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Analytical Method Validation for the Estimation of Fusidic Acid and Hydrocortisone Acetate in Cream Form by RP-HPLC

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ABSTRACT

A simple, accurate, precise and sensitive analytical RP-HPLC method was developed for the determination of fusidic acid and hydrocortisone acetate in cream form. The mobile phase used was a mixture of 10 volumes of methanol, 40 volumes of 0.05M orthophosphoric acid and 50 volumes of Acetonitrile. A stainless steel column of 150mm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5µm) was used, with flow rate 2 ml/min, wavelength 235 nm and injection volume 20 µl. The optimized method was then validated as per ICH guidelines Q2 [R1]. The method was linear over the concentration range 80% -120%.The percentage recoveries of fusidic acid and hydrocortisone acetate were 100.52% and 99.93 % respectively. The assay results obtained by two analysts using two instruments on different days had a statistical RSD less than 2%.The method was also found to be robust and rugged. All of the validation parameters were within the acceptance criteria as per the ICH guidelines. Hence the proposed method can be used for the routine control analysis of fusidic acid and hydrocortisone acetate in cream form.

Key Words: *Fusidic acid, Hydrocortisone acetate, Analytical method validation, HPLC.*

INTRODUCTION

Validation is an integral part of quality assurance; it involves the systematic study of systems, facilities and processes aimed at determining whether they perform their intended functions adequately and consistently as specified. Validation means demonstration, by provision of objective evidence that consistently meets its predetermined requirements [1]. The word "validation" comes from Latin word term *valdius* meaning worth/strong, thus suggesting that something is true, useful, and reliable [2]. The most accurate definition of validation is provided by ISO 900:2000 as the conformation, by means of a thorough examination and obtaining realistic and unequivocal evidences, that the procedure is effectively applicable for its intended purposes [3]. It is the act of providing that any approach, strategy experimental procedure, process, instrumentation and room conditions selected for the method will function in a proper way under a fixed set of conditions. Besides it can be used to individually evaluate the appropriateness of these factors [4].

Method Validation means establishing documented evidence that a specific method and the ancillary instruments included in the method will consistently yield results that accurately reflect the quality characteristics of the product tested. It is the process of demonstrating that an analytical procedure is suitable for its intended purpose [4]. The method validation evaluates the range and conditions of applicability, and checks if every future measurement in routine analysis will provide a concentration of the analyte close enough to the true value [5]. In addition, it can also quantify the degree

of coincidence of a measured concentration and the true value, by the calculation of the bias and the uncertainty associated with the result [6]. Therefore, the validation verifies if the method is suitable to be used as a quality control tool and for research support [7]. It is an essential step in method development, which must be implemented by laboratories to prove they can produce analytical data with high reliability [8]. Method Validation is an important requirement for any package of information submitted to international regulatory agencies to support new product marketing or clinical trials applications. Analytical method should be validated, including methods published in the relevant pharmacopoeia or other organized standard references. The suitability of all test methods used should always be verified under the actual conditions of use and should be well documented [9].

Method development is a continuous process that progress in parallel with the evolution of the drug product. The notion of phase-appropriate method development is critical one if time cost and efficiency are concerns. The goal and purpose of the method should reflect the phase of drug development. During early drug development, the method may focus on API behavior. They should suitable to support pre-clinical safety evolution, pre-formulation studies and pro-type product stability studies [10].

When there are no authoritative methods are available, new methods are being developed for analysis of novel products. To analyze the existing either pharmacopoeial or non-pharmacopoeial products novel methods are developed to reduce the cost besides time for better precision and ruggedness. These methods are optimized and validated through trial runs. Alternate methods are proposed and put into practice to replace the existing procedure in the comparative laboratory data with all available merits and demerits [11,12,13].

DRUG PROFILE

Fusidic Acid

- Drug: Fusidic Acid
- Synonym: Ramycin Fucithalmic
- Drug Category: Steroidal Antibiotic
- Molecular Weight: 516.7 g/mol
- Molecular Formula: $C_{31}H_{48}O_6$
- Description: White to off-white powder
- Solubility: Freely Soluble in ethanol(95%) and chloroform
- Melting point: 192.5 °C

Fusidic acid is an antibiotic isolated from the fermentation broth of *Fusidium coccineum* that belongs to a group of its own, the fusidanes. The molecule has a steroid-like structure but does not possess any steroid activity. The structure is thought to be responsible for the steroid-like high penetration, and for the fact that no cross-resistance or cross-allergy has been seen with other antibiotics in routine clinical use. The anti-microbial activity of fusidic acid is specifically aimed at the most common skin pathogens, including *Staphylococcus aureus*, towards which it is one of the most potent antibiotics. The place of fusidic acid in dermatology is in the treatment of mild to moderately severe skin and soft-tissue infections, e.g. impetigo, folicullitis, erythrasma, furunculosis, abscesses and infected traumatic wounds, whereas it is of less use in conditions such as hidradenitis suppurativa, chronic leg ulcers, burns and pressure sores. playing an important exacerbating role. Fusidic acid acts as a bacterial protein synthesis inhibitor by preventing the turnover of elongation factor G (EF-G) from the ribosome. Fusidic acid is effective primarily on Gram-positive bacteria such as *Staphylococcus* species, and *Coryne bacterium* species. Fusidic acid inhibits

bacterial translation and does not kill the bacteria, and is therefore termed "bacteriostatic" [14,15].

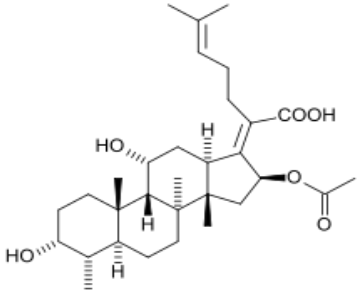


Fig 1: Structure of Fusidic Acid

Hydrocortisone Acetate is naturally occurring corticosteroid hormone secreted by the adrenal cortex and released during times of stress. The synthetic drug is employed in the management of inflammatory and rheumatoid diseases, allergic conditions and autoimmune disorders such as Addison's disease (adrenal insufficiency disease).Hydrocortisone may exist Commercially as the unchanged hormone or as the acetate, cypionate, sodium phosphate, butyrate and sodium succinate forms [16].

Hydrocortisone Acetate

- Drug Name: Hydrocortisone Acetate
- Synonymn: Cortisol 21-acetate,Cortell
- Drug Category: Corticosteroids
- Molecular weight: 404.5 g/mol
- Molecular Formula: $C_{23}H_{32}O_6$
- Descrption: White to off white powder
- Solubility: Freely Soluble in ethanol(95%) and chloroform
- Melting Point: 220°C

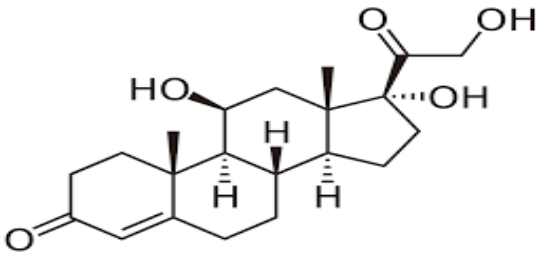


Fig 2: Structure of Hydrocortisone acetate

METHODOLOGY

Materials and Chemicals

List of Instruments

Instrument	Model
HPLC	SHIMADZU Prominence I (LC-2030) and Prominence I (LC-2030C PDA)
Electronic Balance	SHIMADZU
Ultrasonic bath Sonicator	PCI
FTIR	SHIMADZU

List of Chemicals

Chemicals	Source
Fusidic Acid	Indian Pharmacopeia Reference Standard
Hydrocortisone Acetate	Simson Reference Standard
HPLC grade Acetonitrile	Alive Pharmaceutical Pvt. Ltd, Nepal

HPLC Grade Methanol	Alive Pharmaceutical Pvt. Ltd, Nepal
HPLC Grade Water	Alive Pharmaceutical Pvt. Ltd, Nepal
Orthophosphoric Acid	Shree Rani Sati Trading Concern, Nepal
Fuzabact-H (Combination of Fusidic acid and Hydrocortisone Acetate cream with label claim of 2% w/w and 1% w/w respectively)	R&D Department, Alive Pharmaceutical Pvt. Ltd)

METHOD DEVELOPMENT

The mobile phase constituted of: 10 volumes of *methanol*,40 volumes of *0.05M orthophosphoric acid* and 50 volumes of *Acetonitrile* was chosen. The mobile was thoroughly mixed in magnetic stirrer and filtered through 0.45 µm filter paper by using vaccum pump filter and then sonicated for 10 mins. A stainless steel column of 150mm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5µm) was used. Selection of wavelength was done with the help of PDA detector present in Shimadzu Prominence I LC-2030.

Preparation of Standard Solution

50 mg of *Fusidic Acid RS* and 25 mg of *Hydrocortisone acetate RS* was weighed and transferred in 100 ml clean and dry volumetric flask and diluted to the mark with mobile phase to get a final concentration of 0.5mg/ml of *Fusidic Acid* and 0.25 mg/ml of *Hydrocortisone acetate* respectively. The solution was heated on water bath for about 10 minutes to dissolve the drug substances and then filtered through 0.45 µm filters.

Preparation of Sample Solution

The sample was provided by R&D Department, Alive Pharmaceutical Pvt. Ltd, Nepal. The sample has following composition

Each Tube Contains:

Fusidic Acid 2%

Hydrocortisone Acetate 1%

The sample solution was prepared by taking 5 g of cream, transferred into motor and triturated for 5 min, from this, 2.5 g of cream was weighed and transferred to a 100 ml clean and dry volumetric flask. Then 25 ml of mobile phase was added and stirred for 10 min on a magnetic stirrer and diluted to the mark with mobile phase. The solution was heated on water bath for about 10 minutes to dissolve the drug substances and then filtered through 0.45 µm filters.

Method Validation

Specificity

The specificity of the method is determined by checking the interference of blank and placebo with an analyte. Blank, Standard solution, Placebo solution and Test solution were prepared. Mobile phase was used as blank. Placebo solution was prepared by weighing 2.5g of placebo in clean and dry volumetric flask and diluted to the mark with mobile phase and heated on water bath for about 10 minutes and then filtered through 0.45 µm filters.

System Suitability

System suitability tests was performed on HPLC systems to determine the accuracy and precision of the system by injecting six injections of a solution containing analyte at 100% of test concentration. % RSD of area, Resolution and Theoretical plates were analyzed.

Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain

test results which are directly proportional to the concentration (amount) of analyte in sample. Linearity was determined by preparing samples of five different concentrations within the range of 80 % to 120 % of the target concentration. Each of final concentration was prepared in triplicate form. Linearity was plotted for peak area response against concentration

Range
Range was determined by preparing the sample solutions of three different concentrations: 80%, 100% and 120%.They were then compared with the standard solution and mean potency and the RSD% was calculated.

Accuracy
Accuracy was determined by preparing spiked samples at three concentrations over the range of 80 %, 100 % and 120 % of the target concentration. Three individually prepared replicates at each concentration were analyzed. They were then compared with standard solution and then % recovery was found out.

$$\%\ Recovery = \frac{Analytical Result}{True Value} X100\%$$

Precision
The closeness among analytical results i.e. the precision of an analytical method is indicated by relative standard deviation, RSD, which is determined by the equation:

$$RSD(\%) = \frac{100}{\bar{X}}\left[\frac{\sum(X_i - \bar{X})^2}{n-1}\right]^{1/2}$$

Where,
$\bar{X}$ *is the arithmetic mean of total assays or average assay value.*
X_i *is an individual assay value.*
n *is the total number of data, observation or measurement*

Two types of precision were performed: Repeatability and Intermediate Precision

Repeatability
Repeatability was performed by preparing six concentration of 100% concentration of test and their mean value and % RSD were calculated.

Intermediate Precision
Intermediate Precision expresses variations with laboratories, such as different days, different analysts, different equipment and so forth. Triplicate sample of 100% concentration was prepared and then carried on two different days and similarly by two analysts.

Limit of Detection & Limit of Quantification: Not necessary for Assay (As per ICH guidelines)

Robustness
Robustness was performed by varying different factors. The factors that were varied are given below:

- Change in Wavelength: Analytical method was deliberately changed in wavelength by 2 nm (*i.e.* 235 nm to 233 nm)
- Change in Flow rate: Analytical method was deliberately changed in flow rate by 0.2 ml/min (*i.e.* 2 ml/min to 1.8 ml/min)
- Change in Column oven temperature: Analytical method was deliberately changed in oven temperature to 30^0C from Room temperature.
- Change in Column: Analytical method was deliberately changed in Lot. no. of the Column.

Solution Stability
Solution Stability was determined by analyzing solutions of 100% concentration test in comparison to the fresh prepared solutions and original solutions stored at room temperature in auto sampler (at least 24 h) and stored at 2 - 8 °C, in refrigerator (at least 48 hour). The mean value of the standard solutions was compared to the fresh prepared

standard solutions in case of the stability of the standard solution.

RESULT AND DISCUSSION

After selecting suitable mobile phase, Column, diluents and wavelength, the chromatographic condition was selected .The Chromatographic Condition used was:

- **Column**: A stainless steel column of 150mm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5µm)
- **Mobile Phase**: A mixture of 10 volumes of *methanol*,40 volumes of *0.05M orthophosphoric acid* and 50 volumes of *Acetonitrile*
- **Flow rate:** 2 ml/min
- **Wavelength:** 235 nm
- **Injection volume:** 20 µl

Method Validation

The developed RP-HPLC method was validated with reference to ICH guidelines [Q2(R1)].

Specificity

No any interference of blank and placebo was seen in the principal peak of the standard and sample solution. There was no interference with the elution of analyte.

System Suitability

Six replicates injection of same concentration of solution were injected. % RSD of area, resolution, theoretical plates was analyzed.

Table 1: System Suitability Test

Parameters	Limit	Observation	
		Hydrcortisone Acetate	Fusidic Acid
%RSD of Area	NMT 2%	0.594	0.747
Tailing Factor	NMT 2	1.208	1.041
Theretical Plates	NLT 2000	2184	5387

Linearity

Linearity was assessed at five different concentrations. The correlation coefficient for five concentration levels was 0.9995 for Fusidic Acid and 0.999 for Hydrocortisone Acetate. The values found were within limit, hence the linearity meets the requirement for the assay of FA and HA in combination form of cream. The data of Linearity were tabulated below along with their respective calibration curve.

Table 2: Linearity of Fusidic Acid

S.No	Conc. (%)	Area								
		Std.1	Std.2	Std.3	Std.4	Std.5	Std.6	Mean	SD%	RSD %
1	80	2747840	2768664	2793161				2769888	22685.3	0.8190
2	90	3133347	3127537	3122373				3127752	5490.2	0.1755
3	100	3376706	3407732	3428689	3442688	3441134	3435418	3422061	25589	0.7478
4	110	3715943	3764470	3779988	3753467			3753467	33410.2	0.8901
5	120	4115702	4106028	4105828				4109186	5643.91	0.1373

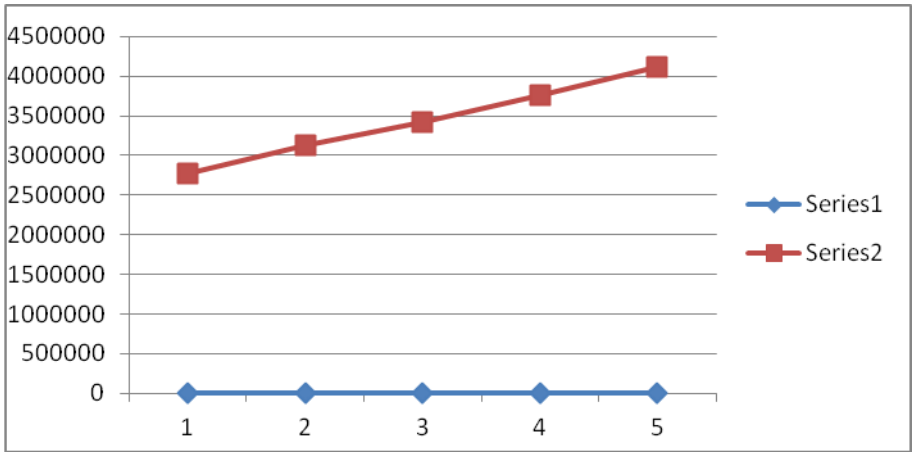


Fig 8: Calibration curve of FA

- *Slope: 33043.1*
- *Intercept: 132161*
- *correlation coefficient: 0.9995274*

Table 3: Linearity of Hydrocortisone Acetate

S.No	Conc. (%)	Area								
		Std.1	Std.2	Std.3	Std.4	Std.5	Std.6	Mean	SD%	RSD %
1	80	4053960	4077617	4068244				4066607	11913.2	0.2930
2	90	4586933	4577636	4573662				4579410	6811.1	0.1487
3	100	5008842	5040758	5079101	5071485	5069847	5064937	5055828	26461.6	0.5234
4	110	5542309	5576934	5581880				5567041	21560.8	0.3873
5	120	6060409	6057766	6064376				6060850	3327.03	0.0549

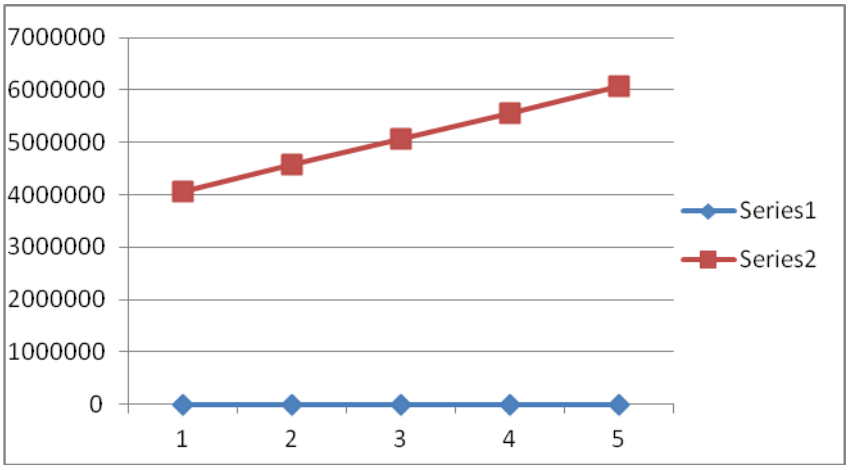


Fig 9: Calibration curve for Hydrocortisone Acetate

- *Slope: 49761.173*
- *Intercept: 89830.066*
- *correlation coefficient: 0.999948*

Accuracy and Range

The content and percentage recovery of Fusidic Acid and Hydrocortisone Acetate were within the limit of ± 2%.The SD and RSD were 0.487%, 0.485% for Fusidic acid and 0.54%, 0.5% for Hydrocortisone Acetate. Hence the accuracy and range meets the requirement for assay of fusidic acid and hydrocortisone acetate in combination form of cream.

Table 4: % Recovery of Fusidic Acid

No. of Test	Level of addition	Amount spiked (%)	% Recovery	% Recovery As per Average
Assay Test1	80%	80.59	100.74	100.21
Assay Test2		80.62	100.78	100.25
Assay Test3		80.59	100.74	100.21
Assay Test1	100%	101.21	101.21	100.68
Assay Test2		100.44	100.44	99.92
Assay Test3		99.49	99.49	98.97
Assay Test1	120%	120.85	100.71	100.18
Assay Test2		120.14	100.12	99.59
Assay Test3		120.60	100.50	99.98
Average				100.52
SD				0.4875
RSD				0.4850
Limit				**NMT-2%**

Table 5: % Recovery of Hydrocortisone Acetate

No. of Test	Level of addition	Amount spiked (%)	% Recovery	% Recovery As per Average
Assay Test1	80%	79.84	99.80	99.87
Assay Test2		79.77	99.71	99.78
Assay Test3		79.48	99.35	99.42
Assay Test1	100%	99.65	99.65	99.72
Assay Test2		99.81	99.81	99.88
Assay Test3		99.66	99.66	99.73
Assay Test1	120%	119.65	99.71	99.78
Assay Test2		121.07	100.89	100.96
Assay Test3		120.97	100.81	100.88
Average				99.93
SD				0.54
RSD				0.5379
Limit				**NMT-2%**

Precision

The assay results obtained by two analysts using two instruments on different days had a statistical RSD less than 2%. Hence the Intermediate precision meets the requirement for the assay of FA and HA in combination form of cream.

Table 6: Intermediate Precision for Fusidic Acid

Parameter	Assay% Test-1	Assay% Test-2	Assay% Test-3	Average
Analyst-1	100.76	101.25	100.33	100.78
Analyst-2	100.97	99.99	99.28	100.08
Instrument-1	100.48	100.44	100.33	100.42
Instrument-2	100.27	101.13	101.08	100.83
Average				100.53
SD				0.3492
RSD				0.3474
Limit				NMT 3%

Table 7: Intermediate Precision for Hydrocortisone Acetate

Parameter	Assay% Test-1	Assay% Test-2	Assay% Test-3	Average
Analyst-1	99.60	99.4	99.79	99.60
Analyst-2	100.65	100.72	100.29	100.55
Instrument-1	99.6	99.4	99.79	99.60
Instrument-2	100.53	101.21	100.58	100.77
Average				100.13
SD				0.6224
RSD				0.6215
Limit				NMT 3%

Robustness

Robustness was carried out by varying wavelength, flow rate of mobile phase, lot no. of the column and column oven temperature. These changes were within the limits that produced acceptable chromatography. The results of Robustness were tabulated below:

Table 8: Robustness for Fusidic Acid

Parameter	Assay % Test1	Assay(%) Test2	Assay (%) Test3	Average %
Wavelength change (233 nm)	99.30	99.51	99.71	99.51
Column Temp. (30°C)	100.01	101.02	101.28	100.77
Change in flow rate (1.8 ml/min)	99.05	98.92	99.23	99.07
Change in Colum 1.5 cm X 4.6 mm	101.15	99.40	99.99	100.18
Average				**99.88**
SD				**0.75**
RSD				**0.7499**

Table 9: Robustness for Hydrocortisone Acetate

Parameter	Assay (%) Test1	Assay (%) Test2	Assay (%) Test3	Average %
Wavelenght change (233 nm)	100.04	100.32	101.12	100.49
Column Temp. (30°C)	101.16	100.94	101.75	101.28
Change in flow rate (1.8 ml/min)	100.38	100.73	101.17	100.76
Change in Colum 1.5 cmX4.6 mm	100.77	99.90	101.27	100.65
Average				**100.80**
SD				**0.34**
RSD				**0.3402**

Solution Stability

The stability of the solutions were analyzed over the specified period of time and compared with freshly prepared solutions. There was no significant difference in the result. The RSD obtained were less than 2 %.The results of Solution stability are tabulated below:

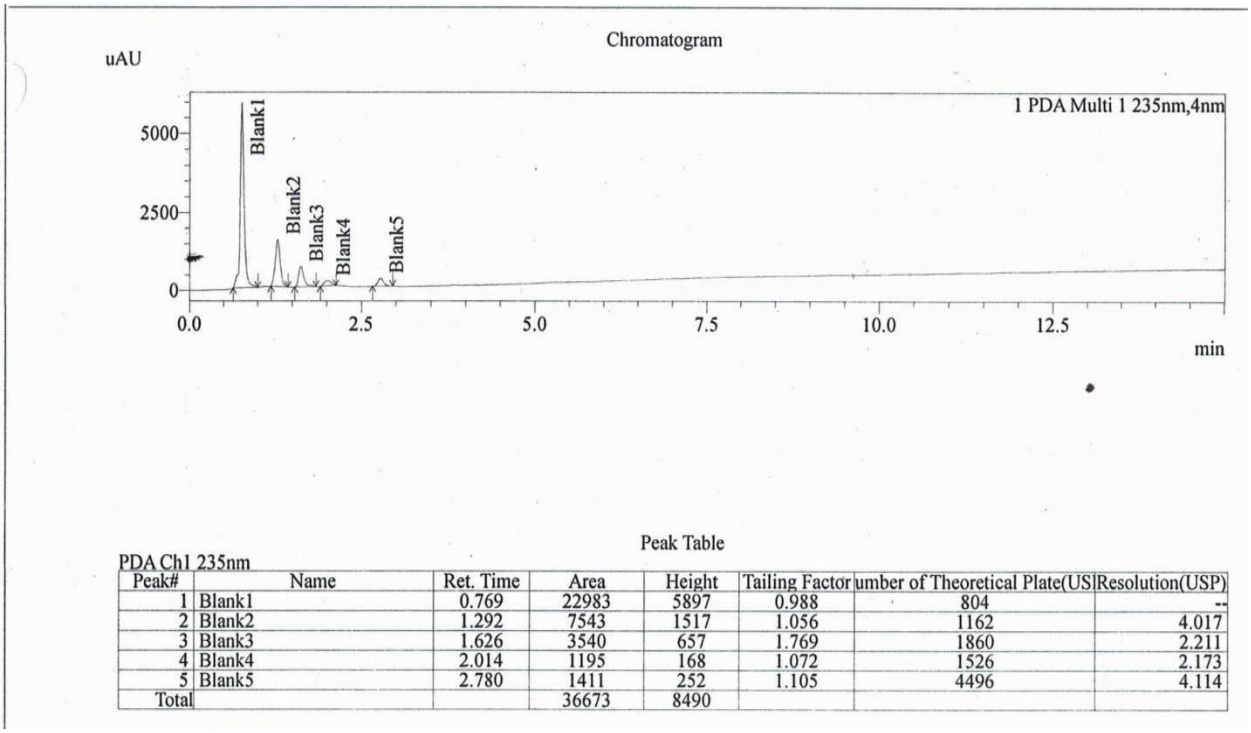
Table 10: Solution Stability of Fusidic Acid

Initially at Room Temperature		**Limit**
Assay Test1	100.76%	There should be no significant difference
Assay Test2	101.25%	
Assay Test3	100.33%	
In refrigerator at 2-8°c for 6 Hours		
Assay Test1	98.37%	
Assay Test2	99.48%	
Assay Test3	98.93%	
In refrigerator at 2-8°c for 24 Hours		
Assay Test1	99.82%	
Assay Test2	100.60%	
Assay Test3	99.76%	

Table 11: Solution Stability of Hyrocortisone Acetate

Initially at Room Temperature		**Limit**
Assay Test1	99.60%	There should be no significant difference
Assay Test2	99.40%	
Assay Test3	99.79%	
In refrigerator at 2-8°c for 6 Hours		
Assay Test1	101.29%	
Assay Test2	101.54%	
Assay Test3	101.20%	
In refrigerator at 2-8°c for 24 Hours		
Assay Test1	101.55%	
Assay Test2	101.90%	
Assay Test3	101.80%	
AVERAGE	100.90%	

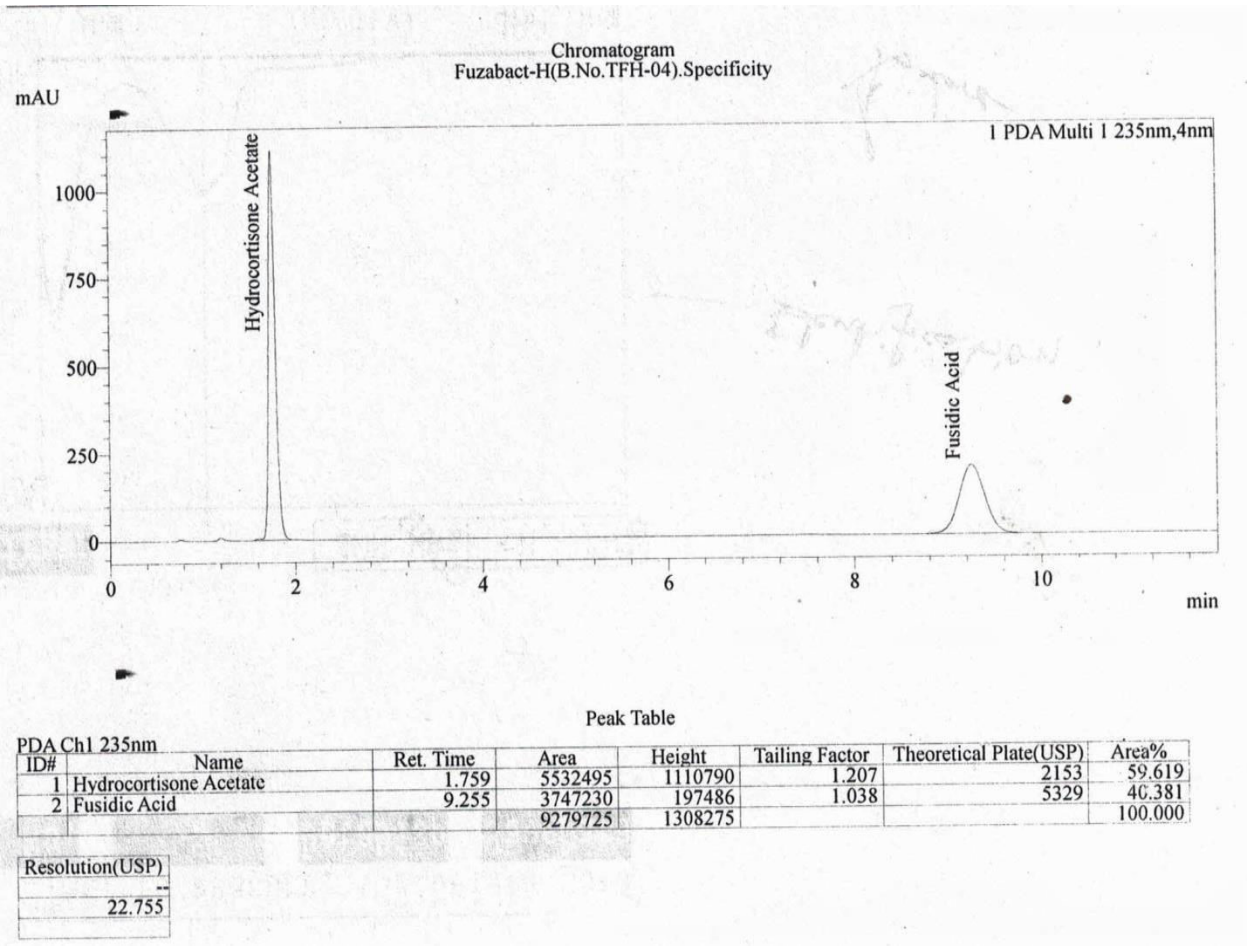
Attachment of Chromatogram



Peak Table

PDA Ch1 235nm

Peak#	Name	Ret. Time	Area	Height	Tailing Factor	umber of Theoretical Plate(US	Resolution(USP)
1	Blank1	0.769	22983	5897	0.988	804	--
2	Blank2	1.292	7543	1517	1.056	1162	4.017
3	Blank3	1.626	3540	657	1.769	1860	2.211
4	Blank4	2.014	1195	168	1.072	1526	2.173
5	Blank5	2.780	1411	252	1.105	4496	4.114
Total			36673	8490			

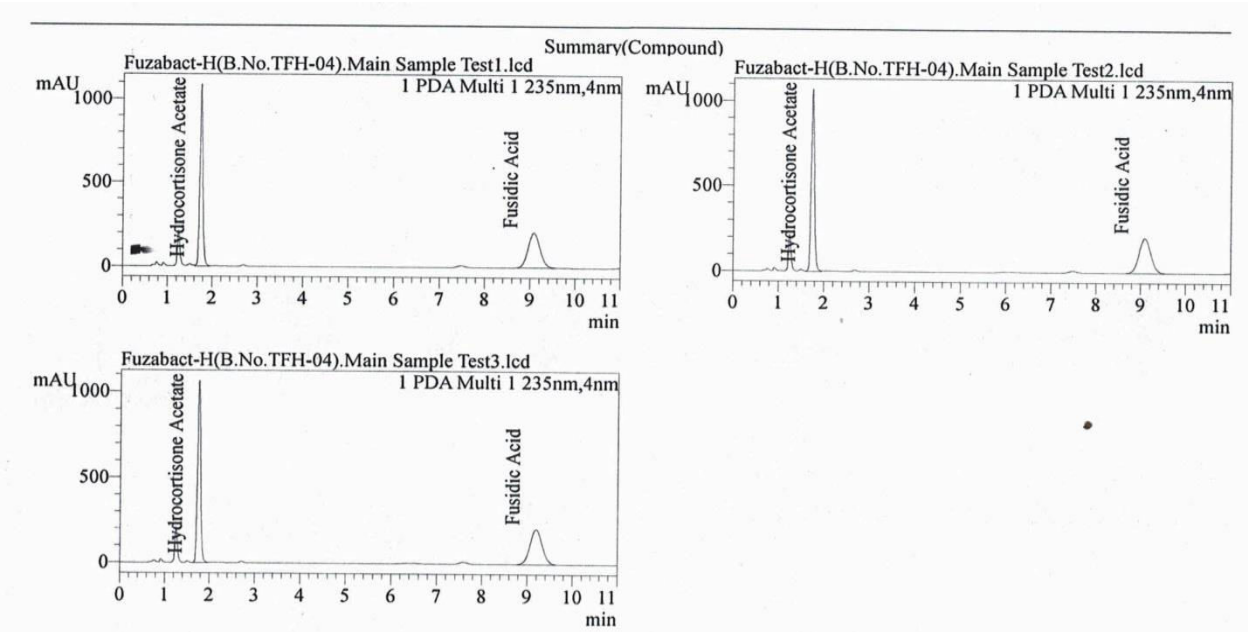


Peak Table

PDA Ch1 235nm

ID#	Name	Ret. Time	Area	Height	Tailing Factor	Theoretical Plate(USP)	Area%
1	Hydrocortisone Acetate	1.759	5532495	1110790	1.207	2153	59.619
2	Fusidic Acid	9.255	3747230	197486	1.038	5329	40.381
			9279725	1308275			100.000

Resolution(USP)
--
22.755



<< PDA >>

ID#1 Compound Name: Hydrocortisone Acetate

Title	Sample Name	Sample ID	Ret. Time
Fuzabact-H(B.No	Fuzabact-H(B.No.TFH-04).Main Sample.TS1	Fuzabact-H.Test	1.747
Fuzabact-H(B.No	Fuzabact-H(B.No.TFH-04).Main Sample.TS2	Fuzabact-H.Test	1.747
Fuzabact-H(B.No	Fuzabact-H(B.No.TFH-04).Main Sample.TS3	Fuzabact-H.Test	1.755
Average			1.750
%RSD			0.280
Standard Deviatio			0.005

Area	Height	Tailing Facto	eoretical Plate(US
5353982	1086167	1.215	2261
5278940	1072608	1.217	2264
5335179	1065014	1.224	2230
5322701	1074596	1.219	2252
0.734	0.997	0.401	0.820
39047	10716	0.005	18

ID#2 Compound Name: Fusidic Acid

Title	Sample Name	Sample ID	Ret. Time
Fuzabact-H(B.No	Fuzabact-H(B.No.TFH-04).Main Sample.TS1	Fuzabact-H.Test	9.088
Fuzabact-H(B.No	Fuzabact-H(B.No.TFH-04).Main Sample.TS2	Fuzabact-H.Test	9.091
Fuzabact-H(B.No	Fuzabact-H(B.No.TFH-04).Main Sample.TS3	Fuzabact-H.Test	9.194
Average			9.124
%RSD			0.663
Standard Deviatio			0.060

Area	Height	Tailing Facto	eoretical Plate(US
3814019	207600	1.065	5473
3786580	206379	1.052	5464
3777295	203970	1.045	5425
3792631	205983	1.054	5454
0.503	0.897	0.988	0.464
19095	1847	0.010	25

CONCLUSION

A simple, accurate, precise and sensitive analytical RP-HPLC method was developed for the determination of fusidic acid and hydrocortisone acetate in cream form. The method isolates the individual peaks of the mixture of drugs and overcomes the problem of merging and interference of mixture peaks with each other. This method was validated as per ICH guidelines for all the parameters and the results passed the criteria set forth by ICH guidelines.

REFERENCES

1) International Conference on Harmonization (ICH), Q2A; Text Validation of Analytical Procedures, March 195
2) Araujo, P. (2009). Key aspects of analytical method validation and linearity evaluation. *Journal of*

chromatography B, *877*(23), 2224-2234.

3) ISO, I. (2004). 9000: 2000—Quality Management Systems—Fundamentals and Vocabulary. *International Organization for Standardization (ISO), Switzerland*.
4) Shabir, G. A. (2003),Validation of high-performance liquid chromatography methods for pharmaceutical analysis: Understanding the differences and similarities between validation requirements of the US Food and Drug Administration, the US Pharmacopeia and the International Conference on Harmonization. *Journal of chromatography A*, *987*(1-2), 57-66.
5) Harisudha, K., Lavanya, G., Eswarudu, M. M., & Sunil, M. (2013). An overview on pharmaceutical process validation. *International Research Journal of Pharmaceutical and Applied Sciences*, *3*(1), 165-168.
6) González, A. G., Herrador, M. Á., & Asuero, A. G. (2005). Practical digest for evaluating the uncertainty of analytical assays from validation data according to the LGC/VAM protocol. *Talanta*, *65*(4), 1022-1030.
7) Us, M. A. (2005). ISO/IEC 17025: 2005, General requirements for the competence of testing and calibration laboratories.
8) Konieczka, P. (2007). The role of and the place of method validation in the quality assurance and quality control (QA/QC) system. *Critical Reviews in Analytical Chemistry*, *37*(3), 173-190.
9) International Conference on Harmonization (ICH), Q2B; Text Validation of Analytical Procedures: May 1997
10) Ravisankar P., and Rao, G. (2014).A review of analytical method development. Indian Journal of research in pharmacy and Biotechnology.2(3):236-247
11) Ravisankar, P., Gowthami, S., & Rao, G. D. (2014). A review on analytical method development. *Indian journal of research in pharmacy and biotechnology*, *2*(3), 1183-1195
12) Ravisankar, P., Rajyalakshmi, G., Devadasu, C., & Devala Rao, G. (2014). Instant tips for right and effective approach to solve HPLC trouble shooting. *Journal of chemical and pharmaceutical sciences*, *7*(3), 259-274.
13) Breaux J,Jones K, and Boulas P.(2003). Development services analytical method development and validation ,*Pharmaceutical Technology*.27(1): 6-13.
14) Wilkinson, J. D. (1998). Fusidic acid in dermatology. *British Journal of Dermatology-Supplement-*, *139*, 37-40.
15) Iwasaki, E. (1987). Hydrocortisone succinate and hydrocortisone simultaneously determined in plasma by reversed-phase liquid chromatography, and their pharmacokinetics in asthmatic children. *Clinical chemistry*, *33*(8), 1412-1415.
16) Pyka, A., Babuska-Roczniak, M., & Bochenska, P. (2011). Determination of hydrocortisone in pharmaceutical drug by TLC with densitometric detection in UV. *Journal of liquid chromatography & related technologies*, *34*(9), 753-769.
17) Nawaz, M., Arayne, M. S., Sultana, N., Haider, A., & Hisaindee, S. (2014). Simultaneous determination of fusidic acid and steroids from bulk drugs and human plasma by reversed phase HPLC. *Acta Chromatographica*, *26*(1), 57-66.
18) Curbete, M. M., & Salgado, H. R. N. (2016). Stability-indicating RP-LC method for quantification of fusidic acid in cream. *Brazilian Journal of Pharmaceutical Sciences*, *52*(3), 447-457.

19) Byrne, J., Velasco-Torrijos, T., & Reinhardt, R. (2015). An RP-HPLC method for the stability-indicating analysis of impurities of both fusidic acid and betamethasone-17-valerate in a semi-solid pharmaceutical dosage form. *Journal of chromatographic science*, *53*(9), 1498-1503.
20) Ibrahim, F., El-Deen, A. K., & Shimizu, K. (2018). Comparative study of two different chromatographic approaches for quantitation of hydrocortisone acetate and pramoxine hydrochloride in presence of their impurities. *journal of food and drug analysis*, *26*(3), 1160-1170.
21) Varaganti, M. S., Chengalva, P., & Gundala, A. (2019). Novel Stability-indicating high-performance liquid chromatographic assay method for simultaneous estimation of hydrocortisone and tetracycline in bulk and pharmaceutical dosage form. *Asian J Pharm Clin Res*, *12*(10), 255-258.
22) Abbas, N., Arshad, M. S., Hussain, A., Irfan, M., Ahsan, M., Rasool, M. F., & ur Rehman, M. H. (2017). Development and validation of a spectroscopic method for the simultaneous analysis of miconazole nitrate and hydrocortisone acetate in pharmaceutical dosage form. *Tropical Journal of Pharmaceutical Research*, *16*(2), 413-420.
23) Harika, M., & Kumar, G. S. (2013). Simultaneous uv-spectrophotometric estimation of hydrocortisone acetate and sulphacetamide sodium in combined dosage form. *International Journal of Pharmaceutical Sciences and Research*, *4*(5), 1901.
24) Adi-Dako, O., Bekoe, S. O., Ofori-Kwakye, K., Appiah, E., & Peprah, P. (2017). Novel HPLC analysis of hydrocortisone in conventional and controlled-release pharmaceutical preparations. *Journal of pharmaceutics*, *2017*.
25) Sudhakar P., Sharma P and Shrivastava B. Validation of stability indicating HPLC method for assay of Fusidic acid, Hydrocortisone Acetate and Potassium Sorbate in topical pharmaceutical formulation. *World Journal of Pharmacy and Pharmaceutical sciences*,2016;5(4):1923-1938.
26) Abbas, N., Arshad, M. S., Hussain, A., Irfan, M., Ahsan, M., Rasool, M. F., & ur Rehman, M. H. (2017). Development and validation of a spectroscopic method for the simultaneous analysis of miconazole nitrate and hydrocortisone acetate in pharmaceutical dosage form. *Tropical Journal of Pharmaceutical Research*, *16*(2), 413-420.
27) Shaikh, S., Muneera, M. S., Thusleem, O. A., Tahir, M., & Kondaguli, A. V. (2009). A simple RP-HPLC method for the simultaneous quantitation of chlorocresol, mometasone furoate, and fusidic acid in creams. *Journal of chromatographic science*, *47*(2), 178-183.
28) Goswami, N., Gupta, V., & Jogia, H. A. (2013). Development and validation of a novel stability-indicating RP-HPLC method for the simultaneous determination of Halometasone, Fusidic acid, Methylparaben, and Propylparaben in topical pharmaceutical formulation. *Scientia pharmaceutica*, *81*(2), 505-518.
29) Vladimirov, S., Fiser, Z., Agbaba, D., & Zivanov-Stakic, D. (1995). Spectrophotometric determination of fusidic acid and sodium fusidate in dosage forms. *Journal of pharmaceutical and biomedical analysis*, *13*(4), 675-678.
30) Iqbal, D. N., Ashraf, A., Iqbal, M., & Nazir, A. (2020). Analytical method development and validation of hydrocortisone and clotrimazole in topical dosage form using RP-

HPLC. *Future Journal of Pharmaceutical Sciences*, *6*(1), 1-7.

31) Ibrahim, F., Sharaf El-Din, M. K., El-Deen, A. K., & Shimizu, K. (2019). A New HPLC-DAD Method for the Concurrent Determination of Hydroquinone, Hydrocortisone Acetate and Tretinoin in Different Pharmaceuticals for Melasma Treatment. *Journal of chromatographic science*, *57*(6), 495-501.

32) Byrne, J., Velasco-Torrijos, T., & Reinhardt, R. (2014). Development and validation of a novel stability-indicating HPLC method for the simultaneous assay of betamethasone-17-valerate, fusidic acid, potassium sorbate, methylparaben and propylparaben in a topical cream preparation. *Journal of pharmaceutical and biomedical analysis*, *96*, 111-117.

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