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Research Paper

Method Development, Validation, And Degradation Studies Of Fosdenopterin

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ABSTRACT

A simple, rapid, precise, sensitive and reproducible reverse phase high performance liquid chromatography (RP-HPLC) method has been developed for the quantitative analysis of Fosdenopterin in pharmaceutical dosage form. Chromatographic separation of Fosdenopterin was achieved on Waters Alliance-e2695, by using X-Bridge Phenyl (250x4.6mm, 5µ) column and the mobile phase containing 0.1% OPA & ACN in the ratio of 70:30% v/v. The flow rate was 1.0 ml/min; detection was carried out by absorption at 220nm using a photodiode array detector at ambient temperature. The number of theoretical plates and tailing factor for Fosdenopterin were NLT 2000 and should not more than 2 respectively. % Relative standard deviation of peak areas of all measurements always less than 2.0. The proposed method was validated according to ICH guidelines. The method was found to be simple, economical, suitable, precise, accurate & robust method for quantitative analysis of Fosdenopterin

INTRODUCTION

Studying anti-cancer medications in this laboratory over the last five years, the author has worked on developing new techniques to monitor their stability. An overview of pharmaceutical manufacturing in terms of quality maintenance, the requirement for stability-indicating test techniques, and validation will be covered thoroughly before the experimental procedures used and the findings obtained are described. Pemetrexed, erlotinib hydrochloride, nilotinib hydrochloride, and dasatinib hydrochloride are among the anticancer medications chosen for stability suggesting studies.

The quality of medications on the market has been the subject of significant scrutiny in recent years. Both the pharmaceutical and the bulk drug businesses face the problem of producing high-quality goods. Each industry's production must be subjected to stringent quality control tests if it is to remain of high quality and pure. Raw ingredients,

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manufacturing methods, and the kind of crystallisation and purifying procedure all have a role in determining the active medicinal ingredient's purity. Analytical chemistry progress is inextricably linked to modifications in the purity concept. The pharmacopoeias establish purity as well as impurity restrictions, some of which may be quite strict. Because they separate and measure components concurrently, modern separation technologies are obviously dominating in scientific research today. This makes it simpler to separate and characterise contaminants. Unwanted compounds that persist in pharmaceuticals or emerge during formulation or develop as a result of ageing of both the active pharmaceutical ingredients (APIs) and formed APIs to medications (1-4). Even at minute concentrations, these unwelcome substances may impair the effectiveness and safety of pharmaceuticals. Impurity levels in APIs and drug formulations are gradually being regulated by many pharmacopoeias, including the British Pharmacopoeia (BP) and the United States Pharmacopoeia (USP). Impurities in novel medicinal compounds, products, and residual solvents have been outlined by the International Conference on Harmonization (ICH) (5-7). One way to describe the contaminants in an API batch is to describe the recognised and undiscovered impurities present in that batch

2. MATERIALS AND METHODS

a) Equipment:

Table No.1: List of Apparatus used in HPLC

S.No	Name	Model	Manufacturer
1.	HPLC	ALLIANCE	Waters e 2695- Empower software2.0versions
2.	pH meter	-	Eutech
3.	Weighing balance	-	Sartouris
4.	UV/VIS spectrophotometer	-	UV-1700
5.	Pipettes, beakers and Burettes	-	Borosil
6.	Ultra sonicator	UCA 701	Unichrome
7.	Pump	Isocratic model	--

(b) Reagents & Chemicals

Table No.2: List of chemicals used in HPLC Method

S.No	Name	Grade	Manufacturer
1.	Acetonitrile	HPLC	Rankem
2.	Water (Milli Q)	HPLC	In house production
3.	Formic acid	HPLC	Analytical reagents
4.	Ortho phosphoric acid	HPLC	Rankem

Determination of Working Wavelength (λ_{max}): In estimation of the drug isobestic wavelength was used. Isobestic point is the wavelength where the

molar absorptivity is the same for the substances that are inter convertible. So this wavelength was used in estimation of drug accurately.

The wavelength of maximum absorption of the solution of the drug in mixture of Acetonitrile and 0.1% OPA (30:70) were scanned using PDA Detector within the wavelength region of 200–400 nm against Acetonitrile and 0.1% OPA (30:70) as blank. The absorption curve shows isobestic point at 220 nm. Thus 220 nm was selected as detector wavelength for the HPLC chromatographic method.

Chromatographic conditions:

During the selection of chromatographic conditions, numbers of trails were carried out and the best trail was selected for optimized method.

Preparation of standard solution

Accurately weigh and transfer 50 mg of Fosdenopterin working standard into a 100 ml clean dry volumetric flask add diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 5 ml of the above stock solutions into a 50 ml volumetric flask and dilute up to the mark with diluent. (50ppm of Fosdenopterin)

Preparation of Sample solution

Accurately weigh and transfer 50 mg of Fosdenopterin sample into a 100 ml clean dry volumetric flask add diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 5 ml of the above stock solutions into a 50 ml volumetric flask and dilute up to the mark with diluent. (50ppm of Fosdenopterin)

Trails in optimization of chromatographic condition

Table No.3: TRIAL-1 Chromatographic conditions

Column	Luna C18 250x4.6 mm, 5µ
Mobile phase ratio	ACN+ 0.1% formic acid (70+30)
Detection wavelength	200-400nm
Flow rate	1ml/min
Injection volume	10µl
Run time	10min
Observation	Splitting of the peak is observed

Table No.4: TRIAL-2 Chromatographic conditions

Column	Luna C18 250x4.6 mm, 5µ
Mobile phase ratio	ACN+ 0.1% formic acid (65+35)
Detection wavelength	220 nm
Flow rate	1ml/min
Injection volume	10µl
Run time	11min
Observation	Peak height is not within the limit

Table No.5: TRIAL-3 Chromatographic conditions

Column	X-Bridge Phenyl (250x4.6mm, 5µ)
Mobile phase ratio	ACN + formic acid (60+40)
Detection wavelength	220 nm
Flow rate	1ml/min
Injection volume	10µl
Run time	10min
Observation	Base line is not sufficient

Table No.6: TRIAL-4 Chromatographic conditions

Column	X-Bridge Phenyl (250x4.6mm, 5µ)
Mobile phase ratio	ACN + 0.1% OPA (20+80)
Detection wavelength	220 nm
Flow rate	1ml/min
Injection volume	10µl
Run time	4min
Observation	Broad peak is observed

Table No.7: TRIAL-5 Chromatographic conditions

Column	X-Bridge Phenyl (250x4.6mm, 5µ)
Mobile phase ratio	Acetonitrile: 0.1% OPA (25:75)
Detection wavelength	220 nm
Flow rate	1ml/min
Injection volume	10µl
Run time	8min
Observation	Extra peak is formed

Table No.8: TRIAL-6 Chromatographic conditions (Optimized)

Column	X-Bridge Phenyl (250x4.6mm, 5µ)
Mobile phase ratio	Acetonitrile: 0.1% OPA (30:70)
Detection wavelength	220nm
Flow rate	1ml/min
Injection volume	10µl
Run time	6min
Observation	This method is suitable for validation

The Fosdenopterin peak was observed at 4.358 min with peak area 3239455, tailing factor 1.05. This trial was optimized.

General preparations

Preparation of Mobile Phase: Mobile phase was prepared by mixing 0.1% OPA and ACN taken in the ratio 70:30. It was filtered through 0.45µ membrane filter to remove the impurities which may interfere in the final chromatogram.

Chromatographic condition:

Use suitable High Performance Liquid Chromatographic equipped with PDA detector.

Column : X-Bridge Phenyl (250x4.6mm, 5µ)

Mobile phase ratio : Acetonitrile and 0.1% OPA (30:70)
Detection wavelength : 220 nm
Flow rate : 1ml/min
Injection volume : 10µl
Run time : 6min

Preparation of Diluent: Mobile phase was used as a diluent.

Preparation of standard solution

Accurately weigh and transfer 50 mg of Fosdenopterin working standard into a 100 ml clean dry volumetric flask add diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)Further pipette 5 ml of the above stock solutions into a 50 ml volumetric flask and dilute up to the mark with diluent. (50ppm of Fosdenopterin)

Preparation of Sample solution

Accurately weigh and transfer 50 mg of Fosdenopterin sample into a 100 ml clean dry volumetric flask add diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)Further pipette 5 ml of the above stock solutions into a 50 ml volumetric flask and dilute up to the mark with diluent. (50ppm of Fosdenopterin)

Procedure:

Inject 10 µL of the standard, sample into the chromatographic system and measure the areas for Fosdenopterin peak and calculate the %Assay by using the formulae.

SYSTEM SUITABILITY:

Tailing factor for the peak due to Fosdenopterin in Standard solution should not be more than 2.0 Theoretical plates for the Fosdenopterin peak in Standard solution should not be less than 2000.

Formula for Assay:

$$\%\ Assay = \frac{AT}{AS} * \frac{WS}{DS} * \frac{DT}{WT} * \frac{Average\ weight}{Label\ Claim} * \frac{P}{100} * 100$$

Where: AT = average area counts of test (sample) preparation.
AS = average area counts of standard preparation.
WS = Weight of working standard taken in mg.
DS = Dilution of working standard in ml.
DT = Dilution of test (sample) in ml.
WT = Weight of test (sample) taken in mg.
P = Percentage purity of working standard
LC = Label Claim mg/ml.

METHOD VALIDATION SUMMARY:

Specificity:

Specificity of an analytical method is ability to measure specifically the analyte of interest without interference from blank and known impurities. For this purpose blank chromatogram, standard chromatogram and sample chromatogram were recorded. The chromatogram of blank shows no response at the retention times of drugs which confirms the response of drug was specific**.**

LINEARITY:

Preparation of stock solution:

Accurately weigh and transfer 50mg of Fosdenopterin working standard into a 100 ml clean dry volumetric flask add diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Preparation of Level – I (12.5ppm of Fosdenopterin):

1.25 ml of above stock solutions has taken in different 50 ml of volumetric flasks, dilute up to the mark with diluent.

Preparation of Level – II (25ppm of Fosdenopterin):
2.5 ml of above stock solutions has taken in different 50 ml of volumetric flasks, dilute up to the mark with diluent.

Preparation of Level – III (37.5ppm of Fosdenopterin):
3.75 ml of above stock solutions has taken in different 50 ml of volumetric flasks, dilute up to the mark with diluent.

Preparation of Level – IV (50ppm of Fosdenopterin):
5 ml of above stock solutions has taken in different 50 ml of volumetric flasks, dilute up to the mark with diluent.

Preparation of Level –V (62.5ppm of Fosdenopterin):
6.25 ml of above stock solutions has taken in different 50 ml of volumetric flasks, dilute up to the mark with diluents.

Preparation of Level – VI (75ppm of Fosdenopterin)
7.5 ml of above stock solutions has taken in different 50 ml of volumetric flasks, dilute up to the mark with diluent**.**

Procedure:
Inject each level into the chromatographic system and measure the peak area.
Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

Range:
The Range of an analytical method is the interval between the upper and lower levels of analyte (including these levels) that have been demonstrated with precision, accuracy and linearity

Acceptance Criteria:
Correlation coefficient should be not less than 0.999.

Preparation Accuracy Sample solutions:

For preparation of 50% solution (With respect to target Assay concentration):
Accurately weigh and transfer 25mg of Fosdenopterin sample into a 100 ml clean dry volumetric flask add diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
Further pipette 5 ml of the above stock solutions into a 50ml volumetric flask and dilute up to the mark with diluent. (25ppm of Fosdenopterin)

For preparation of 100% solution (With respect to target assay concentration):
Accurately weigh and transfer 50 mg of Fosdenopterin sample into a 100 ml clean dry volumetric flask add diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
Further pipette 5ml of the above stock solutions into a 50ml volumetric flask and dilute up to the mark with diluent. (50ppm of Fosdenopterin)

For preparation of 150% solution (With respect to target assay concentration):
Accurately weigh and transfer 75mg of Fosdenopterin sample into a 100 ml clean dry volumetric flask add diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
Further pipette 5 ml of the above stock solutions into a 50ml volumetric flask and dilute up to the mark with diluent. (75ppm of Fosdenopterin)

Procedure:
Inject the standard solution, Accuracy -50%, Accuracy -100% and Accuracy -150% solutions.

Acceptance Criteria:

The % Recovery for each level should be between 98.0 to 102.0%

Precision

Precision is the degree of repeatability of an analytical method under normal operation conditions. Precision is of 3 types

1. System precision
2. Method precision
3. Intermediate precision (a. Intra-day precision, b. Inter-day precision)

System precision is checked by using standard chemical substance to ensure that analytical system is working properly. In this peak area and % of drug of six determinations measured and % RSD should be calculated.

In method precision, a homogenous sample of single batch should be analyzed 6 times. This indicates whether a method is giving constant results for a single batch. In this analyze the sample six times and calculate the % RSD.

The precision of the instrument was checked by repeatedly injecting (n=6) solutions of 50ppm of Fosdenopterin).

Acceptance Criteria:

The % RSD for the absorbance of six replicate injections results should not be more than 2%.

ROBUSTNESS:

As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method.

A. The flow rate was varied at 0.8 ml/min to 1.2ml/min.

Standard solution 50ppm of Fosdenopterin was prepared and analysed using the varied flow rates along with method flow rate. On evaluation of the above results, it can be concluded that the variation in flow rate affected the method significantly. Hence it indicates that the method is robust even by change in the flow rate ±20%.

B. The variation of Organic Phase ratio.

Standard solution of 50ppm of Fosdenopterin was prepared and analysed using the varied in mobile phase ratio.

Limit of detection (LOD) and limit of quantification (LOQ):

The limit of detection (LOD) limit of quantification (LOQ) of the drug carry was calculated using the following equation as per international conference harmonization (ICH) guidelines.

$LOD = 3.3 \times \sigma / S$

$LOQ = 10 \times \sigma / S$

LOD for Fosdenopterin was found to be 1.5µg/mL and LOQ for Fosdenopterin was found to be 5µg/ml.

DEGRADATION STUDIES:

Preparation of stock:

Accurately weigh and transfer 50mg of Fosdenopterin working standard into a 100 ml clean dry volumetric flask add diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Acid degradation:

Pipette 5 ml of above solution into a 50ml volumetric flask and 3 ml of 1N HCl was added. Then, the volumetric flask was kept at 60ºC for 6 hours and then neutralized with 1 N NaOH and make up to 50ml with diluent. Filter the solution with 0.22 microns syringe filters and place in vials.

Alkali degradation:

Pipette 5 ml of above solution into a 50ml volumetric flask and add 3ml of 1N NaOH was added. Then, the volumetric flask was kept at 60ºC for 6 hours and then neutralized with 1N HCl and

make up to 50ml with diluent. Filter the solution with 0.22 microns syringe filters and place in vials.

Peroxide degradation

Pipette 5 ml above stock solution into a 50ml volumetric flask, 1 ml of 3% w/v of hydrogen peroxide added in 50 ml of volumetric flask and the volume was made up to the mark with diluent. The volumetric flask was then kept at room temperature for 15 min. Filter the solution with 0.45 microns syringe filters and place in vials.

Reduction degradation

Pipette 5ml of Stock solution transferred into 50ml volumetric flask to this add 1ml of 10% Sodium Bisulphate and kept on bench top for 10min then the remaining procedure is same as the test preparation.

Hydrolysis degradation

Pipette 5ml above stock solution into a 50ml volumetric flask, 1 ml of water added in 50 ml of volumetric flask and the volume was made up to the mark with diluent. The volumetric flask was then kept at room temperature for 15 min. Filter the solution with 0.45 microns syringe filters and place in vials.

3. RESULTS AND DISCUSSION

3.1 RP-HPLC METHOD

Determination of Working Wavelength (λ_{max}):

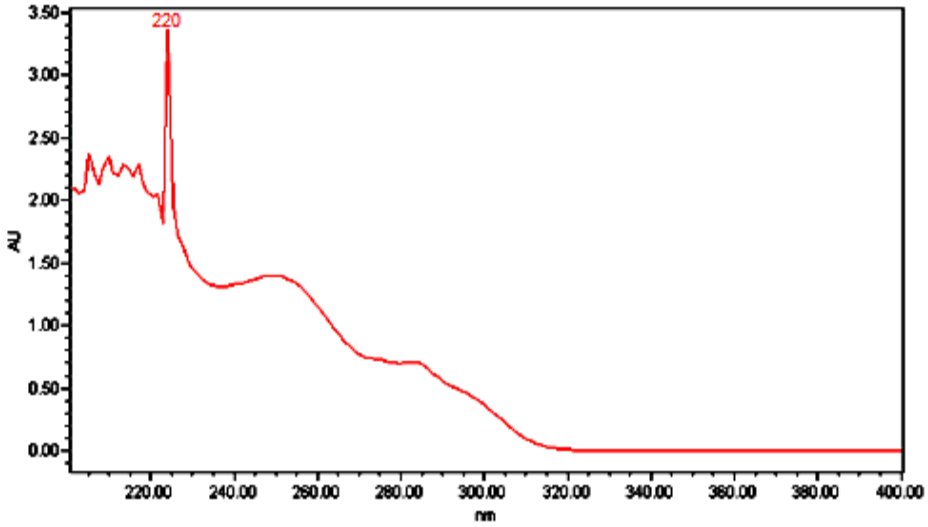

Fig No.:1PDA - Spectrum of Fosdenopterin

3.2. Optimization of chromatographic conditions

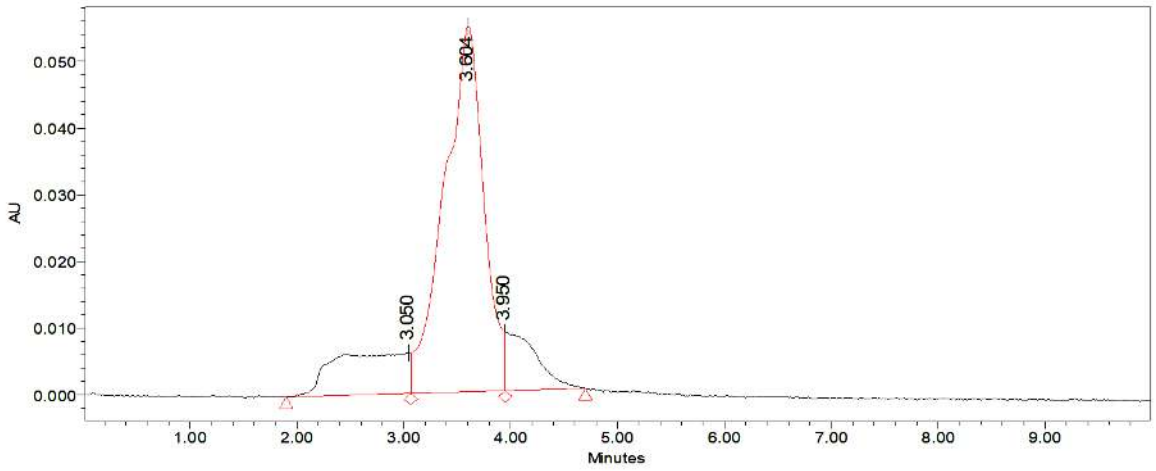

Fig No-2: chromatogram of Trial-1

Retention Time	Area	% Area	USP Resolution	USP Tailing	USP Plate Count
3.050	304177	16.11		2.65	215
3.604	1408290	74.57	0.34	1.87	462
3.950	176137	9.33	0.42	2.24	964

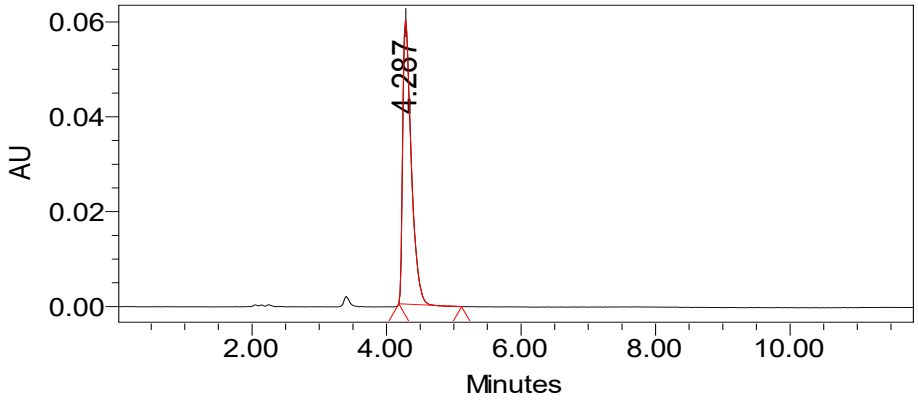

Fig No-3: chromatogram of Trial-2

Retention Time	Area	% Area	USP Tailing	USP Plate Count
4.287	192149	100	1.14	2541

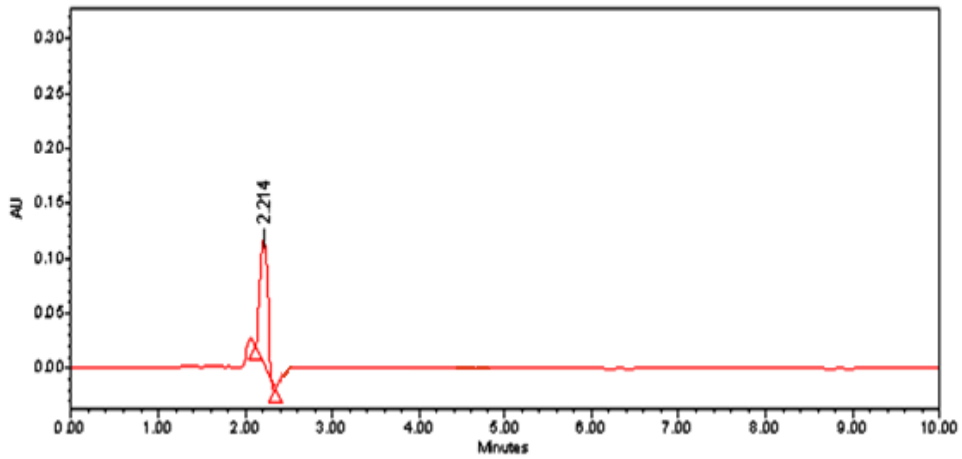

Fig No-4: chromatogram of Trial-3

Retention Time	Area	% Area	USP Resolution	USP Tailing	USP Plate Count
2.214	224981	100.0		2.05	2013

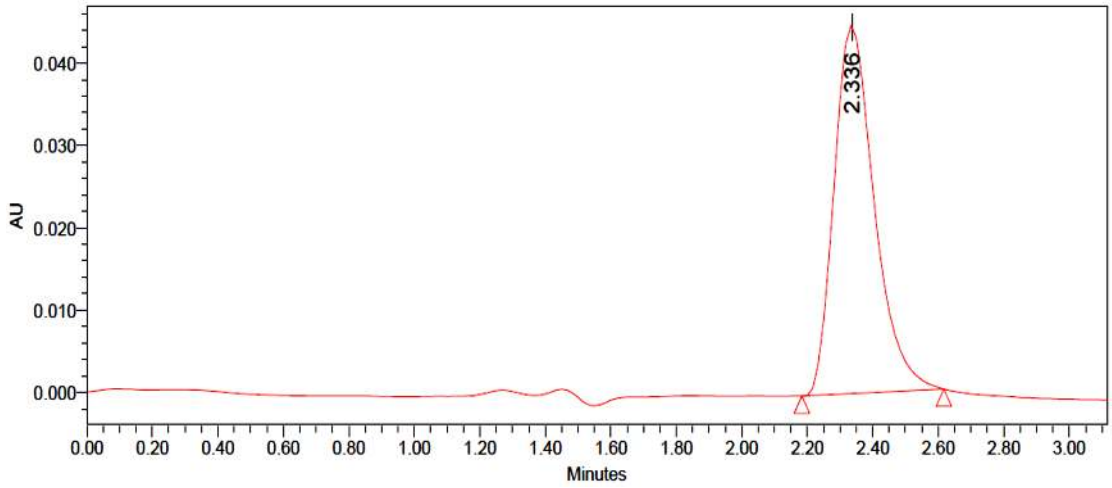

Fig No-5: chromatogram of Trial-4

	Name	Retention Time	Area	USP Resolution	USP Tailing	USP Plate Count
1		2.336	382244		1.32	2771

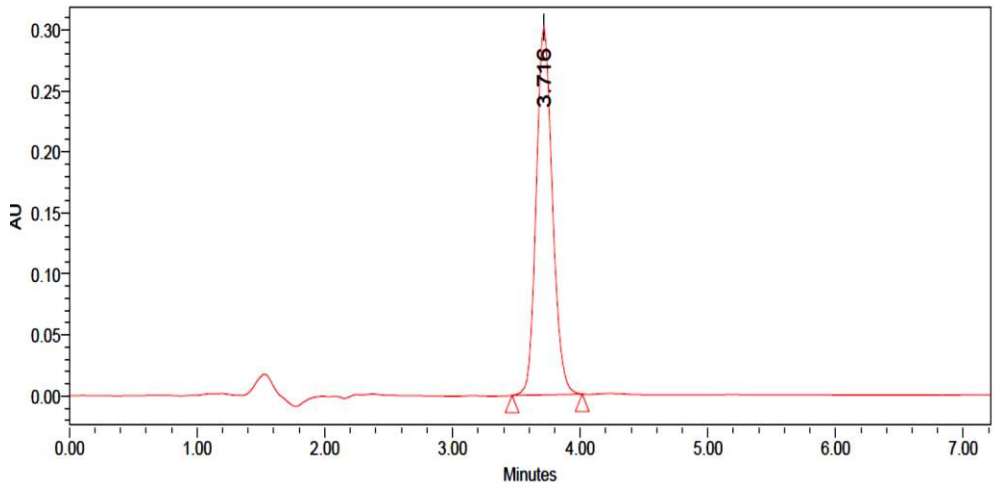

Fig No-6: chromatogram of Trial-5

Retention Time	Area	% Area	USP Resolution	USP Tailing	USP Plate Count
3.716	2655952	100.12		1.44	4148

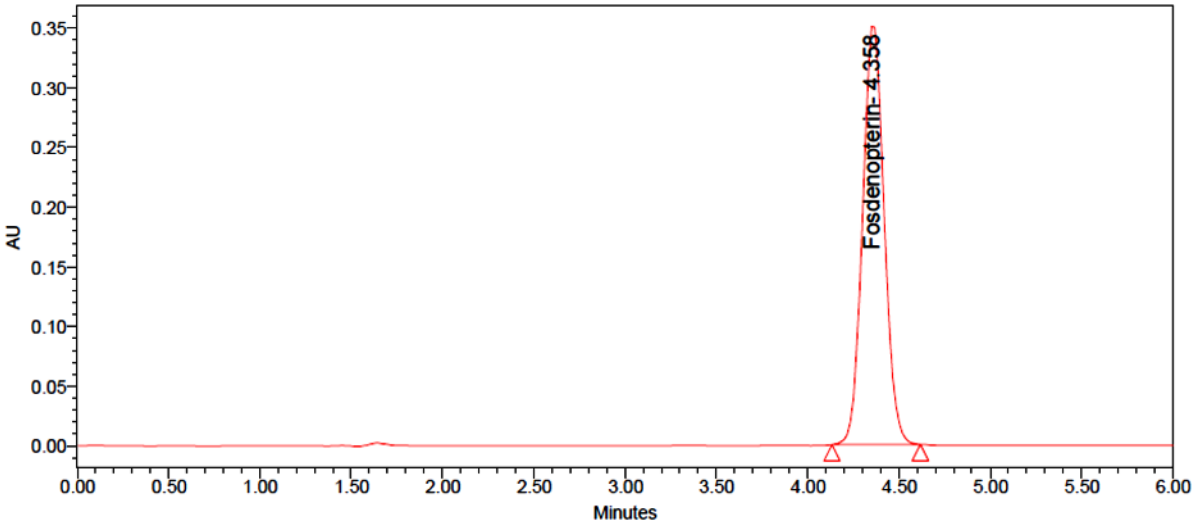

Fig No-7: chromatogram of Trial-6

S.No	Name	RT	Area	% Area	USP Resolution	USP Tailing	USP Plate Count
1	Fosdenopterin	4.358	3239455	100.00		1.05	6527

Table 11: Optimized chromatographic conditions

PARAMETERS	OBSERVATION
Instrument used	Waters HPLC with auto sampler and UV detector.
Injection volume	10µl
Mobile Phase	Acetonitrile and 0.1% OPA (30:70)
Column	X-Bridge Phenyl (250x4.6mm, 5µ)
Detection Wave Length	220nm
Flow Rate	1 mL/min

Runtime	6min
Temperature	Ambient(25° C)
Mode of separation	Isocratic mode

Specificity:

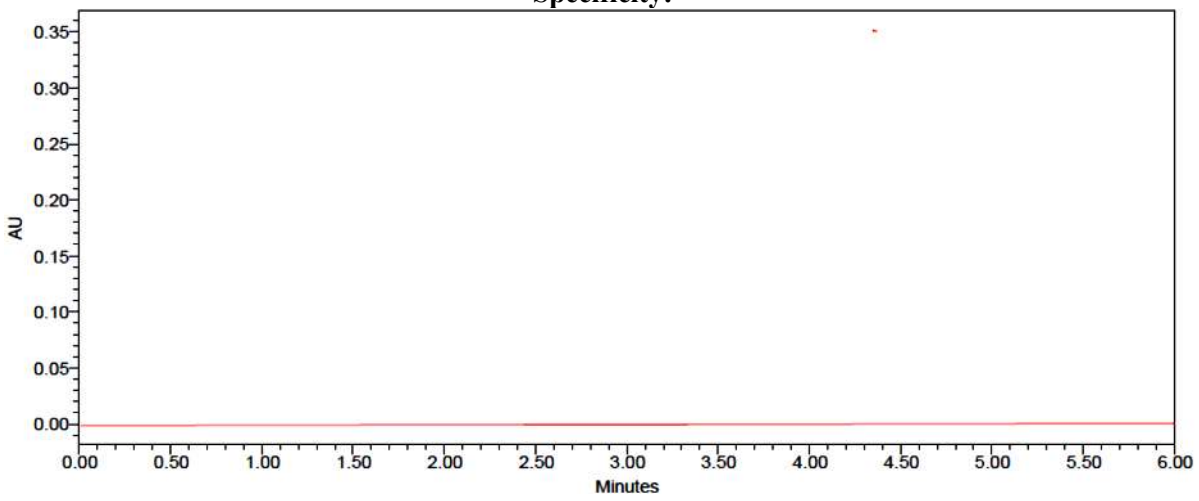

Fig No.10: Chromatogram of blank

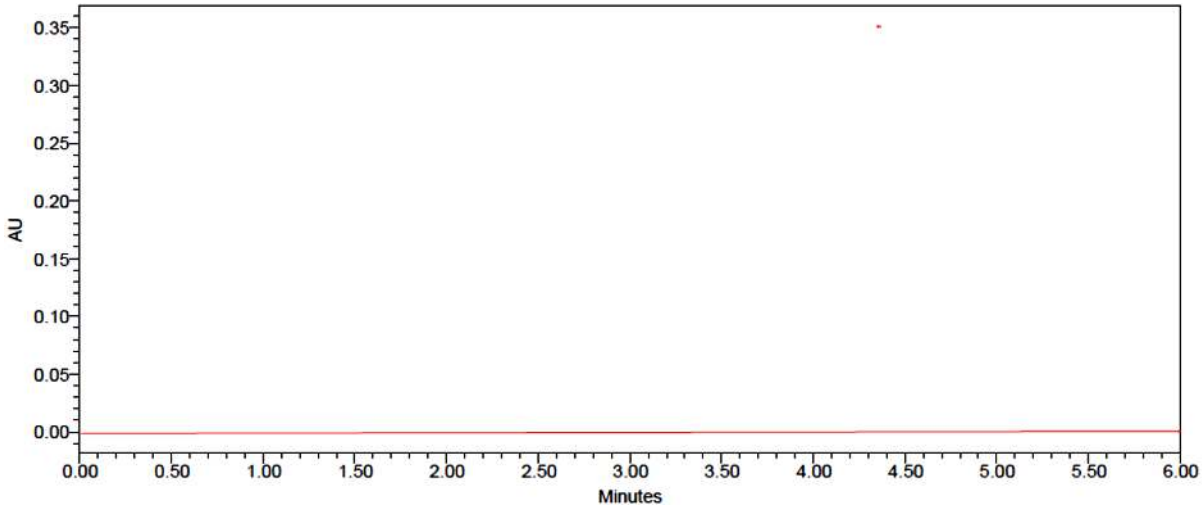

Fig No.11: Chromatogram of placebo.

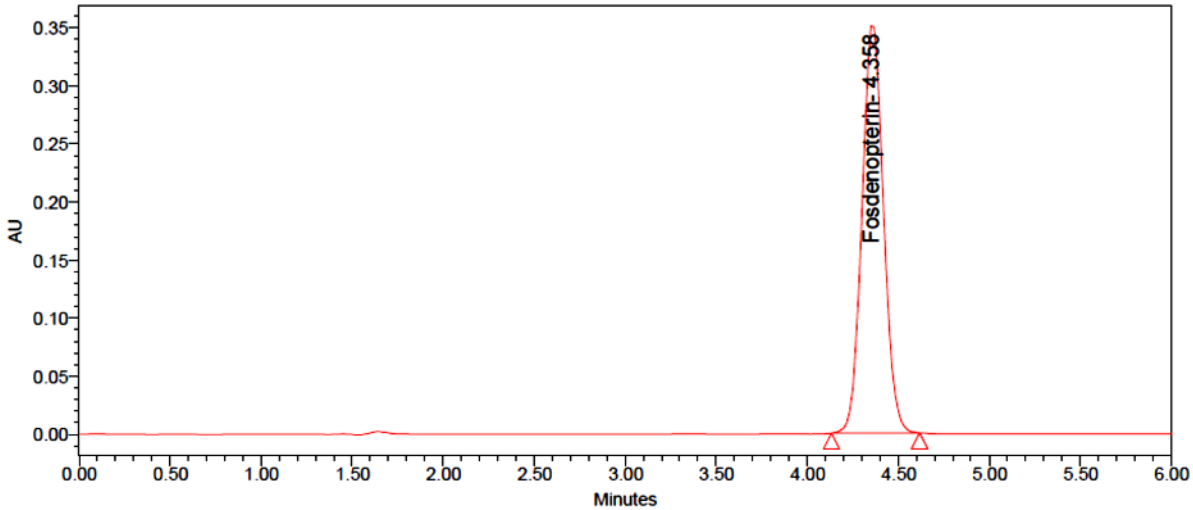

Fig No.12: Chromatogram of standard

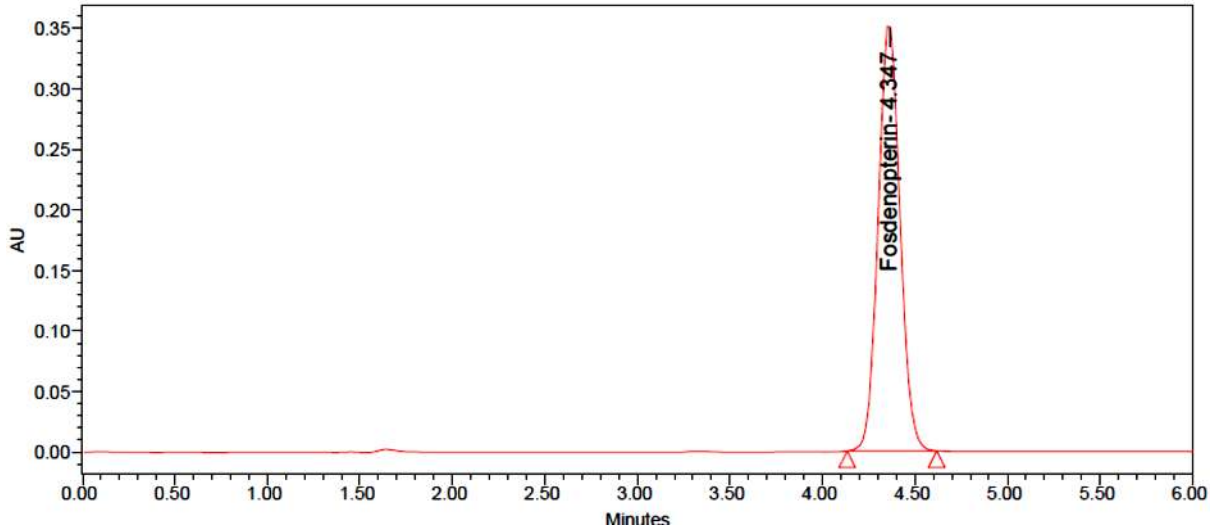

Fig No.13: Chromatogram of sample

ANALYTICAL METHOD VALIDATION (HPLC)

The method was validated for its linearity range, accuracy, precision, and specificity. Mevalidation was carried out as per ICH guidelines.

Linearity:

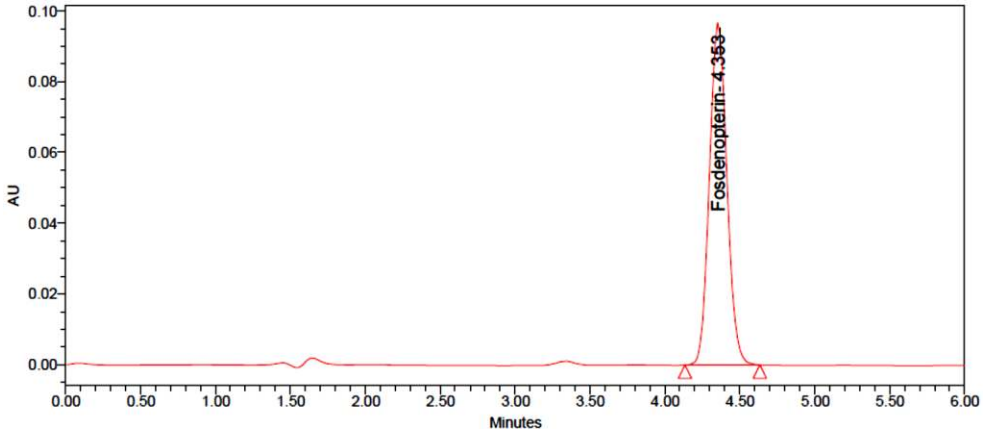

Fig No.14: Chromatogram of Linearity-1

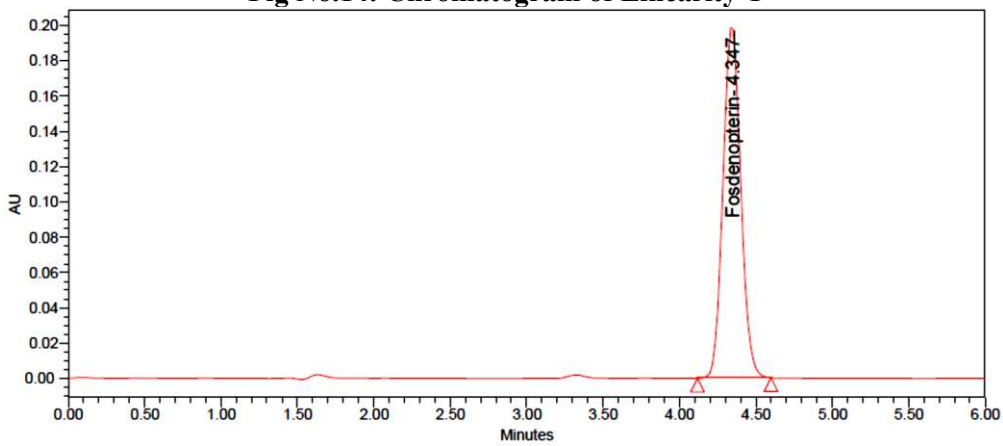

Fig No.15: Chromatogram of Linearity-2

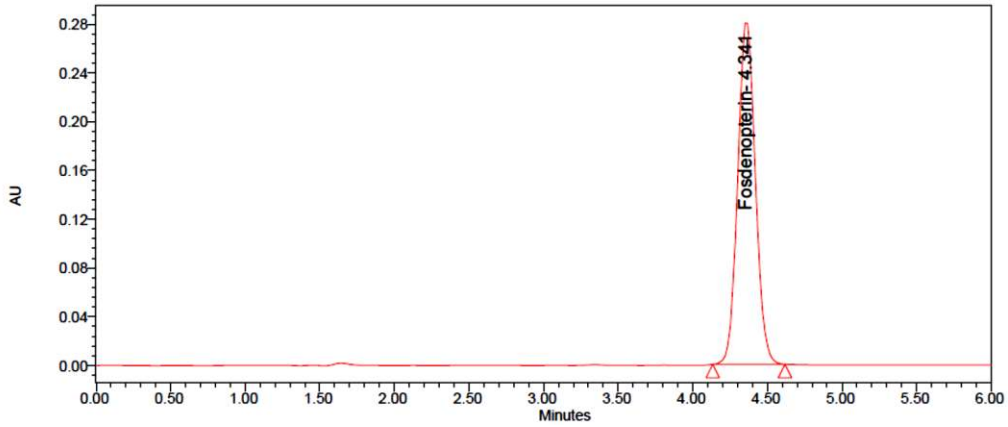

Fig No.16: Chromatogram of Linearity-3

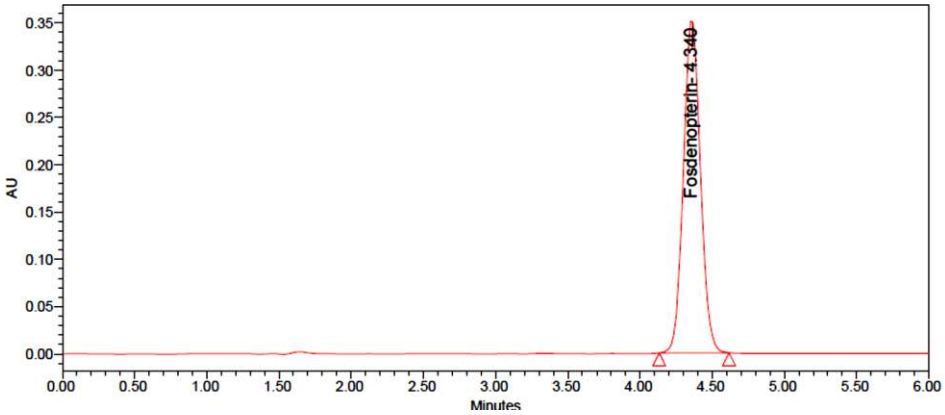

Fig No.17: Chromatogram of Linearity-4

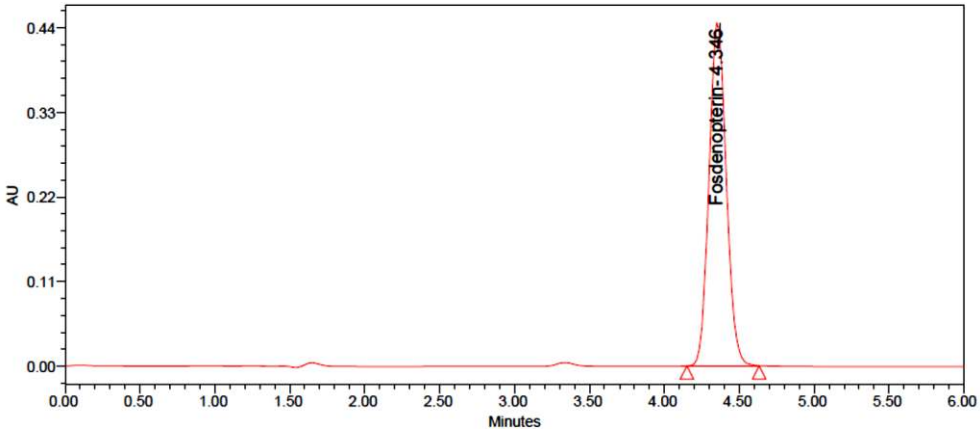

Fig No.18: Chromatogram of Linearity-5

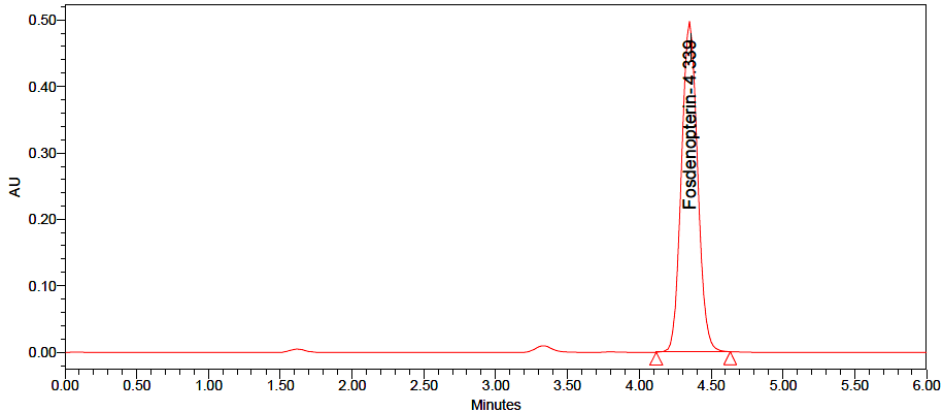

Fig No19: Chromatogram of Linearity-6

Table No.12: Results of linearity for Fosdenopterin

S.NO	Fosdenopterin	
	Conc.(µg/ml)	Peak area
1	12.5	843514
2	25	1606209
3	37.5	2486332
4	50	3234505
5	62.5	4088517
6	75	4855617
Regression equation	y = 64814.72x +14404.18	
Slope	64814.72	
Intercept	14404.18	
R^2	0.9998	

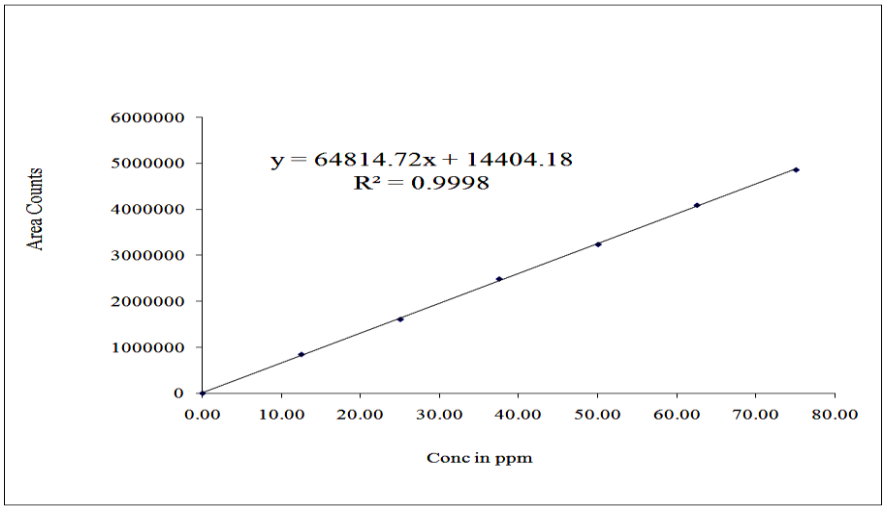

Fig No.20: Calibration curve for Fosdenopterin at 220 nm

Accuracy:

Table No.13: Accuracy results of Fosdenopterin by RP-HPLC method

%Concentration(at specification Level)	Area	Amount of API Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	1645742	25	25.19	100.8	100.3
100%	3299312	50	50.51	101.0	
150%	4881148	75	74.72	99.6	

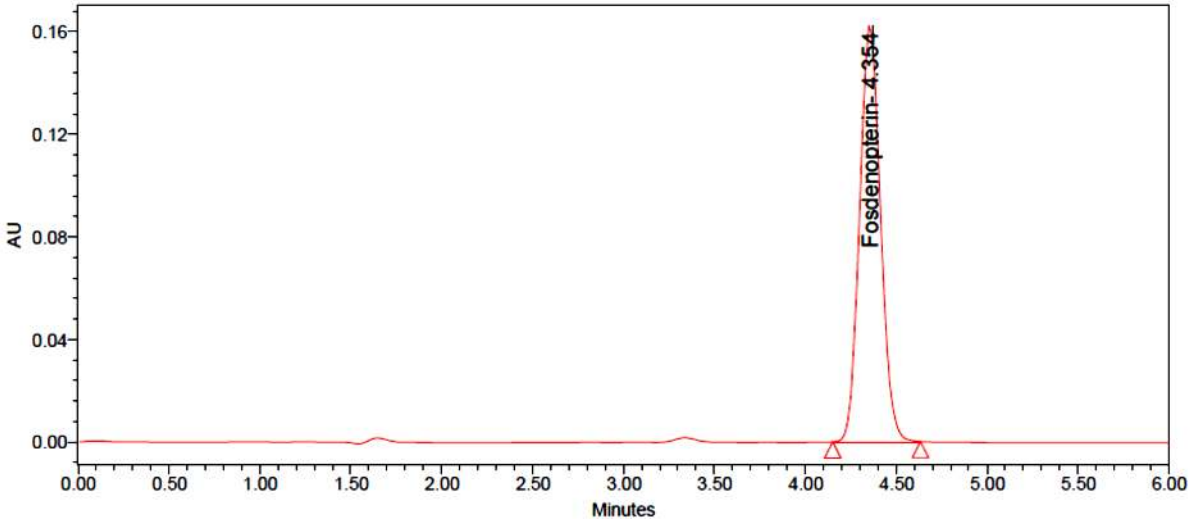

Fig No.21: Chromatogram for Accuracy 50%

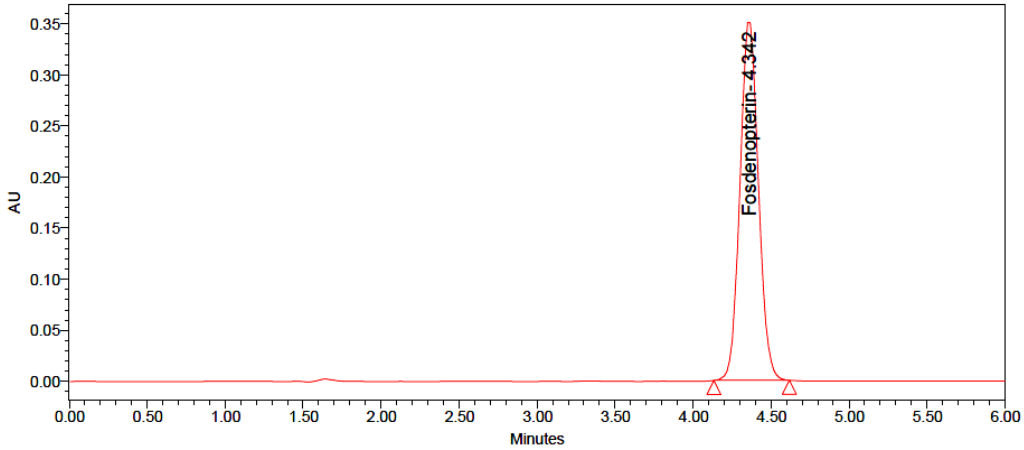

Fig No.22: Chromatogram for Accuracy 100%

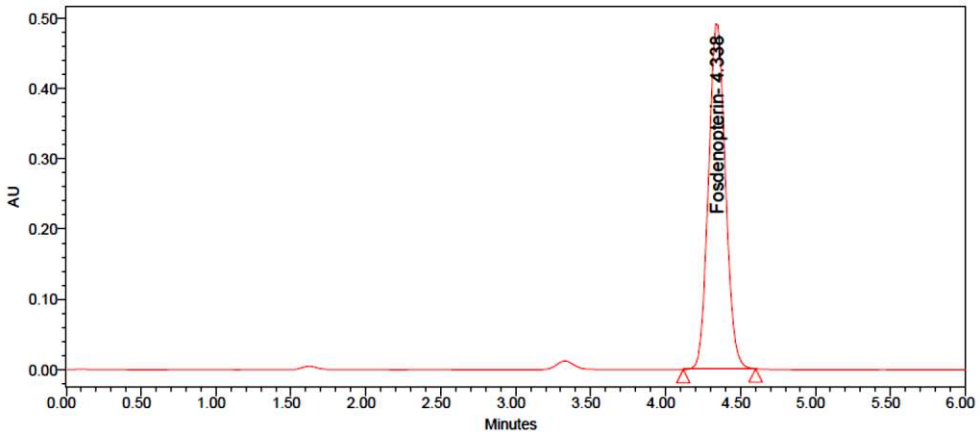

Fig No.23: Chromatogram for Accuracy 150%

Precision:

Table No.14: Standard results for Fosdenopterin by RP-HPLC method

Injection	Area for Fosdenopterin
Injection-1	3239455
Injection-2	3261501
Injection-3	3254080
Injection-4	3280398
Injection-5	3281624
Injection-6	3279900
Average	3266160
Standard Deviation	17386.39
%RSD	0.53

Table No.15: Method Precision for Fosdenopterin by RP-HPLC method

Parameter	Area for Fosdenopterin
Method precision-1	3250839
Method precision -2	3242476
Method precision -3	3284127
Method precision-4	3257333
Method precision -5	3266629
Method precision -6	3252396
Average	3258966
Standard Deviation	14666.951
%RSD	0.45

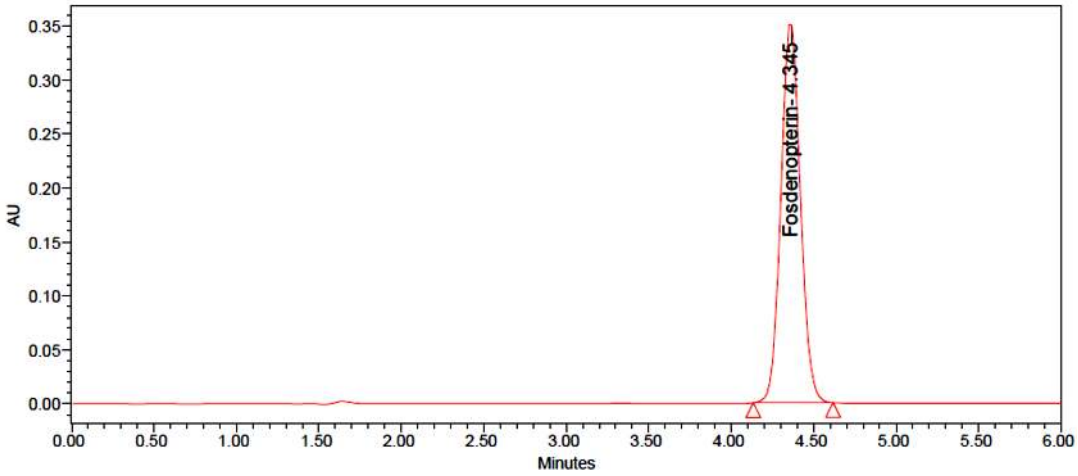

Fig No.24: Chromatogram of Method Precision

Acceptance Criteria: The % RSD for the area of six standard injections results should not be more than 2%**.**

Table No.16: Intermediate Precision for Fosdenopterin by RP-HPLC method

Parameter	Area for Fosdenopterin
Intermediate precision-1	3222841
Intermediate precision -2	3260823
Intermediate precision -3	3299252
Intermediate precision -4	3246210
Intermediate precision -5	3263629
Intermediate precision -6	3270798
Average	3260592
Standard Deviation	25455.902
%RSD	0.78

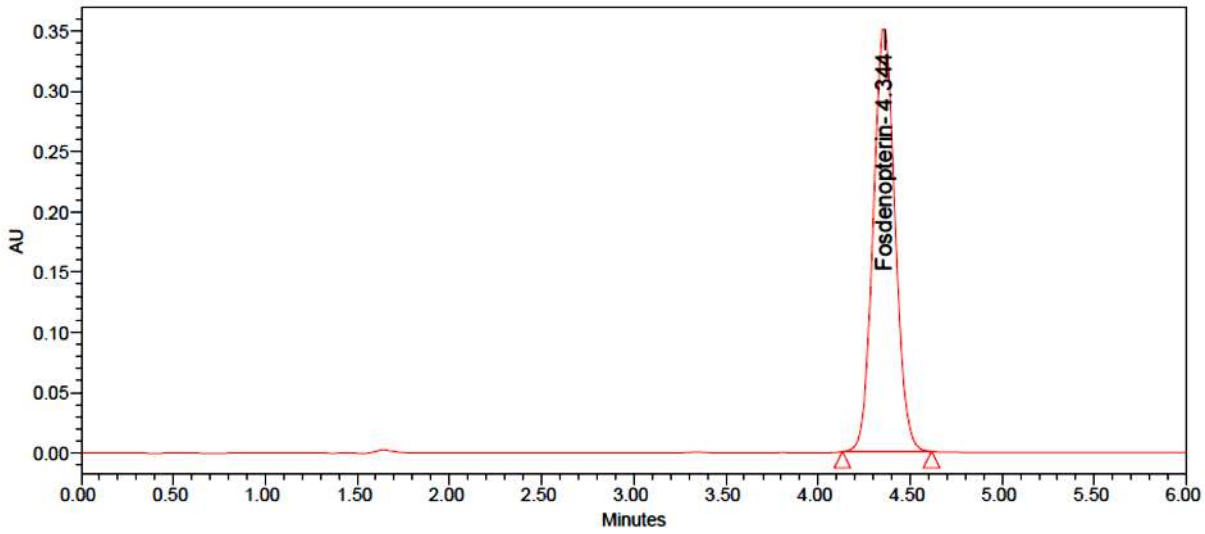

Fig No.25: Chromatogram of Intermediate Precision

Acceptance Criteria: The % RSD for the area of six standard injections results should not be more than 2%.

Robustness:

Table No.17: Robustness results of Fosdenopterin by RP-HPLC

Parameter	Fosdenopterin					
	Condition	Retention time(min)	Peak area	Resolution	Tailing	Plate count
Flow rate Change (mL/min)	Less flow(0.8ml)	5.383	3458897		1.08	6649
	Actual(1ml)	4.358	3239455		1.05	6527
	More flow(1.2ml)	3.623	3087620		1.02	6491
Organic Phase change	Less Org (27:73)	4.939	3500196		1.09	6603
	Actual(30:70)	4.355	3261501		1.07	6530
	More Org (33:67)	3.860	2842723		1.00	6512

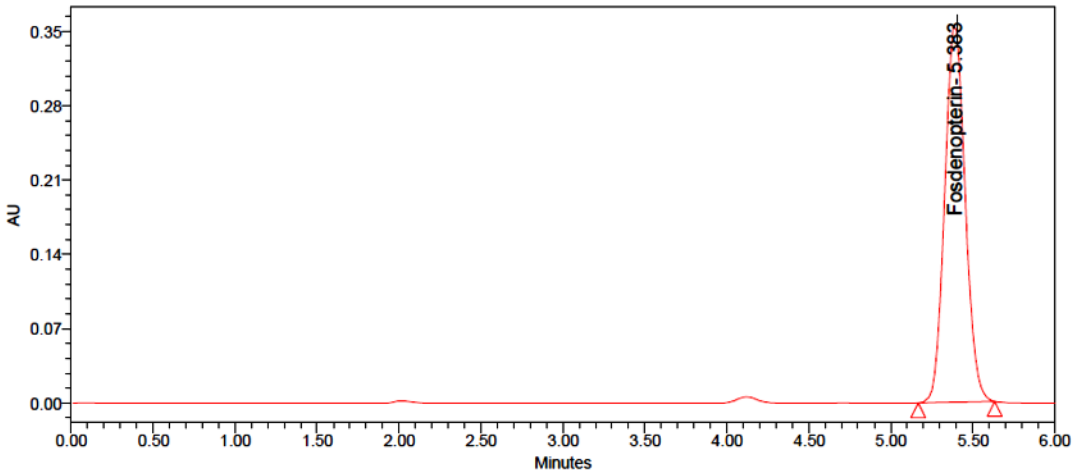

Fig No.26: chromatogram for less flow rate (0.8 ml)

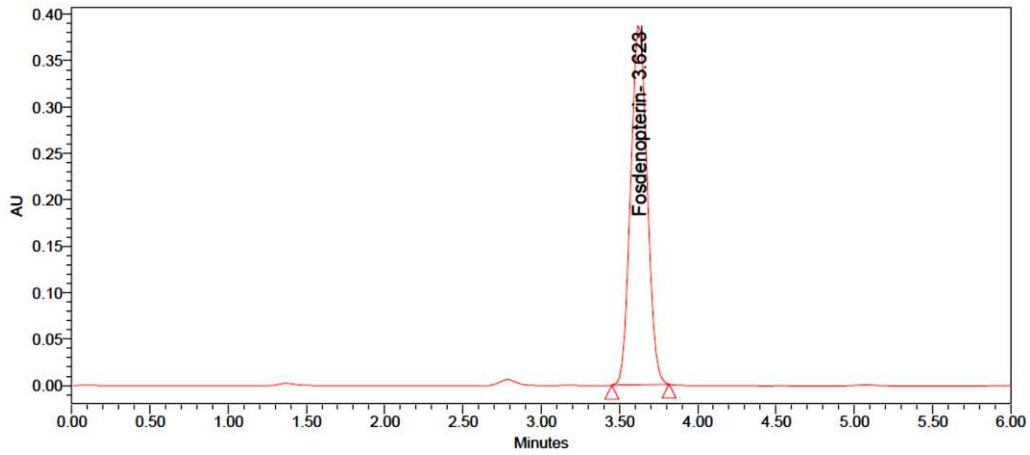

Fig No.27: chromatogram for more flow rate (1.2mL)

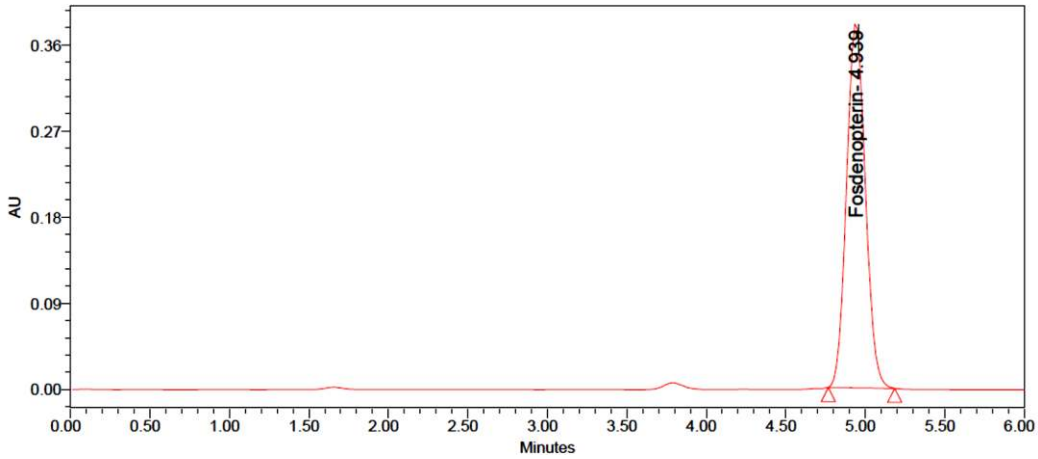

Fig No.28: chromatogram for less Organic Phase (27:73)

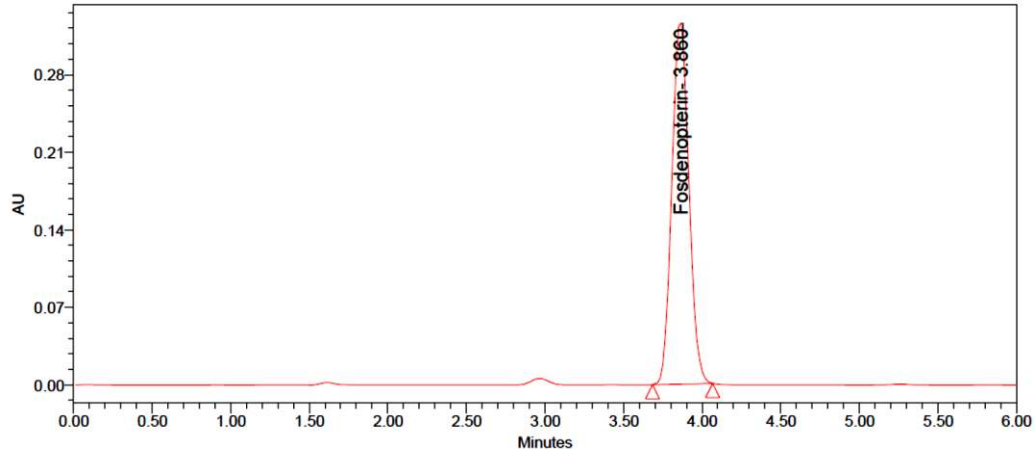

Fig No.29: chromatogram for more Organic Phase (33:67)

LOD and LOQ:

Table No.18: Sensitivity parameters (LOD & LOQ) by RP-HPLC

Name of drug	LOD (µg/ml)	LOQ (µg/ml)
Fosdenopterin	1.5	5

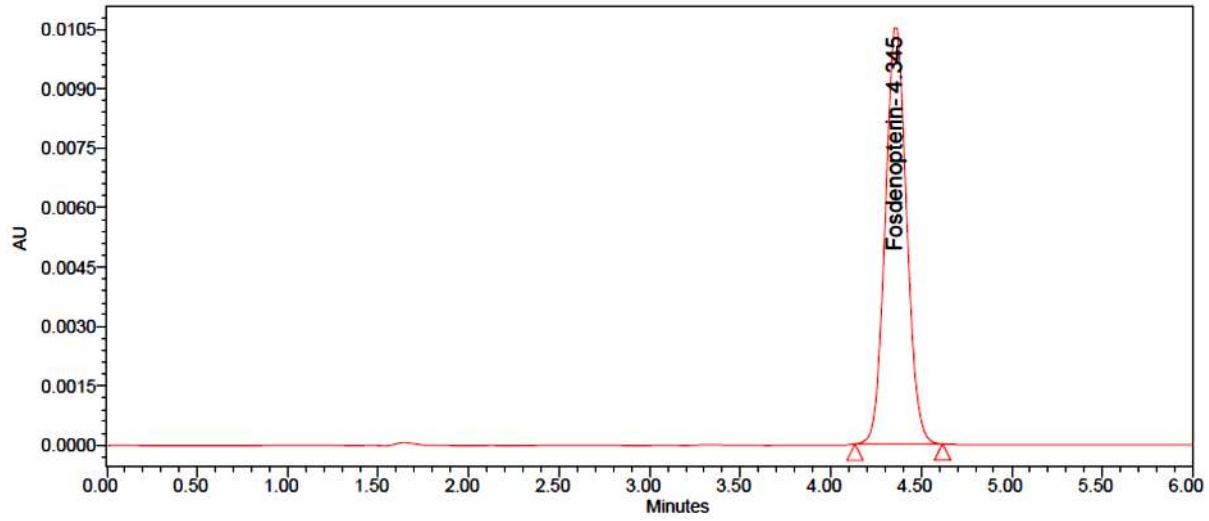

Fig No.30: chromatogram of LOD

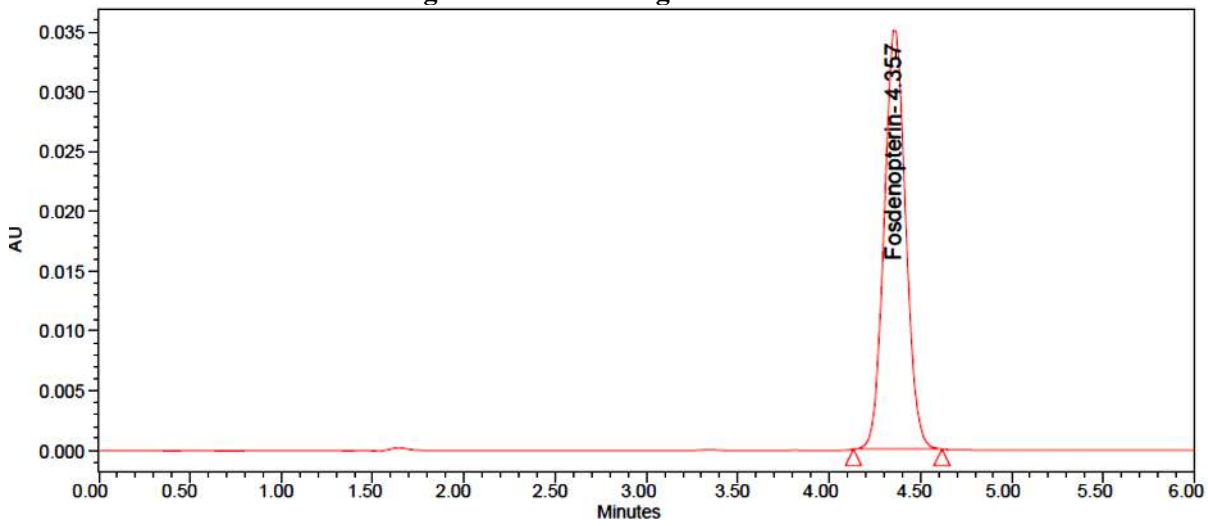

Fig No.31: chromatogram of LOQ

DEGRADATION STUDIES:

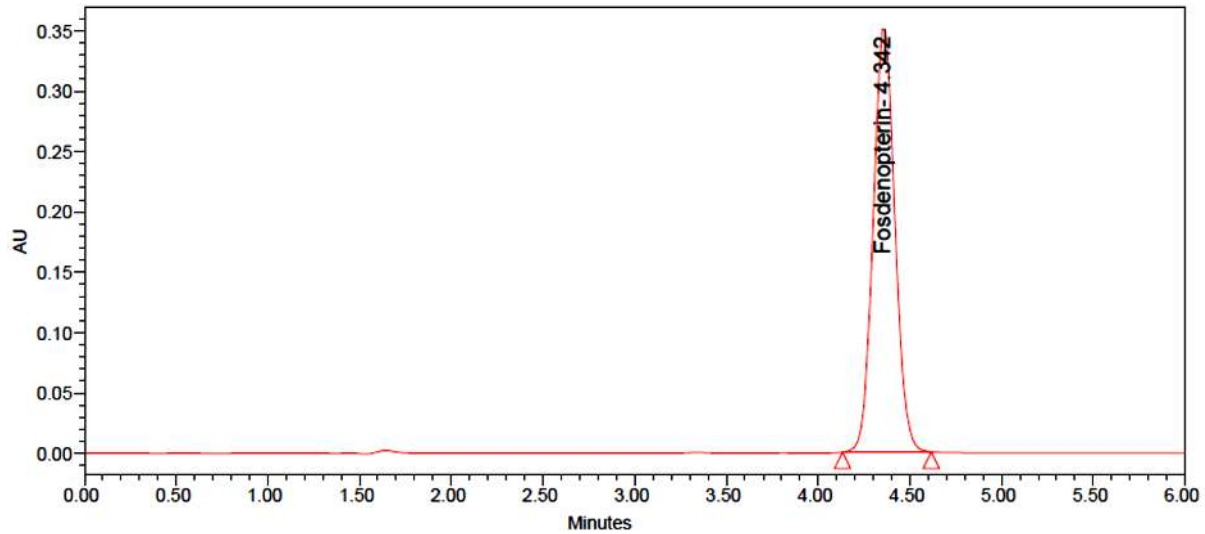

Fig No.32: Chromatogram of Control degradation

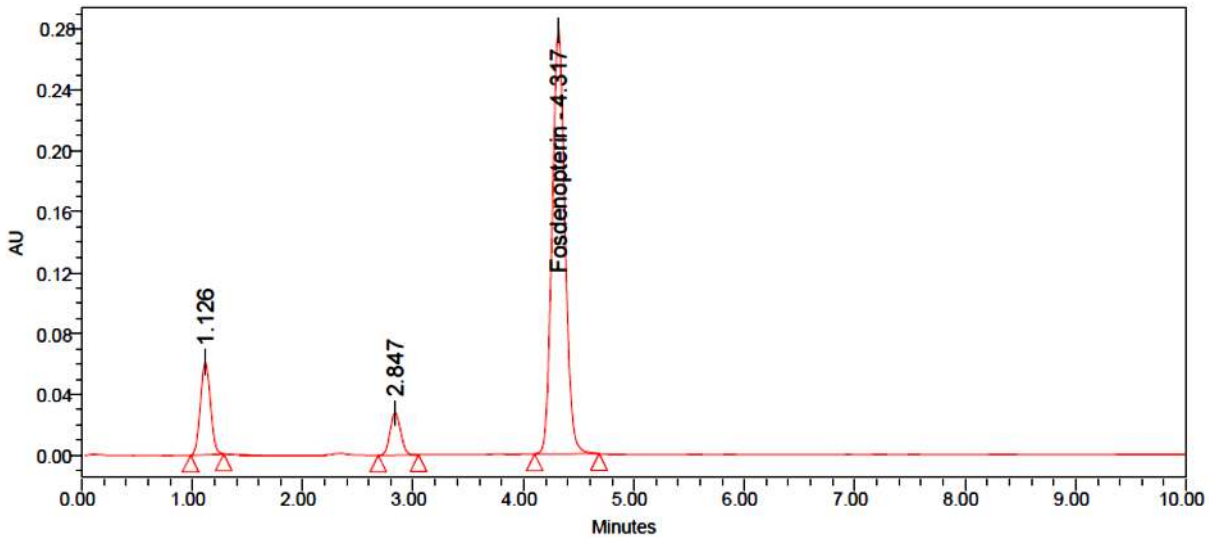

Fig No.33: Chromatogram of Acid degradation

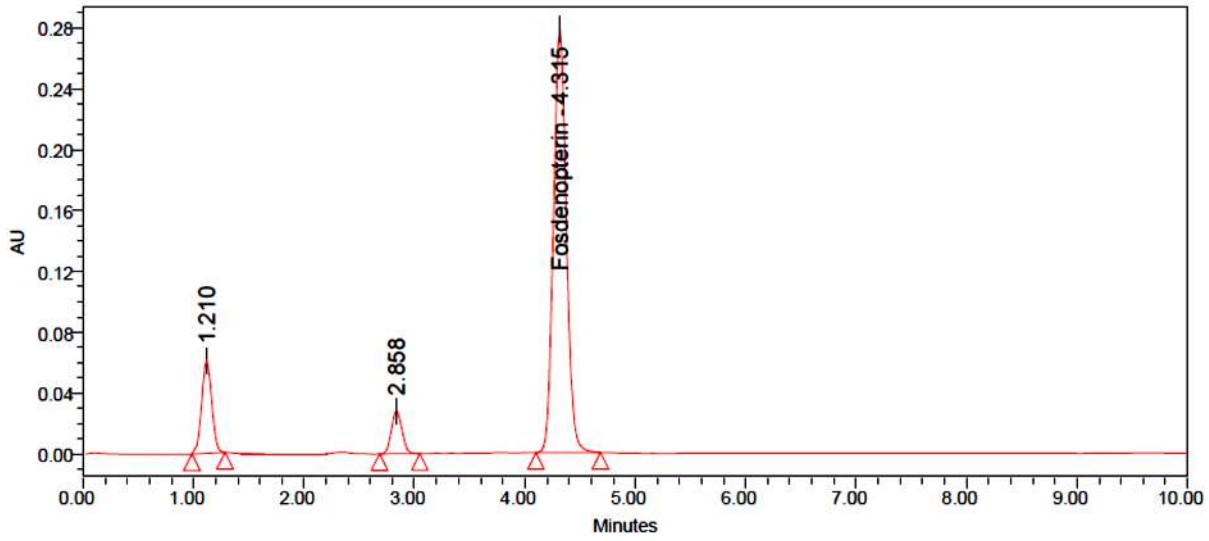

Fig No.34: Chromatogram of Alkali degradation

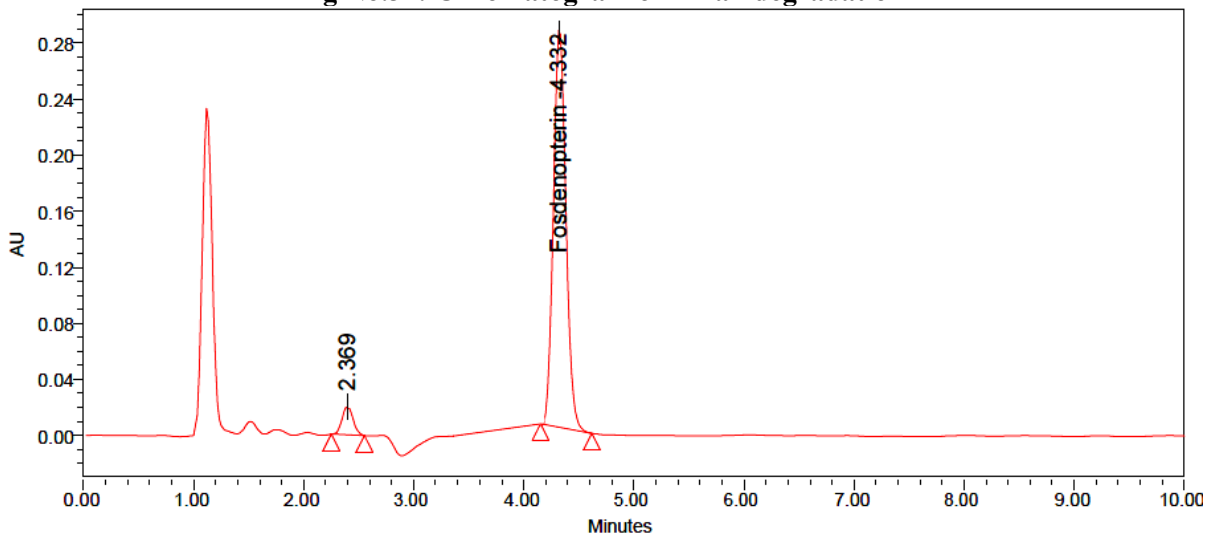

Fig No.35: Chromatogram of Peroxide degradation

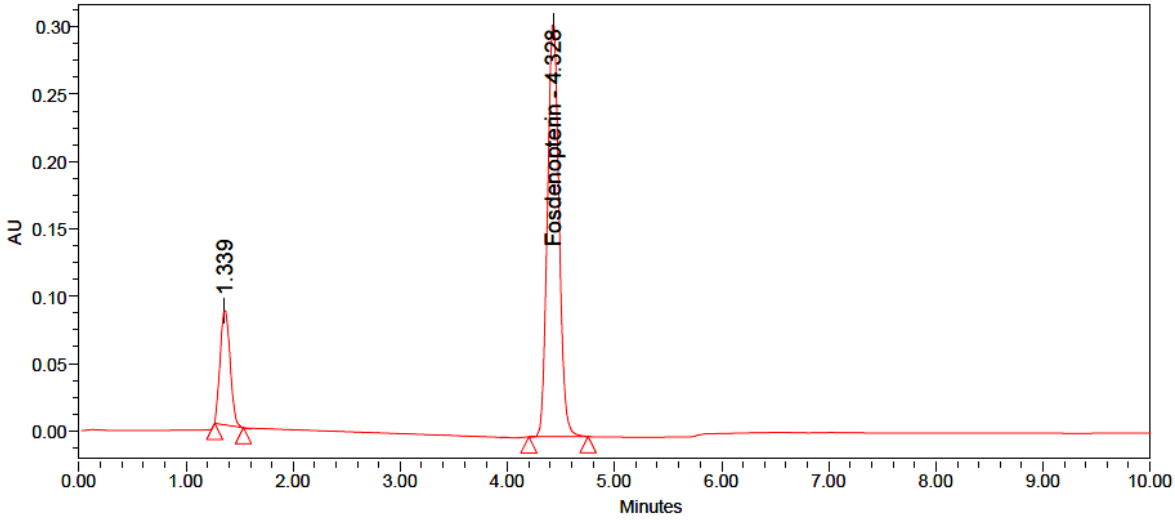

Fig No.36: Chromatogram of Reduction degradation

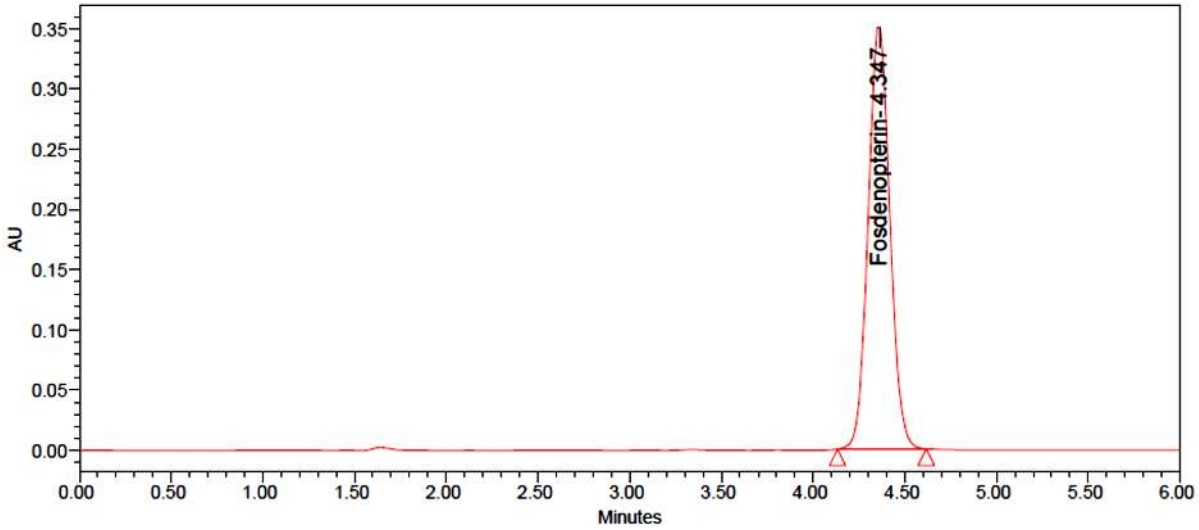

Fig No.37: Chromatogram of Hydrolysis degradation

Table No.19: Forced Degradation results for Fosdenopterin

Results: % Degradation results	Fosdenopterin	
	Area	% Degradation
Control	3264196	0
Acid	2726528	16.5
Alkali	2751953	15.7
Peroxide	2808415	13.9
Reduction	2770521	15.1
Hydrolysis	3235506	0.9

CONCLUSION

An attempt has been made to develop a validated stability indicating RP-HPLC method for the estimation of Fosdenopterin. Literature survey revealed that no analytical methods have been reported individually or in combination with other drugs. However, no method was reported for estimation of drug by HPLC method. The developed HPLC method for the estimation of selected drug is simple, rapid, accurate, precise, robust and economical. The mobile phase and solvents are simple to prepare and economical, reliable, sensitive and less time consuming.

The sample recoveries were in good agreement with their respective label claims and they suggested non interference of formulation excipients in the estimation and can be used in laboratories for the routine analysis of selected drugs.

Since the system validation parameters of HPLC method used for estimation of selected drug in pure and have shown satisfactory, accurate and reproducible results (without any interference of excipients) as well, it is deduced that the simple and short proposed methods be most useful for analysis purpose. The present work concluded that stability indicating assay method by RP-HPLC was simple, accurate, precise, and specific and has no interference with the placebo and degradation products. Hence these can be used for routine analysis of Fosdenopterin.

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