Method Development and Validation of Finerenone by RP-HPLC Method

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Abstract— A simple, sensitive, accurate, rapid and specific RP-Hplc method was developed for estimation and validation of Finerenone in pure drug and tablet dosage form. chromatographic separation was achieved on an symmetry ODS C analytical column using mobile phase composition of methanol and phosphate buffer in ratio of 35:65% that was set at the flow rate of 1.0 ul/min with detection of 235 nm. The presentation of method was validated according to present ICH guideline for accuracy precision and robustness, linearity, limit of quantification, limit of detection linearity.

Index Terms- Finerenone, RP-HPLC, Method development, Accuracy, Precision.

I. INTRODUCTION

Finerenone is a nonsteroidal MRA, developed using the chemical structure of a dihydropyridine channel blocker but optimized to create a bulky MRA without any activity at the L-type calcium channel. Finerenone has strong selectivity for the MR while maintaining very low affinity for androgen, glucocorticoid, and progesterone receptors.

The objective of these research are as follow:

- To develop method for Anti-mineralcorticoid receptor Antagonists drug in bulk and solid dosage form.
- To validate accuracy, precision, linearity, robustness as per ICH guidelines.
- The objective of the present study is to establish and generate inheriting Validation data for Finerenone used the wavelength of uv spectrophotometer.

The need of the research is:

- Method development and validation are extremely important in the development of drug material.

For many drug products, Scientists working on Investigational New Drug (IND), New Drug Application (NDA), and Abbreviated New Drug Application (ANDA) used to characterize API'S and excipients have not been sufficiently developed and validated.

DRUG PROFILE

Finerenone

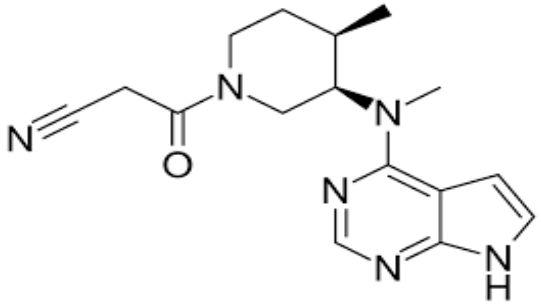

Fig. No.1 Structure of Finerenone

Table No.1 Classification of Chromatographic methods

Molecular formula	$C_{21}H_{22}N_4O_3$
Molecular weight	378.432 g/mol
Chemical name	4*S*)-4-(4-Cyano-2-methoxyphenyl)-5-ethoxy-2,8-dimethyl-1,4-dihydro-1,6-naphthyridine-3-carboxamide
Description	White crystalline powder
Category	Mineralcorticoid Receptor antagonists

Mechanism of action:

Finerenone is a selective mineralocorticoid receptor (MR) antagonist with no significant affinity or activity for androgen, progesterone, estrogen, and glucocorticoid receptors. Animal studies have shown that Finerenone binding to MR reduces inflammation and fibrosis, and phase 2 clinical trials have shown a reduction in albuminuria. Aldosterone is a mineral corticoid hormone involved in the regulation of blood pressure, sodium reabsorption, and potassium excretion. In 1943, hypersalt-associated MR agonism was shown to be associated with malignant hypertension, which can progress to organ inflammation and fibrosis. Binding of aldosterone, an MR agonist, to the MR causes a conformational change that dissociates the receptor from inactivating chaperone proteins. Active MR travels to the nucleus along with a complex of other co-activators to induce transcription of several genes.

Pharmacokinetics:
Absorption, Distribution and Excretion
Absorption
In healthy individuals, the mean absolute bioavailability of finerenone following subcutaneous injection at a dose of 1-2 mg/kg is around 100%. Finerenone has linear absorption, meaning that its absorption is proportionate to the dosage. After a subcutaneous injection, the typical maximal plasma anti-Xa activity is obtained three to five hours later.9,13 Maximum anti-Factor Xa levels were 1.16 IU/mL after an immediate 1 mg/kg SC given twice a day after a 30 mg IV bolus. After 3–4 days9 of therapy, steady-state is attained with a Cmax of 1.2 IU/mL.Thirteen 305 +/- 48.8 was the area under the thrombin generation curve (AUC).

Route of Elimination:
The M2, M3 (47.8%), and M4 metabolites accounted for the bulk of the dosage recovered in urine; the parent molecule was unaltered for less than 1.3% of the dose recovered in urine.1. Just 0.2% of the dosage was removed as the unaltered parent chemical, with the rest of the amount recovered in the feces being as the M5 metabolite.1. Less than 1.5% of the recovered dosage in urine and feces was composed of the M1 metabolite.

Volume of Distribution:
The volume of distribution of finerenone as steady state is 52.6L.

Clearance:
The clearance of is estimated to be 0.74L/h.
More common side effects

- Confusion.
- Irregular heartbeat.
- Nausea or vomiting.
- Nervousness.
- numbness or tingling in the hands, feet, or lips.
- stomach pain.

II. MATERIAL AND METHODS

Selection and Procurement of Drug

List of reagents & chemicals used

Table 2: List of Reagents and Chemicals used

Sr. No.	Name of chemicals	Manufacturer.
1.	Acetonitrile (HPLC grade)	Merck Ltd., India
2.	Methanol (HPLC grade)	Merck Ltd., India
3.	0.1% OPA (HPLC grade)	Merck Ltd., India
4.	water (HPLC grade)	Merck Ltd., India

Selection of formulation:

Marketed Preparation

Table No.3: List of brand names of combined formulations of Finerenone

Sr. No	Brand name	Formulation	Available strength	Address of manufacturer
1.	Kerendia	Tablet	Finerenone 10 mg	Bayer Zydus Pharma

The marketed concoction, which goes by that name in this thesis, was purchased at a local market.

Selection of Analytical Technique
The analytical method of choice for finerenone estimation was HPLC.

- Instruments:

Agilent Tech was utilized for the drug analysis. Gradient System featuring Gradient Detector, Auto Injector, and DAD. outfitted with UV730D Absorbance detector, Reverse Phase (Agilent) C18 column (4.6 x 250 mm; 5µm), and Chemstation 10.1 software.

- Stock preparations:
- Stock I: Standard Sample Preparation

Std. Finerenone 10 mg in 100 ml Methanol = 100 µgm/ml

- Stock II: Tab solution Preparation*:* -
- Take 25 mgs in 100 ml Methanol i.e= 100 µgm/ml
- For Accuracy Solution Preparations: -
- Take 10 µgm/ml TAB SOLUTION FOR ACCURACY,

80 % = 0.1 ML TAB SOLUTION and ADD 1.6 µgm/ml STD FRN
AND MAKE UP VOL 10 ML WITH Mobile PHASE
100 % =0.1 ML TAB SOLUTION and ADD 2 µgm/ml STD FRN
AND MAKE UP VOL 10 ML WITH Mobile PHASE
120 %= 0.1 ML TAB SOLUTION and ADD 2.4 µgm/ml STD FRN
AND MAKE UP VOL 10 ML WITH Mobile PHASE

Instruments and Equipments

Table. 4: Instrument (HPLC) Details used during Method Development

	Name of Instrument	Company Name
1	HPLC Instrument	Agilent Tech. Gradient System with Auto injector (Chemstation software)
2	UV-Spectrophotometer	Analytical Technologies Limited
3	Column(C_{18})	Agilent C_{18} (250mmX 4.6mm,5µm)
4	pH meter	VSI pH meter(VSI 1-B)
5	Balance	WENSAR™ High Resolution Balance.
6	Sonicator	Ultrasonics electronic instrument

EXPERIMENTAL WORK

HPLC:

Selection of Analytical Technique

- HPLC was selected as analytical technique for estimation of Finerenone
- Instruments:

Using an Agilent Technologies Gradient System with Auto Injector and DAD Detector, the drug was analyzed. outfitted with a UV730D absorbance detector, a 250 x 4.6 (5µm) Reverse Phase C18 (Agilent) detector, and Chemstation 10.1 software.

Table No.5: chromatographic conditions (HPLC) details used during method Development.

1.	HPLC	Agilent Tech. Gradient System with Auto injector
2.	Software	*chemstation 10.1*
3.	Column	(Agilent) C18 column (4.6mm x 250mm
4.	Particle size packing	*5 µm*
5.	Stationary phase	C18 (Agilent)
6.	Mobile Phase	*Methanol : water (0.1 % OPA)* *45 : 55*
7.	Detection Wavelength	*230 nm*
8.	Flow rate	*1 ml/min*
9.	Temperature	*Ambient*
10.	Sample size	*20 µl*
11.	pH	*3.0*
12.	Run Time	15 min
13.	Filter paper	0.45 µm

Study on the selection of uv spectrum use in uv-vis spectrometer of Finerenone :

Weigh 10 mg of the finerenone working standard precisely, then transfer it into a 100 ml volumetric flask as much as possible to dilute the Methanol prepared in full and bring the volume up to the mark using the same solvent to obtain a 100 µg/ml standard

(stock solution). Sonicate the solution for 15 minutes to dissolve it, then transfer 0.5 ml of the resulting solution to a 10 ml volumetric flask and add Methanol to bring the volume up to the mark

Study on the chromatographic conditions of Finerenone:
Precisely weigh and transfer 10 mg of the working standard finerenone into a 100 ml volumetric flask. Dilute the methanol prepared completely and adjust the volume to the mark using the same solvent to obtain a 100µg/ml standard (stock solution). Sonicate the solution for 15 minutes to dissolve it. Subsequently, transfer 0.1 ml of the resultant solution to a 10 ml volumetric flask. Adjust the volume to the mark using the mobile phase Methanol:(0.1%OPA) Water solvent. Chromatographic studies were performed on the resultant 10µg/ml solution using various strengths of mobile phases under the chromatographic conditions

- Analytical column : Agilent C18 Column (250mm x 4.6mm), 5µm particle size.
- Injection volume : 20µl
- Flow rate : 1 ml/min
- Detection : 230 nm
- Run Time : 10 min

- Following Mobile phase were tried:

METHOD DEVELOPMENT OF HPLC:

- List of Mobile Phase:

Table No.6: Selection of mobile Phase.

Sr.No.	Mobile Phase
1.	Methanol+ 0.1% (OPA)Water, (90+10% v/v) 20 Mcg, C_{18} (Agilent) (4.6mm x 250mm)
2.	Methanol+ 0.1% (OPA)Water, (70+30% v/v) 20 Mcg, C_{18} (Agilent) (4.6mm x 250mm)
3.	Methanol+ 0.1% (OPA)Water, (50+50% v/v) 0.7 20 Mcg, C_{18} (Agilent) (4.6mm x 250mm)
4.	Methanol+ 0.1% (OPA)Water, (40+60% v/v) 20Mcg C_{18} (Agilent) (4.6mm x 250mm)0.7
5.	Methanol+ 0.1% (OPA)Water, (20+80% v/v) 0.7 20 Mcg, C_{18} (Agilent) (4.6mm x 250mm)
6.	Methanol+ 0.1% (OPA)Water, (20+80% v/v) 20 Mcg, Flow 0.7 C_{18} (Agilent) (4.6mm x 250mm)
7.	Methanol+ 0.1% (OPA)Water, (50+50% v/v) 20 Mcg, Flow 0.7 C_{18} (Agilent) (4.6mm x 250mm)
8.	Methanol+ 0.1% (OPA)Water, (45+55% v/v) 20 Mcg, Flow 1 C_{18} (Agilent) (4.6mm x 250mm)

Calibration Experiment:

- RP-HPLC Method :

a) Preparation of Calibration curve standard:
Mobile phase was used to dilute the aforementioned standard stock solution (100 µg/ml) of finerenone, resulting in five calibration curve (cc) standards with finerenone concentrations of 2, 4, 6, 8, and 10 µg/ml

b) Calibration runs and regression analysis:
Under the chromatographic conditions listed below, these calibration standard solutions were examined in three duplicates.

- Analytical column : C18 Column (250mm x 4.6mm, 5µm partical size).
- Injection volume : 20µl.
- Flow rate : 0.7 ml/min.
- Mobile phase : Methanol: Water (0.1% OPA) (45: 55 % V/V).

- Detection : 230 nm.

III. RESULT AND DISCUSSION

Preliminary studies on Finerenone

Melting point

It was discovered that the obtained finerenone reference standard melted between 211-2160 C.

Solubility

The drug was found to be

- Freely soluble in Acetonitrile, methanol.
- Practically *soluble* in water, but freely *soluble in organic solvents*.

UV Spectroscopy

The 200–400 nm range of standard solutions was scanned against a volume made using a water solvent system and 10 ml of methanol as a reference. The wavelength at which finerenone in water was discovered to be chosen is 230 nm.

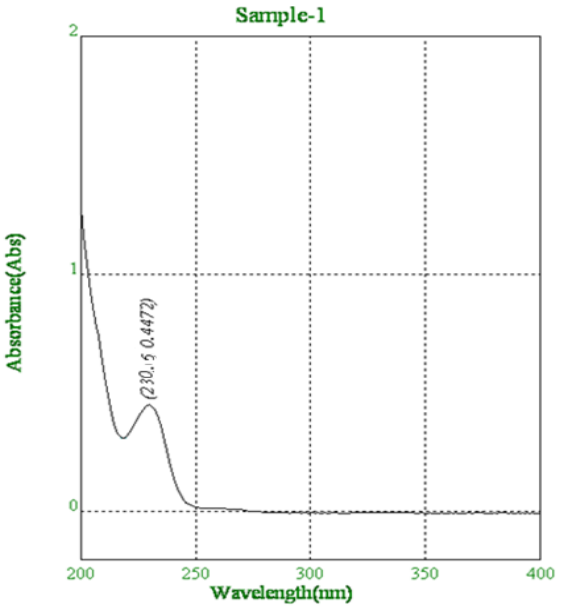

Fig No.2: UV Spectrum of Finerenone

TABLE NO-7: Chromatographic behavior of Finerenone mobile phase of various compositions.

Fig No	Column used	Mobile phase, Flow Rateand Wavelength	Inj. Vol.	Observation	Conclusion
1.	C_{18} (Agilent) (4.6 mm x 250 mm), 5.0µ)	Methanol+ 0.1% OPA)Water, Ph3, (90+10),230nm 20 Mcg, Fl. 0.7,	20 µl	Sharp Peaks were not obtained	Hence rejected
2.	C_{18} (Agilent) (4.6 mm x 250 mm), 5.0µ)	Methanol+ 0.1% OPA)Water,Ph3 (70+30),230nm 20 Mcg, Fl. 0.7,	20 µl	Sharp Peaks were not obtained	Hence rejected
3.	C_{18} (Agilent) (4.6 mm x 250 mm), 5.0µ)	Methanol + 0.1% OPA)Water(50+50)PH3.0, 230nm, Flow rate 0.7mL	20 µl	Sharp Peaks were not obtained	Hence rejected
4.	C_{18} (Agilent) (4.6 mm x 250 mm), 5.0µ)	Methanol + 0.1% OPA)Water,(40+60)PH3.0, 230nm, Flow rate 0.7mL	20 µl	Sharp Peaks were not obtained (larger RT)	Hence rejected
5.	C_{18} (Agilent) (4.6 mm x 250 mm), 5.0µ)	Methanol + 0.1% OPA)Water(20+80)PH3.0, 230nm, Flow rate 0.7mL	20 µl	Peaks were not obtained	Hence rejected

6.	C_{18} (agilent) (4.6 mm x 250 mm), 5.0µ)	Methanol + 0.1% OPA)Water (20+80 %v/v)PH3.0, 230nm, Flow rate 1 mL	20 µl	Sharp Peaks were not obtained (Larger RT)	Hence rejected

Thus, it can be seen from the foregoing that a flow rate of 1 ml and a mobile phase of meoh+0.1% OPA)water, (45:55 % v/v), PH 3.0, 230 nm, provided sufficient retention at 3.945 min with a decent peak shape (Theoretical plates: Finerenone 11477)

Chromatogram of Trial 1:

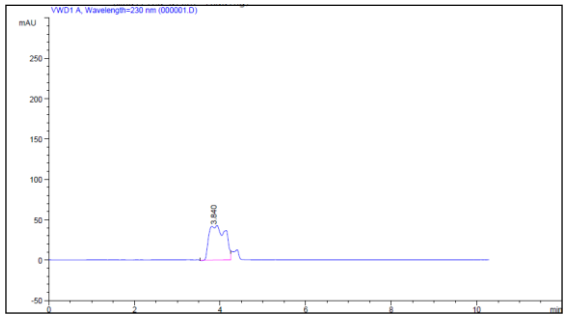

Fig No 3: Chromatogram of Trial 1

Table.No.8. Trial-1 of chromatogram of Finerenone

No.	RT [min]	Area[mV*s]	Area %	TP	TF
1	3.840	1133.2940	100.00	350	0.35

Observation: peak Splitting was observed, sharp peak were not obtained, so method was rejected.

Chromatogram of Trial 2:

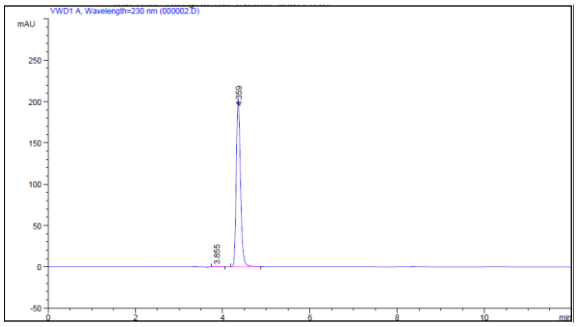

Fig No 4: Chromatogram of Trial 2

Table.No.9. Trial-2 of chromatogram of Finerenone

No.	RT[min]	Area[mV*s]	Area %	TP	TF
1	4.359	1372.2932	100.00	9521	0.83

Observation: peak Splitting was observed, sharp peak were not obtained, so method was rejected.

Chromatogram of Trial 3:

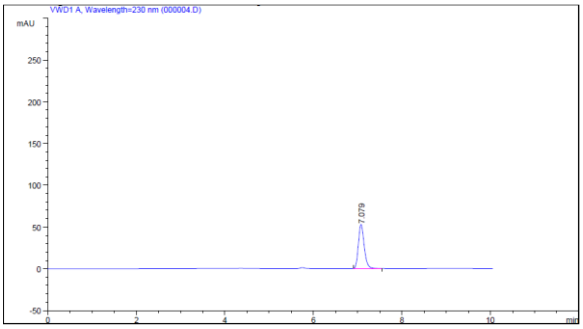

Fig No 5: Chromatogram of Trial 4

Table.No.10. Trial-3 of chromatogram of Finerenone

No.	RT[min]	Area[mV*s]	Area %	TP	TF
1	7.079	482.9403	100.00	14342	0.82

Observation: Larger RT was observed, so method was rejected

Chromatogram of trial 4:

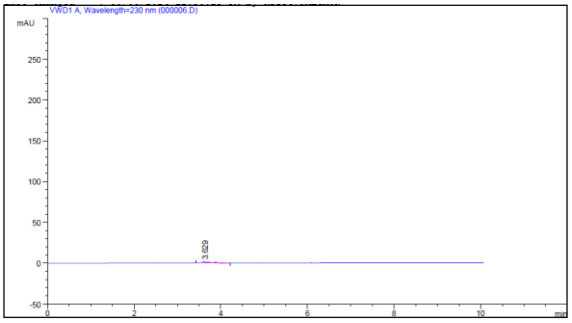

Fig No 6: Chromatogram of Trial 5

Table.No.11 Trial-4 of chromatogram of Finerenone

No.	RT[min]	Area[mV*s]	Area %	TP	TF

1	3.629	20.4069	100.00	663	0.38

Observation: peak was not found so method was rejected

Chromatogram of Trial 5:

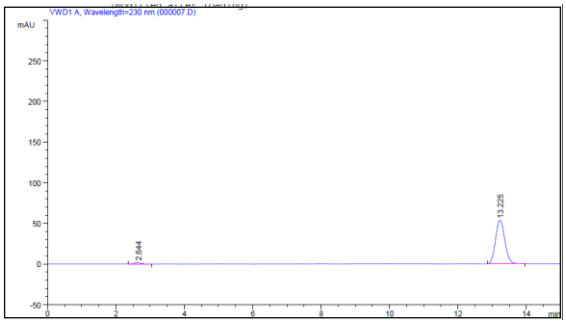

Fig No 7: Chromatogram of Trial 6 (20Methanol +80%Buffer flow 1ml/min)

Table.No.12. Trial-5 of chromatogram of Finerenone

No.	RT[min]	Area[mV*s]	Area %	TP	TF
1	13.225	1002.9291	100.00	11620	0.89

Obsrvation: Larger RT was found, time consuming method, so method was rejected.

Chromatogram of Trial 6:

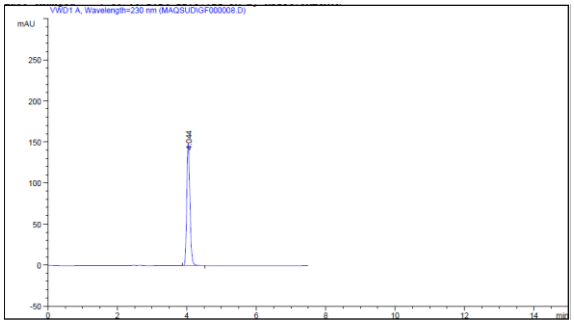

Fig No 8: Chromatogram of Trial 7 (50methanol+50 buffer, 1 ml)

Table.No.13. Trial-6 of chromatogram of Finerenone

No.	RT[min]	Area[mV*s]	Area %	TP	TF
1	4.044	957.9613	100.00	8723	0.86

Chromatogram of final Trial 7:

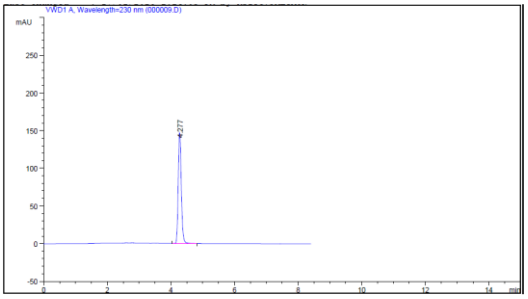

Fig No 9: Chromatogram of final Trial 8 (55Methanol+45buffer,1 ml)

Table.No.14. Final Trial-7 of chromatogram of Finerenone

No.	RT[min]	Area[mV*s]	TP	TF	Resolution
1	4.277	1001.13354	9164	0.84	0.0000

Observation: sharp peak were obtained, theoretical plate count is more than 2000 so method was selected.

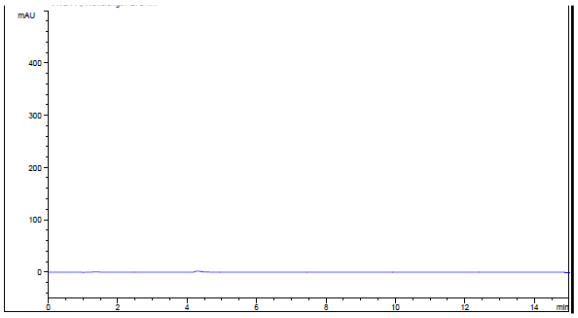

Fig No 10: Chromatogram of blank

- The final chromatographic conditions selected were as follow:
- Analytical column : Agilent C18 Column (250mm x 4.6mm, 5µm partical size).
- Injection volume : 20µl.
- Flow rate : 1 ml/min.
- Mobile phase : Methanol : water (55: 45% V/V)
- Detection : 230 nm
- Run Time : 15 min.
- Preparation of Standard chromatogram of Finerenone

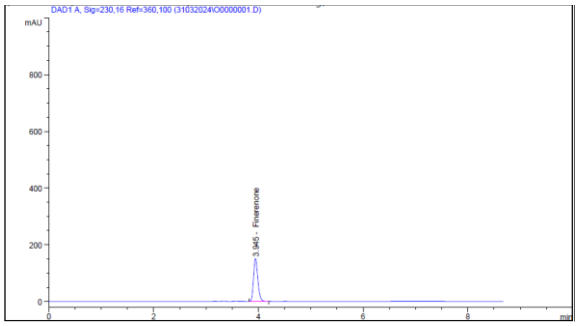

Fig No.11: Chromatogram of standard Finerenone

Table.No.15. Details of chromatogram of standard Fi nerenone

No.	RT[min]	Area[mV*s]	TP	TF	Resolution
1	3.945	905.14233	11477	0.79	0.0000

Theoretical plates for Finerenone were discovered above 2000 in the standard; that is, for Finerenone 11477, at least RT 3.945.

Calibration experiment

- RP-HPLC Method :

When the data from the calibration experiments were subjected to linear regression analysis, the results for Finerenone (Table No. 21) demonstrated a linear connection between peak areas and concentrations in the range of 2–10µg/mL. For Finerenone, the corresponding linear equation was Y= 428.2x+59.02, where x represents concentration and y represents peak area. There was a 0.999 correlation coefficient. (Fig No.18) shows the Finerenone calibration curve.

Table No 16: Linearity data for Finerenone

Method	Conc µg/ml	Peak area(µV.sec)		Average peak area (µV.sec)	S.D. of Peak Area	% RSD of Peak Area
		1	2			
RP-HPLC Method	2	906.1423	905.343	905.7428	0.57	0.06
	4	1800.2926	1801.02	1800.6603	0.52	0.03
	6	2610.3544	2608.36	2609.3576	1.41	0.05
	8	3474.3530	3476.52	3475.4382	1.53	0.04
	10	4351.7192	4349.40	4350.56	1.63	0.04
	Equation		Y= 428.2x+59.02			
	R^2		0.999			

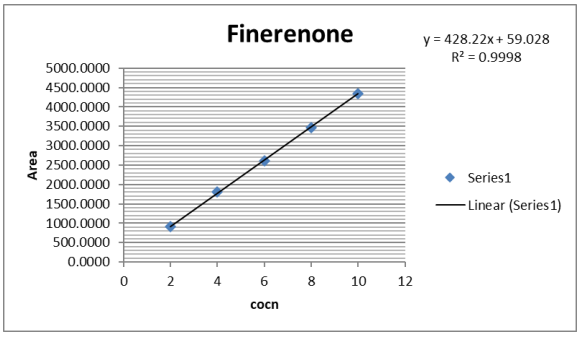

Fig.No.12: Calibration curve of Finerenone (HPLC)

The corresponding linear equation for finerenone, as determined by the RP-HPLC method, was y = 428.2 x +59.02, where x represents concentration and y represents peak area. There was a 0.999 correlation coefficient. Fig. 18 shows the Finerenone calibration curve.

Analysis of tablet formulation:-
Procedure:
Finerenone is a selective mineralocorticoid receptor (MR) antagonist with no significant affinity or activity for androgen, progesterone, estrogen, and glucocorticoid receptors. Animal studies have shown that Finerenone binding to MR reduces inflammation and fibrosis, and phase 2 clinical trials have shown a reduction in albuminuria. Aldosterone is a mineral corticoid hormone involved in the regulation of blood pressure, sodium reabsorption, and potassium excretion. In 1943, hypersalt-associated MR agonism was shown to be associated with malignant hypertension, which can progress to organ inflammation and fibrosis. Binding of aldosterone, an MR agonist, to the MR causes a conformational change that dissociates the receptor from inactivating chaperone proteins. Active MR travels to the nucleus along with a complex of other co-activators to induce transcription of several genes.

Brand Name: *Kerendia 10 MG (Zydus pharma)*
Total weight of 20 tab wt = 0.50 gms
Avgr Weight = 0.025 gms. /Tab
Eq.wt for 10 mg= 10 X 0.025 / 10 = 25 mg
Take 25 mgs in 100 ml Methanol sonicate 10 min
i.e. 100 µgm/ml Finerenone ----- STOCK -I

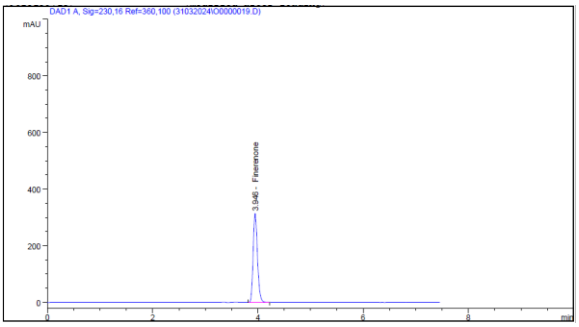

Fig No.13: Chromatogram for Marketed Formulation 4mcg

Table.No.17. Details of chromatogram of Marketed Formulation 4mcg

No.	RT[min]	Area[mV*s]	TP	TF	Resolution
1	3.946	1794.65698	11486	0.78	-

Table.18. Analysis of marketed formulation

Assay	Drug	Lable Claimed	Amt.Found	%Lable Claim	SD	%RSD
Rp-HPLC Method	FRN	4	4.05	101.33	0.13	0.414
		4	4.058	101.45	0.42	0.414

- Analysis of marketed formulation were also %Lable Claim was found to be 98-101% Satisfactory are concluded.

SUMMARY AND CONCLUSIONS

Clinical applications for finerenone include anti-mineralcorticoid receptor antagonists. The current study focuses on developing and validating the RP-HPLC technique for measuring finerenone in both pure and tablet dose forms.

Summary of RP-HPLC method:

An RP-HPLC technique for estimating finerenone from tablets was attempted to be developed. Agilent (Autosampler) GradientSystem DAD Detector and C18 (Agilent) with 250 mm x 4.6 mm i.d. and 5µm particle size are required for the RP HPLC procedure. The procedure employed methanol:water 0.1% OPA (45:55v/v) pH 3.0 as the mobile phase. The flow rate was 1 ml/min and the detecting wavelength was 230 nm. The retention period of finerenone in the developed technique was determined to be 3.9 minutes.

The ICH recommendations were followed in the validation of the devised approach. The ICH recommendations' limitations were met by the linearity, accuracy, range, and robustness. The approach was therefore determined to be straightforward, accurate, precise, affordable, and repeatable.

Thus, it is valuable that the suggested techniques may be used to the regular quality control analysis of finerenone in both bulk and formulation medication forms.

CONCLUSION

For the regular analysis of finerenone in API and tablet dosage forms, RP-HPLC has been designed and verified to be simple, fast, accurate, and precise. Without interfering with one another, both approaches are appropriate for determining the amount of finerenone in single-component formulations. It is advised to use the developed procedures for regular and quality control examination of the pharmaceutical preparations containing the medications under investigation. The quantity discovered using the suggested techniques agreed well with the formulation's label claim. Furthermore, the computed coefficient of variation and standard deviation values were both adequately low, suggesting that the suggested approaches are appropriate for routine tablet dosage form determination.

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