol in the ratio 50:50
Results	**:**	**Fineranone** drug peak has good resolution, tailing Factor, theoretical plate count and resolution.

Degradation: To conduct the forced degradation experiment, standard stock solutions finerenonewas exposed to various stress conditions, including 1 mL of 20% H_2O_2 (for oxidative degradation), 1 mL of 2N HCL (for acidic degradation), and 1 mL of 2N NAOH (for acidic degradation) (for basic degradation). The produced solutions were refluxed for 30

minutes at 60^{o}C. To examine the descent, the standard solutions were also subjected to UV radiation and temperature conditions. The resulting solutions were diluted to yield 50µg/ml finerenone for degradation studies. To examine sample stability, 10µl samples were fed into the system and chromatograms were obtained.

Method Validation: The method was validated by ICH recommendations Q2R1. System appropriateness, specificity, linearity, accuracy, precision, LOD& LOQ, and robustness are among the validation parameters.

RESULTS AND DISCUSSION

System suitability parameters: The system suitability parameters were assessed by making standard solutions finerenone (50µg/ml) and injecting them six times. Peak tailing, resolution, and USP plate count were all determined. For three medications in combination, the USP Plate count exceeded 2000 and the tailing factor was less than 2. All of the system's appropriate parameters were passed and remained within the limitations. Table 1 shows the results.

Specificity: In the optimized method, the interference is checked. Fineranone, had retention time of 2.346 minutes. We did not find any interfering peaks in the chromatograms of blank and placebo samples during the retention periods of the drug in our approach. As a result, this procedure was stated to be particular. Figures 3, 4, and 5 show the chromatograms for specificity.

Linearity: Six linear concentrations finerenone (12.5-75µg/ml) was injected in triplicate manner. Correlation coefficient obtained was 0.999 for all the three drugs. The results were shown in table 2 and fig 6.

Precision:

Repeatability: Multiple samples were taken from a sample stock solution, and six working sample solutions of the same concentrations (50µg/ml Finerenone) were created. Each injection was given from each working sample solution, and the results are shown in table 3. The average area, standard deviation, and % RSD for the medication were computed and found to be 0.5% for Lamivudine. The system precision was passed for this procedure since the precision limit was less than "2 %." Table 3 shows the information results.

Intermediate Precision: Multiple samples were taken from a sample stock solution, and six working sample solutions of the same concentrations (50µg/ml finerenone) were prepared. Each injection from each working sample solution was given on the following day of the sample preparation, and the obtained areas are listed in table 4. The average area, standard deviation, and % RSD for the medication were computed and found to be 0.7% for Fineranone. Because the precision limit was less than "2%" the intermediate precision was used for this procedure. Table 4 shows the information results.

Accuracy: The conventional addition procedure was used to create three levels of accuracy samples. Triplicate injections were administered at each degree of accuracy, and the mean % recovery for Finerenone was found to be 99.99 %. Tables 5 show the outcomes. Because satisfactory recover values were achieved, the accuracy of this approach was passed.

Robustness: Robustness conditions such as flow minus (0.9ml/min), flow plus (1.1ml/min), mobile phase minus (65:35 v/v), mobile phase plus (55:45 v/v), temperature minus (25°C), and temperature plus (35°C) were maintained, and samples (50µg/ml Fineranone) was injected in duplicate. The % RSD was computed and determined to be within the acceptable range. Table 6 shows the data.

Assay: Lyvelsa tablets had a label claiming Finerenone 20mg per unit formulation. The aforementioned formulation was used for the assay. The average % assay achieved for Fineranone was 100.08%.

Degradation Studies: Degradation studies were performed with the stock standard solution and the degraded samples were analyzed using proposed method. Assay % finerenonein the injected samples was calculated and all the samples passed the limits of degradation. The results were shown in table 7. The purity plots obtained in degradation studies are shown in fig 7,8,9,10 and 11.

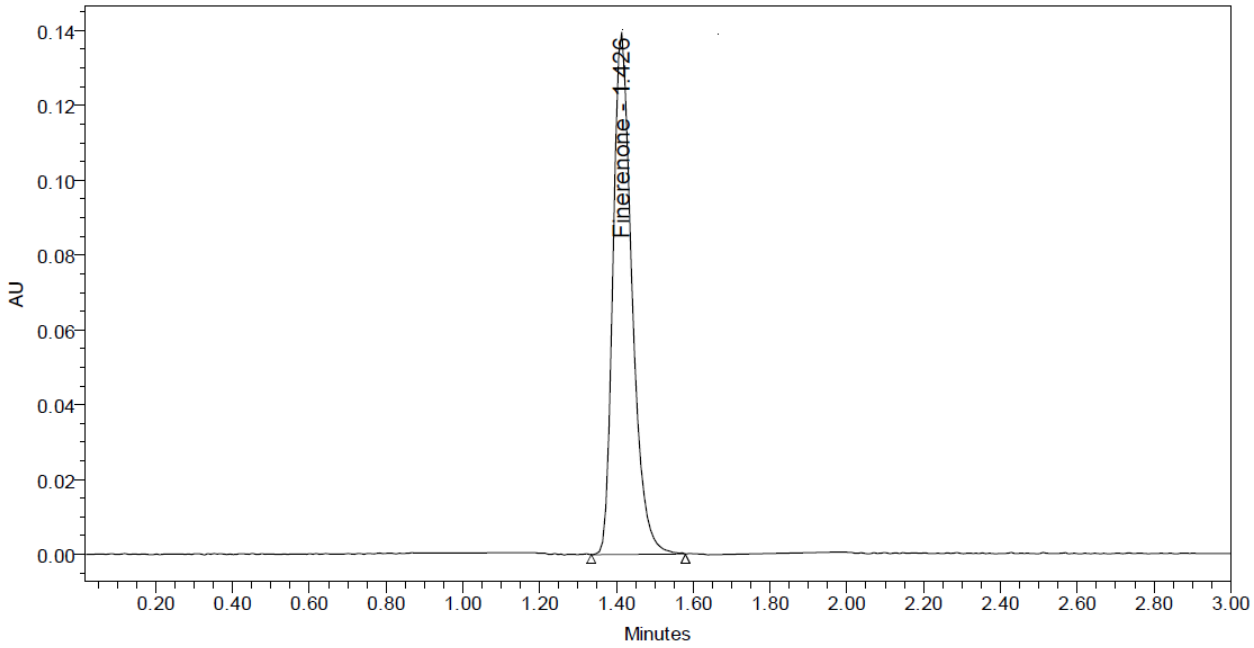


Fig No.1: Optimized Chromatogram

Table No.1: System suitability parameters

S no	**Finerenone**		
Inj	RT(min)	USP Plate Count	Tailing
1	2.434	4848	1.16
2	2.434	4708	1.15
3	2.435	4789	1.15
4	2.435	4657	1.16
5	2.435	4578	1.14
6	2.437	4609	1.17

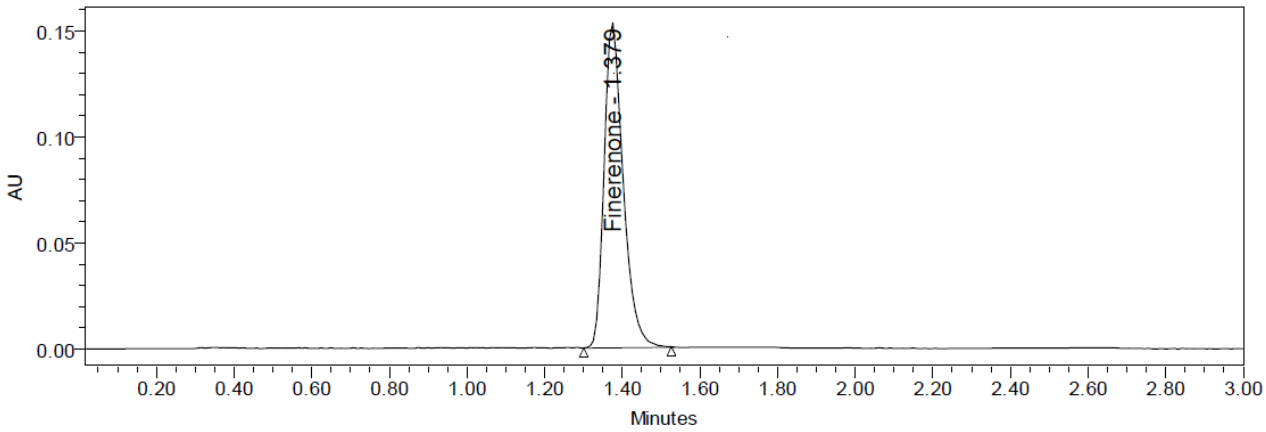


Fig No.2: Standard solution chromatogram

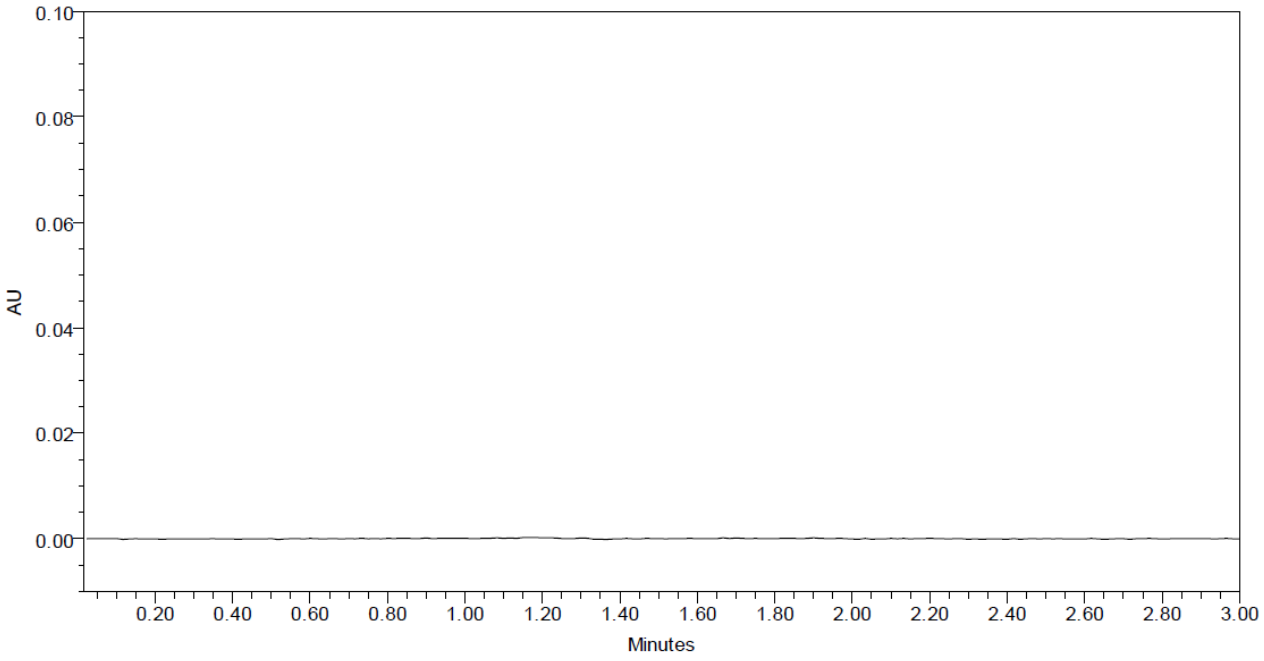


Figure No.3: Blank chromatogram

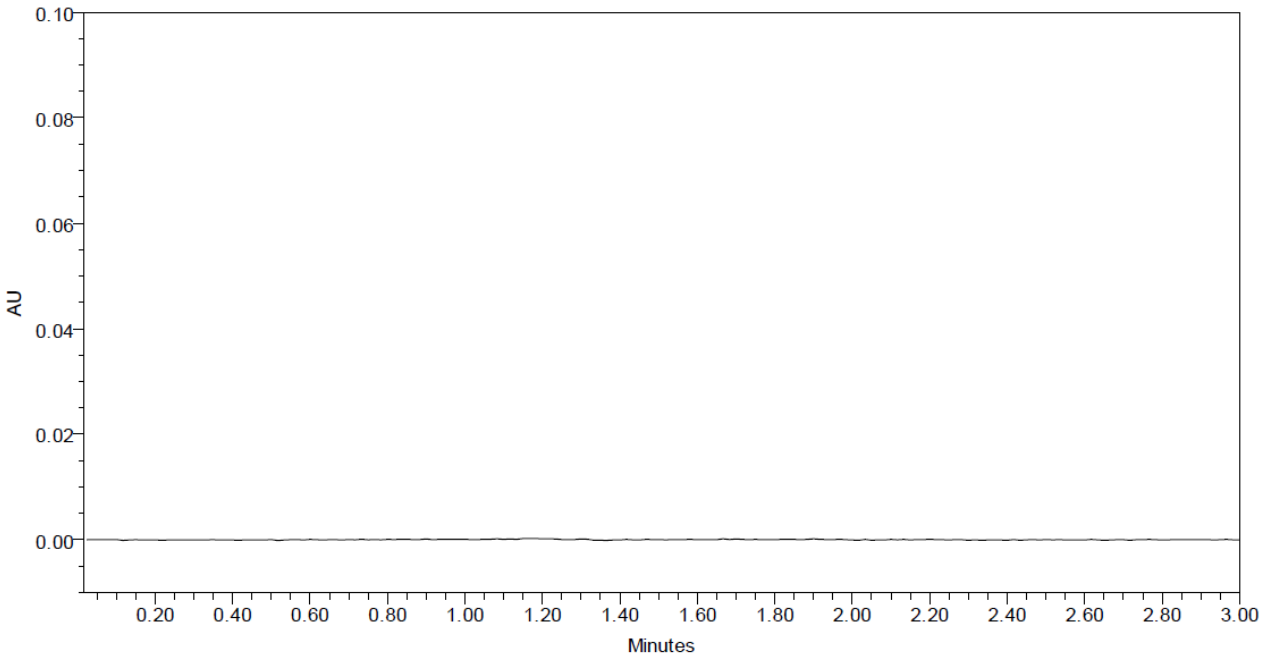


Fig No.4: Placebo chromatogram

Table No.2: Linearity table for Fineranone,

Finerenone	
Conc (µg/mL)	**Peak area**
0	0
10	303519
20	562411
30	821646
40	1133848
50	1366777
60	1691273

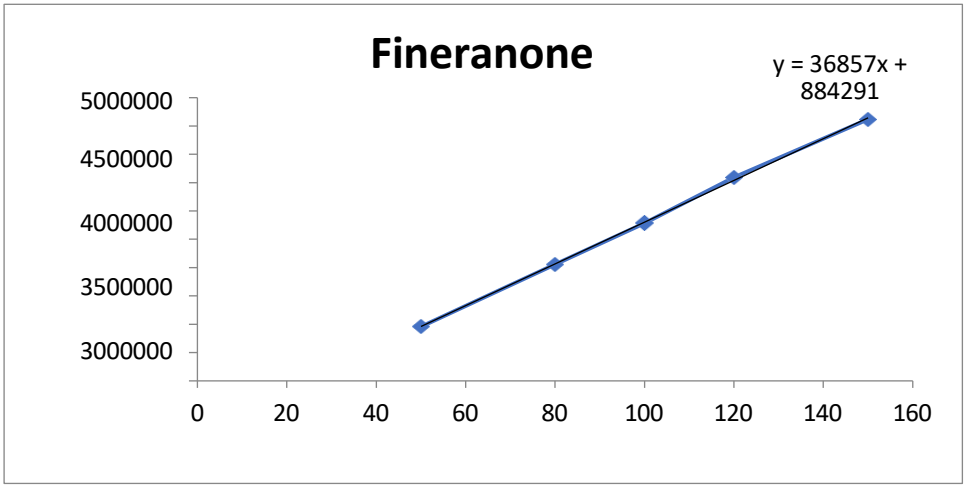


Fig No 5: Calibration curve finerenone

Table No.3: Repeatability for Fineranone

S. No	Area of Finerenone
1.	1129853
2.	1142738
3.	1133946
4.	1124501
5.	1142684
6.	1122964
Mean	1132781
S.D	8626.4
%RSD	0.8

Table No.4: Intermediate Precision for Fineranone

S. No	Area finerenone
1.	1111893
2.	1108797
3.	1126677
4.	1134395
5.	1123308
6.	1131298
Mean	1122728
S.D	10363.1
%RSD	0.9

Table No.5: Accuracy for Fineranone

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	20	40.68	101.69	99.97%
	20	40.02	100.04	
	20	40.42	101.05	
100%	40	39.39	98.49	
	40	40.21	100.51	
	40	39.77	99.42	
150%	60	39.89	99.74	
	60	39.74	99.34	
	60	39.80	99.49	

Table No.6: Robustness Data

S.no	Condition	%RSD finerenone
1	Flow rate (-) 0.3ml/min	0.4
2	Flow rate (+) 0.4ml/min	0.9
3	Mobile phase (-) 75B:25A	0.8
4	Mobile phase (+) 85B:15A	0.7
5	Temperature (-) 27°C	0.4
6	Temperature (+) 33°C	0.9

Table No.7: Degradation Data

S.NO	Degradation Condition	% Drug Undegraded
1	Acid	99.00
2	Alkali	99.60
3	Oxidation	99.60
4	Thermal	98.90
5	UV	98.10
6	Water	99.00

CONCLUSION:

The purpose of fineranone in drug quantity methods is discussed using a straightforward and selective LC approach. On an Inertsil C8 (150 mm x 4.6 mm x 5.0 m) column, chromatographic departure was skillful by a mobile phase made up of 10 mM phosphate cushion pH 3.0: Methanol (80:20) %v/v prepared with detection at 227 nm. For Fineranone, breadth was understood in the attention range of 50–150 g/ml (r2 = 0.9995), indicating that the amount of pharmaceuticals calculated by the suggested methods was reasonably consistent with the label claim.

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