Biosci. Biotech. Res. Asia Vol. **3,** No.(**1**) 151-152 (2005)

IDENTIFICATION OF FUSIDIC ACID BY CHROMATOGRAPHY

Charitra K. Mishra, Manik Sharma and Jagrati Tripathi

Vidisha Science Academy, Vidisha - 464 001 (India)

(Received January 29, 2005; Accepted March 17, 2005)

ABSTRACT

The chromatogram obtained with the test solution were showed a peab with the same reteution time as those of the fusidic acid. Potency of Fusidic acid in cream was found to 20.62 mg/g by HPLC.

Keywords: Fuscidic acid, chromatography and potency.

INTRODUCTION

Fuscidic acid is ent-16a-acetoxy-36, 116-dihydroxy-4b, 8b, 14 a-trimethyl-18-nor-56, 10a-cholesta (172) 24-dien-21 oic acid hemi hydrate, an antimicrobial substance produced by the growth of certain strains of *Fusidium coccineum*. It is an antibiotic particularly useful in the treatment of *Staphylococcal* infections[1].

MATERIAL AND METHODS

Fusidic acid quantified by chromatography in a reverse phase HPLC system using UV detection (235 nm) medroty progesterone was used as an internal reference substance material.

Material:

- Diethanolamine fusidate reference substance*
- Potassium sorbate reference substance*
- Medroxyprogesterone acetate*
- Phosphoric acid
- Acetonitrile
- Methanol, purified water, glass fiber paper - whatman filter paper 1.6 um

Column:

Merck lichrosphere 125 x 4 mm.

Preparation of phosphoric acid 0.05 M

70 ml phosphoric acid .....1000 ml (1M)
.....1000 ml (0.05 M)

Mobile Phase:

Acetonitrile: Methanol: 0.05 M Phosphoric acid
50% : 10% : 40%

Operating condition: Retention time

Excepients	< 0.05 min
Sorbic acid	0.6 min
Fusidic acid	5.3 min.
IR	Internal reference solution

1.5 mg/ml solution of medroxy progesterone acetate in mobile phase.

Reference solution

1. 40.0 mg + 4.0 mg (a^1 mg potency c^1 %) of potassium sorbate reference substance was dissolved in mobile phase to make 50 ml.
2. 36.0 mg + 4.0 mg (a^2 mg potency c^2 %) of Diethanolamine fusidate reference substance was dissolved in 5.0 ml reference solution 1, 1.0 ml of IR solution and mobile phase to make 100ml.

Test solution

0.7605/0.7595g + 0.08 g of the samples were dissolved in 5.0 ml of IR solution and 45 ml of mobile phase. This mixture was heated until the cream was melted and shaked vigorously for 15 min. The mixture was allowed to cool at a temperature less than 10°C and was filtered through glass fiber paper, the first few ml of the filtrate was discarded. This filtrate was allowed to room temperature before injection into the chromatograph.

System suitability test

20 ul of the reference solution 2 was injected and chromatogram was examined.

Retention time

A retention time for the sorbic acid peak of about 0.6 min, the medroxy progesterone acetate peak of about 3.0min and fusidic acid peak 5.3min.

Table 1

Marked	Explanation	
(P/IR)	Peak response ratio of sorbic acid & internal reference obtained	As per readings
(F/IR)	Peak response ratio of fusidic acid & internal reference obtained	As per readings
a^2	Mass of reference substance used	36.0 mg
b	Mass of the text substance used	0.7605 g & 0.7595 g
c^2	Potency in percentage of the reference substance used	99.0%
516.7	Molecular mass of Fusidic acid anhydrous	...
621.9	Molecular mass of Diethalonamine fusidate	...

Table 2

S. No.	STD Peak Area Ratio Fusidic acid	Peak No.	Test Peak Area Ratio Fusidic acid I	Fusidic Acid II
01	0.7433			
02	0.7429	01	0.7864	0.7858
03	0.7422			
04	0.7430			
05	0.7435	02	0.7865	0.7859
06	0.7428			
Mean	0.7430	Mean	0.7865	0.7859

Resolution

1. A minimum resolution of 10.0 between the sorbic acid peak and the medroxy progesterone acetate peak.
2. A minimum resolution of 5.0 between the medroxy progesterone acetate peak and Fusidic acid peak.

Therotical plates

A minimum of 2000 therotical plates for the fusidic acid peak.

Symmetry factor

A maximum symmetry factor of 2.0 for the fusidic acid peak.

Repeatability

5 replicates 20 ul injection of the reference solution 2 was injected (as per Leo's the system is not suitable unless the relative standard deviation for the areas or the heights of the fusidic acid peak in the chromatogram is less than 2.0%).

Separate Perry

Equal volumes (20 ul = 6ug of fusidic acid and 0.8 ug of potassium sorbate) of the reference solution 2 were injected separately and of the test solution into the chromatograph. The chromatograms were recorded and the area or the height of the principle peaks were measured.

Result

Readings of the chromatograms of reference and test solution as per given in Table-1.

Calculation:

Mg fusidic acid per g =

$$\frac{(P/IR)\ test}{(P/IR)\ Ref.} \times \frac{a^2}{b} - \times \frac{516.7}{621.9} \times \frac{c^2}{2} \times \frac{1}{100}$$

Fusidic acid 1st =

$$\frac{0.7865}{0.7430} \times \frac{36.0}{0.7605} \times \frac{516.7}{621.9} \times \frac{99.0}{2} \times \frac{1}{100}$$

= 20.61 mg/g

Fusidic acid 2nd =

$$\frac{0.7859}{0.7430} \times \frac{36.0}{0.7595} \times \frac{516.7}{621.9} \times \frac{99.0}{2} \times \frac{1}{100}$$

= 20.62 mg/g

Avg. = 20.62 mg/g

Result:

Potency of fusidic acid in cream was found to 20.62 mg/g by PHLC.

REFERENCES

1. Hibino, S., Asada, Arihara and Tahimoto, Fusidic acid. A steroidal antibiotic. *Chem. Pharm. Bull. T.,* **20,** 1067-1069 (1972)