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ORIGINAL ARTICLE **OPEN ACCESS**

Assay of Fusidic acid 1% w/w Viscous Eye Drops by HPLC

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ABSTRACT
A simple, precise and accurate method was developed and validated for analysis of Fusidic Acid 1% w/w Viscous eye drop formulation. An isocratic HPLC analysis was performed on Inertsil ODS column (4.6mm × 150 mm × 3.5 µ or equivalent). The compound was separated with the mixture of Methanol, Ortho-phosphoric acid and Acetonitrile as mobile phase ,at flow of 1.5 mL/min. UV detection was performed at 235 nm using photo diode array detection. The retention time was found to be 19.5 min. The system suitability parameters such as theoretical plate count, tailing and percentage RSD between six standard injections were within the limit. The method was validated according to ICH guidelines. The developed method can be applicable for routine quantitative analysis.
Keywords: *Fusidic acid, HPLC, Methanol, Ortho-phosphoric acid and Acetonitrile*

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INTRODUCTION

Fusidic acid (FA) is a bacteriostatic antibiotic used in the treatment of primary and secondary skin infections caused by sensitive strains of *S. aureus*, Streptococci species, and *C. Minutissimum*. Fusidic acid is the steroidal antibiotic used to treat Gram positive infections. It acts by preventing the translocation of peptidyl tRNA. Fusidic acid2 (2), chemically is 2-(16-acetyloxy-3,11-dihydroxy-4,8,10,14-tetramethyl-2,3,4,5,6,7,9,11,12,13,15,16-dodecahydro-1H-cyclopenta[a]phenanthren-17-yelidene)-6-methyl-hept-5-enoic acid. [1,2]

Fusidic acid is active in vitro against *Staphylococcus aureus*, most coagulase-positive staphylococci, Beta-hemolytic streptococci, Corynebacterium species, and most clostridium species. Fusidic acid has no known useful activity against enterococci or most Gram-negative bacteria (except Neisseria, Moraxella, Legionella pneumophila, and Bacteroides fragilis). Fusidic acid is active in vitro and clinically against Mycobacterium leprae but has only marginal activity against Mycobacterium tuberculosis.[3,4]

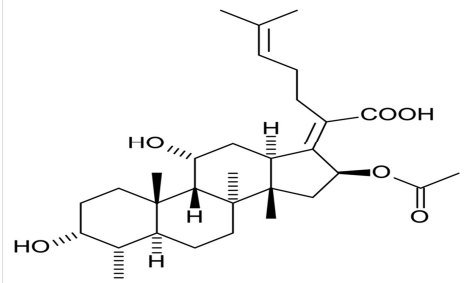


Figure1:- Chemical structure of fusidic acid

MATERIAL AND METHODS

Fusidic Acid standard:

Use the standard as such and use % potency on as is basis for calculations. Protect from light, preserve at a temperature of 2°C-8°C.

Reagents and Material:

Name of Reagent and Material	Grade	Make
Water	HPLC	--
Methanol	HPLC	Rankem
Acetonitrile	HPLC	Rankem
Ortho Phosphoric Acid 88%	Emparta®	Merck
0.45µ Nylon membrane syringe filter	--	Millipore Milex-HN

Instrument and Equipment:

High performance liquid chromatography
Sonicator
Analytical Balance
Centrifuge

Preparation of 5 g/L solution of Ortho-phosphoric acid:

Dilute 5 gm of Ortho-phosphoric acid to 1000 mL with water, mix well.

Preparation of Mobile Phase A:

Prepare a mixture of Methanol, 5 g/L solution of Ortho-phosphoric acid solution and Acetonitrile in the ratio of 20:40:40 v/v/v respectively. Mix well, sonicate to degas it.

Preparation of Mobile Phase B:

Prepare a mixture of Methanol, 5 g/L solution of Ortho-phosphoric acid solution and Acetonitrile in the ratio of 20: 10:70 v/v/v respectively. Mix well, sonicate to degas it.

Preparation of Solvent Mixture:(Diluent)

Prepare a mixture of Methanol, 5 g/L solution of Ortho-phosphoric acid solution and Acetonitrile in the ratio of 10:40:50 v/v/v respectively. Mix well.

Chromatographic condition:

Column	Inertsil ODS, 4.6 mm x 150 mm, 3.5µ or equivalent
Flow Rate	1.0 mL/min
Injection Volume	20 µL
Wavelength	235 nm
Column Temp	30°C
Sample Temp	10°c
Retention time	19.5 minutes
Seal wash	Water : Acetonitrile (90: 1 0)
Needle wash	Water: Acetonitrile (10:90)

Preparation of gradient:

Time in Minutes	Mobile Phase A%	Mobile Phase B%
0	100	0
3	100	0
28	0	100
33	0	100
35	100	0
45	100	0

Preparation of Standard Solution:

Weigh accurately about 80 mg of Fusidic Acid standard and transfer in to 100 mL volumetric flask. Add 60 mL of solvent mixture, sonicate to dissolve and dilute up to the mark with solvent mixture. Note: Prepare standard solution in duplicate as lst standard solution and 2nd standard solution. (Concentration of Fusidic Acid: 800 ppm).

Preparation of Sample Solution:

Weigh accurately 2000 mg of sample (Equivalent to 20 mg of Fusidic acid) in to 25 mL volumetric flask, add 15 mL of solvent mixture, sonicate for 60 minutes, dilute up to the mark with solvent mixture. Centrifuge the sample at 3000 rpm for 5 minutes and then filter through 0.45µ Nylon membrane syringe filter. (Concentration of Fusidic Acid: 800 ppm).

Procedure:

Separately inject equal volumes of Blank (diluent), 1st Standard solution of Fusidic Acid (Six replicates) and 2nd Standard solution of Fusidic Acid (Two replicates) and Sample solution in duplicate.

Evaluation of System Suitability:

Inject the Fusidic Acid I st Standard solution six times.

The relative standard deviation for peak areas of Fusidic Acid in six replicate injections should not be more than 2.0%.

The Tailing factor for Fusidic Acid peak in standard should not be more than 1.5.

The Theoretical plates for Fusidic Acid in standard should not be less than 2000.

The Correlation between I st standard solution and 2nd standard solution should be range of 98.0% to 102.0%.

Correlation formula is mention below.

$$\text{Correlation} = \frac{AS_2}{AS_1} \times \frac{WS_1}{WS_2} \times 100$$

Where,

AS1 WS2

AS2 : Average area from two replicates of2"d standard preparation.

AS1 : Average area from six replicates of 1 st standard preparation.

WS1 : Weight of standard in mg taken for preparation of I st standard.

WS2 : Weight of standard in mg taken for preparation of 2nd standard.

Calculation:

Calculate the %Assay ofFusidic Acid in Eye drop as given below:

$$\%\text{Assay} = \frac{AT}{AS} \times \frac{WS}{100} \times \frac{25}{SW} \times \frac{P}{100} \times \frac{100}{LC} \times \text{Avg wt.}$$

Where,

AT: Peak area of Fusidic Acid in the chromatogram of sample solution

AS: Average Peak area of Fusidic Acid in the chromatogram of Standard solution.

WS: Weight of Fusidic Acid standard in mg.

LC: Label claim of Fusidic Acid in %

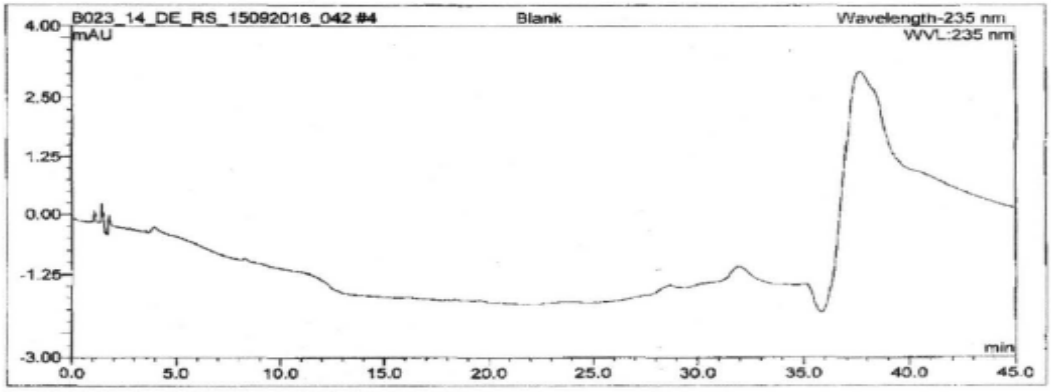
P: % Potency of Fusidic Acid standard on as is basis.

SW: Weight of Sample in mg.

Avg Wt: Average Weight in mg

RESULTS AND DISCUSSION

Chromatogram for Blank

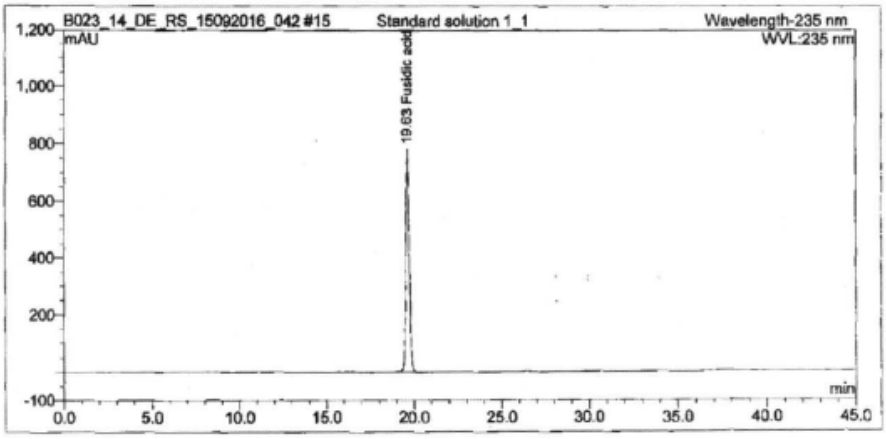


SYSTEM SUITABILITY RESULT

No.	Ret.Time min	Peak Name	Type	Plates USP	Tailing USP	Resolution USP	K'	Area mAU*min
Total:								0.000

Figure 2:- HPLC Chromatogram of Blank Sample

Chromatogram for Standard

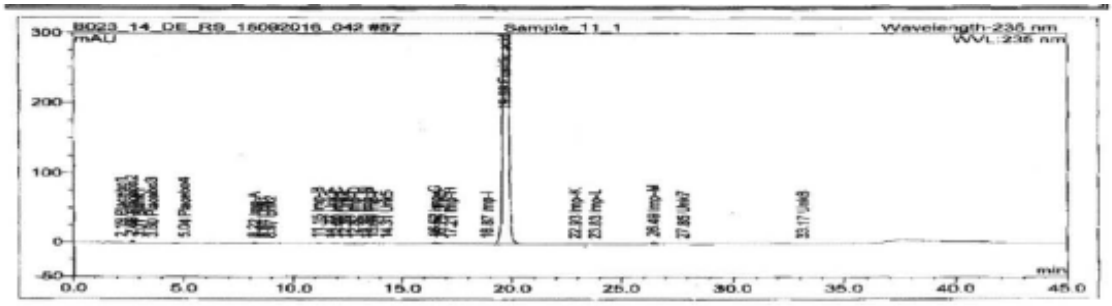


SYSTEM SUITABILITY RESULT

No.	Ret.Time min	Peak Name	Type	Plates USP	Tailing USP	Resolution USP	K'	Area mAU*min
1	19.63	Fusidic acid	BMB	43441	1.16	n.a.	27.04	189.059
Total:								189.059

Figure 3:- HPLC Chromatogram of Standard Sample of Fusidic acid

Chromatogram for Samples



SYSTEM SUITABILITY RESULT

No.	Ret.Time min	Peak Name	Type	Plates USP	Tailing USP	Resolution USP	K'	Area mAU*min
1	2.19	Placebo1	BMB	3965	1.11	n.a.	2.13	0.043
2	2.69	Placebo2	BMB	4432	0.93	3.32	2.85	0.326
3	3.01	Blank1	BMB	7878	1.19	2.11	3.30	0.027
4	3.80	Placebo3	BMB	6272	0.95	3.74	4.14	0.016
5	5.04	Placebo4	BMB	8658	1.26	7.23	6.20	0.007
6	8.22	Imp-A	BM	12188	1.07	12.36	10.74	0.227
7	8.61	Unk1	MB	13986	n.a.	1.34	11.30	0.074
8	8.97	Unk2	BMB	25539	1.16	1.38	11.81	0.010
9	11.16	Imp-B	BMB	15879	1.00	7.60	14.92	0.229
10	11.77	Unk3	BM	8762	n.a.	1.45	15.81	0.069
11	12.00	Imp-C	Mb	13368	n.a.	0.51	16.14	0.109
12	12.39	Unk4	bMB	46939	1.27	1.20	16.70	0.012
13	12.82	Imp-D	BMB	17833	1.02	1.41	17.31	0.124
14	13.39	Imp-N	BMb	26469	0.80	1.59	18.12	0.084
15	13.69	Imp-F	bMB	32149	1.74	0.96	18.55	0.098
16	14.31	Unk5	BMB	35160	1.24	2.06	19.45	0.023
17	16.52	Imp-G	BM	33506	n.a.	6.62	22.60	0.654
18	16.75	Unk6	M	n.a.	n.a.	n.a.	22.93	0.143
19	17.21	Imp-H	MB	25719	n.a.	n.a.	23.58	0.063
20	18.87	Imp-I	BM	31877	n.a.	3.91	25.96	0.175
21	19.69	Fusidic acid	MB	44810	1.17	2.05	27.12	186.714
22	22.93	Imp-K	BMB	54684	0.86	8.48	31.75	0.061
23	23.83	Imp-L	BMB	62011	0.94	2.34	33.05	0.067
24	26.49	Imp-M	BMB	85814	1.10	7.13	36.84	0.706
25	27.85	Unk7	BMB	114508	1.16	3.94	38.78	0.040
26	33.17	Unk8	BMB	111156	1.15	14.65	46.39	0.031
Total:								189.130

Figure 4:- HPLC Chromatogram of Different Samples of Fusidic acid

METHOD VALIDATION

Linearity

The linearity parameter was confirmed in the concentration range of 1%, wherein the calibration equation and correlation coefficient were also determined. In the statistical analysis, results showed that the method had satisfactory linear regression because the p-value was also good.

Precision

In analyzing the method by repeatability and intermediate precision, the RSDs of areas obtained in all concentrations analyzed were no more than 2%, indicating good precision of the chromatographic method.

Accuracy

The recovery test showed that the method had satisfactory accuracy to measure fusidic acid in eye drops, since the percentage value of the average recovery was close to 100%, as shown in figure.

Discussion

The proposed HPLC method for the quantification of fusidic acid in the form of pharmaceutical eye drops is fast, simple, efficient, economical, and sensitive compared to other methods described in the literature, and is stability indicating. While co-relating it with works done by different scientists, we found that all parameters were in accordance with the acceptance criteria for the validation of methods established by the ICH and FDA. Therefore, the validated method could easily be applied to the routine analysis of fusidic acid in the quality control of pharmaceutical sciences.

CONCLUSION

A HPLC method was successfully developed for the Assay of Fusidic acid 1%w/w viscous eye drops.The HPLC method was is quite simple, accurate, precise, reproducible and sensitive. It is also advised for possible use in routine quality control analysis.

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