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Development And Validation of Bioanalytical Method for the Quantification of Finerenone in Rat Plasma by RP-HPLC

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KEYWORDS

Finerenone

Protein Precipitation method,

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Pharmacokintic parameters,

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ABSTRACT:

Introduction: Bioanalytical method development involves designing and optimizing methods to quantify drugs, metabolites, or biomarkers in biological samples (such as blood, plasma). This process is critical in various fields, including pharmacokinetics, toxicology, clinical research, and drug development. Finerenone is a selective non-steroidal mineralocorticoid receptor antagonist, is used primarily for the treatment of chronic kidney disease (CKD) in patients with type 2 diabetes, it helps to reduce the risk of kidney function by blocking the effects of aldosterone, a hormone that promotes sodium retention and contributes to CKD.

Objectives: To optimize the chromatographic condition for the selected approved protocol.To develop a suitable bioanalytical method for the assessment of Finerenone in rat plasma by RP-HPLC method. To validate the developed method as per ICH M10 guideline for intended bioanalytical application. To estimate the *in vivo* pharmacokinetic parameter for Finerenone in rat plasma by RP-HPLC.

Methods: Chromatographic separation was conducted using an HPLC system from Shimadzu (LC 20 AD) equipped with a PDA detector, controlled by Lab Solution software. Phenomenex Luna C18 column (250mm × 4.6mm, 5µm) was utilized for the separation process. Sample preparation was carried out using an ultra-cooling centrifuge. pH measurement was performed using an ELICO pH meter (LI 120). Solutions were degassed using a sonicator from Lifecare. Micropipettes with a capacity of 100-1000 µl were utilized for measurements. Plasma samples underwent filtration using syringe filters with a pore diameter of 0.25 µm.Finerenone was procured from Hangzhou Jinlan Pharm-Drugs Technology CO., Ltd. China. Niacinamide (IS) was procured from Dhamtec pharma pvt limited Mumbai, India. Blank plasma was collected from Animal House, KMCH College of Pharmacy, Coimbatore. All additional chemicals utilized were of high quality (HPLC grade) and were procured from Rankem Chemicals, India.

Results: The HPLC-PDA method with simple and economical protein precipitation extraction was developed and validated for the estimation of Finerenone in wistar rat plasma. The developed method has a shorter retention time (7.3 mins) using water and acetonitrile (15:85 % v/v). In this method, the validated HPLC technique was utilized to simultaneously determine finerenone levels in rats post-oral administration of 10 mg/kg. Graphical depictions illustrating mean plasma concentration over 4 hours with pharmacokinetic parameters. The maximum concentrations (C_{max}) of finerenone were found to be 75 ng/mL and the time observed for maximum drug concentration (T_{max}) being 75 mins. The half-lives ($T_{1/2}$) of finerenone were determined to be 2.58 h. Additionally, the area under the curve for 4 hours (AUC_{0-t}) and the elimination rate constant (K_e) were also evaluated.

Conclusions: A robust and sensitive RP-HPLC method was successfully developed for the accurate quantification of finerenone in rat plasma, ensuring selectivity and reproducibility. The developed method underwent validation according to ICH standards (M10), meeting all

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validation criteria within specified limits. The results indicated that the method was accurate and precise. The validated method is suitable for application in pharmacokinetic and preclinical studies of finerenone, supporting further drug development and research.

1. Introduction

Finerenone

Finerenone is a structurally novel non-steroidal mineralocorticoid receptor antagonist (MRAs), which exhibits outstanding effect on cardio-renal protection. The mineralocorticoid receptors (MRs) are widely distributed in the heart, kidney, brain, lung, colon, skin, liver, skeletal muscle, saliva, sweat gland, and fat.[1] MRs are mainly expressed in the cardiovascular system and kidney, and play vital role in ventricular remodelling and chronic heart failure (CHF) progression. [2]

Aldosterone, the MR, maintains the sodium/potassium homeostasis and the electrolyte balance of the body. In addition, an increasing number of studies have shown that inflammatory and fibrotic effect is mediated by excessive activation of MRs, leading to the adverse cardiac and renal outcomes. It could be an important therapeutic target for chronic kidney disease (CKD) induced by T2DM.

Finerenone, a third-generation highly selective MRA, can directly and specifically block MR hyper activation, and promote the anti-inflammatory and anti-fibrotic effects. In this way, finerenone exhibits cardiovascular and renal double-benefits, and is used in the treatment of T2DM-related CKD (diabetic kidney disease, DKD) to reduce the risk of persistent decline in glomerular filtration rate (eGFR) and the progression of end stage renal disease (ESRD). [3]

Finerenone has been shown to reduce the urinary albumin-to-creatinine ratio in patients with CKD treated with an RAS blocker, while having smaller effects on serum potassium levels than spironolactone.[4] The Finerenone in Reducing Kidney Failure and Disease Progression in Diabetic Kidney Disease (FIDELIO-DKD) trial was designed to test the hypothesis that finerenone slows CKD progression and reduces cardiovascular morbidity and mortality among patients with advanced CKD and type 2 diabetes.

HPLC is widely used in biochemistry and analytical chemistry for separating, identifying, and quantifying active compounds.[5] The modification in migration rates across the column as an outcome of the sample's division into stationary and mobile phases assists as the foundation for sample separation [6].

Development and Validation of an analytical method

When there are no definitive methods available, new techniques are being created to evaluate the invention. New methods are developed to investigate the possibility of either pharmacopoeial or non-pharmacopoeial items to reduce the value other than time for enhanced strength and precision.[7] Method validation was performed through laboratory testing that an showing or proves the accuracy, reliability, and suitability of a method and meets the specifications necessary for the intended analytical use [8].

Various analytical techniques exist for quantifying drugs. Based on the literature survey there is no *in vivo* Pharmacokinetic study for determining Finerenone by RP-HPLC. Hence, a pharmacokinetic study is needed to ensure the product quality through its lifecycle. This study aims to develop and validate an analytical method per the ICH guidelines, and to evaluate the in vivo pharmacokinetic parameters.

2. Objectives

To optimize the chromatographic condition for the selected approved protocol. To develop a suitable bioanalytical method for the assessment of Finerenone in rat plasma by RP-HPLC method. To validate the developed method as per ICH M10 guideline for intended bioanalytical application. To estimate the *in vivo* pharmacokinetic parameter for Finerenone in rat plasma by RP-HPLC.

3. Methods

Chromatographic separation was conducted using an HPLC system from Shimadzu (LC 20 AD) equipped with a PDA detector, controlled by Lab Solution software. Phenomenex Luna C18 column (250mm × 4.6mm, 5µm) was utilized for the separation process. Sample preparation was carried out using an ultra-cooling centrifuge. pH measurement was performed using an ELICO pH meter (LI 120). Solutions were degassed using a sonicator from Lifecare. Micropipettes with a capacity of 100-1000 µl were utilized for

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measurements. Plasma samples underwent filtration using syringe filters with a pore diameter of 0.25 µm.

Finerenone was procured from Hangzhou Jinlan Pharm-Drugs Technology CO., Ltd. China. Niacinamide (IS) was procured from Dhamtec pharma pvt limited Mumbai, India. Blank plasma was collected from Animal House, KMCH College of Pharmacy, Coimbatore. All additional chemicals utilized were of high quality (HPLC grade) and were procured from Rankem Chemicals, India.

4. Results

Chromatographic Method development

The optimal wavelength for detecting finerenone was identified as 251 nm. Separation was achieved using a mobile phase composed of acetonitrile and water in a 85:15% v/v ratio. Effective resolution was observed for at a pH of 7. Since acetonitrile demonstrated the highest recovery when compared to methanol, it was chosen as a suitable separating agent for finerenone. Finerenone had respective retention times of 7.3 minutes. The chromatogram showed no mutual interference and was highly resolved. Furthermore, there were acceptable tailing or fronting values in the peaks.

Based on the literature survey, the C_{18} column was used in most of the methods developed for the Finerenone. Different types of mobile phases and different ratios of the mobile phase were used in the determination. While mass chromatographic techniques provide advantages such as sensitivity, specificity, and shorter analysis times, they often entail significant costs and may not be accessible to many laboratories. Furthermore, there is currently no available HPLC-PDA method combined with protein precipitation extraction for detecting and quantifying Finerenone in rat plasma. Consequently, a cost-effective, dependable, and readily accessible HPLC-PDA method with a simple and economical protein precipitation extraction procedure was established and validated for the simultaneous detection and quantification of Finerenone in Wistar rat plasma. The developed method exhibits shorter retention times (7.3 minutes) using a mixture of acetonitrile and water (85:15 % v/v).

Method validation

Linearity:

The linear range extended from 25 to 500 ng/ml for finerenone encompassing seven data points. The regression coefficient (r^2) was found to be 0.9969 for finerenone.

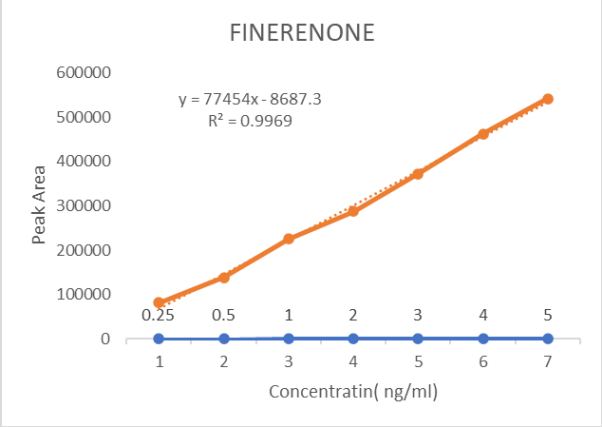

Figure 1: Structure of Finerenone

Accuracy and Recovery:

Accuracy evaluations involved spiking samples at three different levels: 50%, 100%, and 150%. The percentage recovery for fenerenone was found to be 98.33 to 99.4 % confirming the accuracy of the developed method. The results verified the reliability and effectiveness of the deproteination method in achieving satisfactory recovery outcomes for finerenone analysis (Table.1).

Table 1: Accuracy Studies of Finerenone

S. no	Name of drug	Spike Level (%)	Amount of drug added (ng/ml)	Amount of drug recovered (ng/ml)*	Percentage Recovery (%)	% RSD*
1	Finerenone	50	100	98	98.33%	0.2
2		100	200	196	99.00%	0.4
3		150	300	97	99.40%	0.3

*n=6 (Means of 6 values)

Sensitivity:

LOD and LOQ were assessed based on the standard curve data. The determined values for finerenone were 0.004 ng/ml (LOD) and 0.01 ng/ml (LOQ). These findings demonstrate the method's good sensitivity for accurately quantifying the analytes.

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Table 2. Limit of Detection and Limit of Quantification

S.no	Name of drug	LOD	LOQ
1	Finerenone	0.0004 ng/ml	0.001 ng/ml

Specificity:

The proposed method displayed specificity, with no endogenous interferences observed at the retention times of finerenone. Well-defined, symmetrical peaks were evident in the chromatogram, appearing at 7.3 minutes.

Precision:

According to the "ICH guidelines," precision was evaluated for both intra-day and inter-day analyses. Intra-day precision yielded % RSD values of 0.015% for finerenone, while inter-day precision resulted in %RSD values of 0.0021% for finerenone (Table 2). These %RSD results fell within the acceptable threshold of less than 2%, indicating precision in quantifying Finerenone in rat plasma.

Table 2: Precision Studies of Finerenone (Intra-day)

S.no	Name of drug	No. of injections	Conc. of drug added (ng/ml)	Peak area*	% RSD*
1	Finerenone	3	300	371710	0.015

*n=3 (Means of 3 values)

Table 3: Precision Studies of Finerenone (Inter-day)

S.no	Name of drug	Day	Conc. of drug added (ng/ml)	Peak area*	%RSD*
1	Finerenone	Day 1	300	371692	0.0022
2		Day 2	300	371702	0.0028
3		Day 3	300	371689	0.0021

Ruggedness:

The developed method was assessed by conducting the assay using two different analysts. The results presented in Table 1 indicated that the % RSD between the two analysts was ≤ 2.0% and system suitability parameters were shown in Table 5.

Table 4. Summary of validation parameters

S.No	Parameter	Finerenone
1	Range for Linearity (ng/ml)	25 – 500
2	Co efficient of determination (r^2)	0.9969
3	Accuracy (%)	98.33-99.40
4	Precision (%RSD)	0.0021-0.015
5	LOD (ng/ml)	0.004
6	LOQ (ng/ml)	0.01
7	Robustness (%RSD)	0.0018 - 0.0021

Table 5. System suitability parameters for finerenoe

S.No.	Parameters	Finerenone
1	Theoretical plate	9679.81
2	Tailing Factor	1.15
3	HETP	15.49
4	Peak purity index	0.9998
5	Retention time	7.083

Pharmacokinetic studies:

In this method, the validated HPLC technique was utilized to simultaneously determine finerenone levels in ratspost-oral administration of 10 mg/kg. Graphical depictions illustrating mean plasma concentration over 4 hours are shown in Figures 2, with pharmacokinetic parameters outlined in Tables 6. The maximum concentrations (C_{max}) of finerenone were found to be 75 ng/mL and the time observed for maximum drug concentration (T_{max}) being 75 mins. The half-lives ($T_{1/2}$) of finerenone were determined to be 2.58 h. Additionally, the area under the curve for 4 hours

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(AUC_{0-t}) and the elimination rate constant (K_e) were also evaluatedand shown in Table 3.

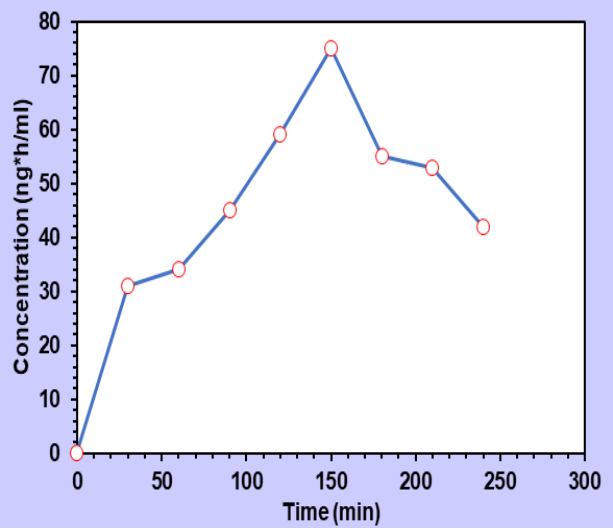

Figure 2: Plasma concentration of Finerenone (vs) time profile

Table 6: Pharmacokinetic Parameters of Finerenone in Rat Plasma

S.No	Pharmacokinetic Parameter	Values
1	AUC_{0-t} (ng. h/ml)	178.5
2	$AUC_{0-\infty}$ (ng. h/ml)	334.50
3	C_{max} (ng/ml)	75
4	T_{max} (h)	150 mins
5	$T_{1/2}$	2.58
6	K_e (h^{-1})	0.269

5. Discussion

The bio-analytical method was developed and validated for estimation of finerenone in rat plasma. The method was validated for all parameters such as linearity, precision, and ruggedness as per ICH M10 guidelines.

Therefore, a cost-effective, robust, and readily available HPLC-PDA method with simple and economical protein precipitation extraction was developed and validated for the estimation of Finerenone in wistar rat plasma. The developed method has a shorter retention time (7.3 mins) using water and acetonitrile (15:85 % v/v).This method was successfully employed in a pharmacokinetic investigation to assess plasma concentrations of Finerenone in wistar rats. The developed method holds potential for application in therapeutic drug monitoring, bioequivalence, bioavailability studies, preclinical and clinical studies and toxicology research involving Finerenone.

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