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Stability indicating method development and validation for the simultaneous estimaion of fluorometholone and sulfacetamide sodium in bulk and pharmaceutical dosage forms by RP-HPLC

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ABSTRACT

An accurate, simple, reproducible and sensitive method for the simultaneous determination of Fluorometholone and Sulfacetamide sodium was developed and validated as per ICH Guidelines. Fluorometholone and Sulfacetamide sodium were separated by HPLC using a Shimadzu RP-18 column (5µm,250mm×4.6mm i.d) and isocratic elution with a flow rate of 1 mL/min. Mixture of Acetonitrile and 1% Ortho Phosphoric (pH=5.0) (70:30) was used as mobile phase. The detection was at 254 nm wavelength. The retention time of FLUO and SULF was found to be 3.614 and 2.578 min respectively. The linearity of developed method was achieved in the range of 10-50 µg/mL (r^2 = 0.998) and 5-25µg/mL (r^2 = 0.998) for Fluorometholone and Sulfacetamide sodium respectively. LOD of both the drugs were 0.0510µg/mL and 0.0031µg/mL and LOQ was found to be 0.1547µg/mL and 0.0095µg/mL for Fluorometholone and Sulfacetamide sodium respectively. Recovery and assay studies of Fluorometholone and Sulfacetamide sodium were within 99% to 102% indicating that the proposed method is suitable for routine analysis of ophthalmic formulation.

Keywords: Fluorometholone, Sulfacetamide sodium, RP-HPLC, Validation.

INTRODUCTION

Fluorometholone (FLUO) is chemically known as (1*R*,2*S*,8*S*,10*S*,11*S*,14*R*,15*S*,17*S*)-14-acetyl-1-fluoro-14-17-dihydroxy-2,8,15-Trimethyltetracyclo [8.7.0.0 [2,7]. 0 [11,15] heptadeca-3, 6-dien-5-one. Fluorometholone is a synthetic glucocorticoid and it is used for the treatment of allergic and inflammatory eye conditions. Fluorometholone thought to act by the induction of phospholipase A2 inhibitory proteins, collectively called as lipocortin's. These lipocortin's controls the biosynthesis of mediators in inflammation, especially prostaglandins and

leukotrienes, via inhibiting the release of the precursor molecule arachidonic acid. In ocular medicines, these actions inhibit edema, capillary dilations, migration of leukocytes, fibrin and collagen deposition, and scar formation associated with inflammation

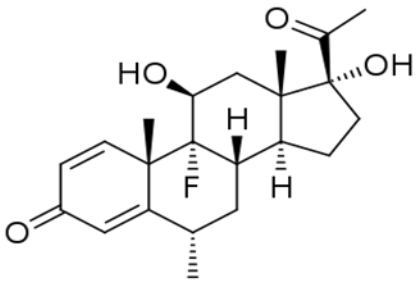

Fig 1: Structure of Fluorometholone

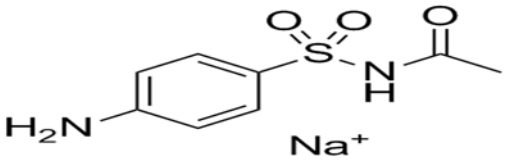

Fig 2: Structure of Sulfacetamide Sodium

Sulfacetamide Sodium (SULF) is chemically known as N-(4-aminophenyl) sulfonyl acetamide. Sulfacetamide Sodium is a antibiotic which inhibits bacterial folic acid synthesis by competing with para amino benzoic acid. With a broad spectrum of action, it is used as an anti-infective topical agent to treat skin infections and as an oral agent for urinary tract infections. Sulfacetamide sodium is a sulfonamide antibiotic. Sulfonamides are synthetic bacteriostatic antibiotics, that are active against gram-positive and gram-negative bacteria.

The combination of Fluorometholone and Sulfacetamide sodium is used for treatment of bacterial eye infections, conjunctivitis and also it reduces the severity of allergy symptoms such as inflammation, irritation and itchiness.

Literature survey reveals that Fluorometholone and Sulfacetamide sodium is a new combination. No method is available for simultaneous estimation of Fluorometholone and Sulfacetamide sodium. This study makes an attempt to establish simple, sensitive and accurate method for the simultaneous estimation of Fluorometholone and Sulfacetamide sodium in bulk and in combined dosage forms. In the view of the need for a suitable method for routine analysis in combined formulations, attempts are being made to develop simple, precise & accurate analytical methods for simultaneous estimation of titled drugs & extend it for their determination in formulation.

MATERIALS AND METHODS

Instrument

A high performance liquid chromatographic system (SHIMADZU Corporation, LC-20 AD), a Shimadzu SPD-20A UV/VIS detector was used for analysis. The data was recorded using Lab Solutions Software.

Chemicals and reagents

Acetonitrile (HPLC grade) and 1%Ortho Phosphoric Acid (HPLC grade) procured from Merk ltd, Mumbai, India were used as Mobile phase. All other chemical reagents were of analytical grade.

Drug sample

Fluorometholone was obtained as gift sample from Micro labs Ltd, Bangalore and Standard Sulfacetamide Sodium and was obtained from Yarrow Chem Products, Mumbai.

Preparation of mobile phase

A mixture of HPLC grade acetonitrile and Ortho Phosphoric Acid in the ratio of 70:30 v/v was prepared and pH was adjusted to 5.0 with triethyelene amine and filtered through 0.45 µm membrane filter paper and sonicated for 20 mins.

Preparation of standard stock solution

100 mg each of FLU and SULF were weighed separately and transferred into two different 100 mL volumetric flasks. Both the drugs were dissolved in 50 mL of mobile phase by sonication

and then volume was made up to the mark with mobile phase to get a concentration of 1000 µg/mL of each component (stock A and A' solution).

From the above stock A solution 10 mL of aliquot was pipetted out into a 100 mL volumetric flask and the volume was made up to the mark with mobile phase to obtain a concentration of 100 µg/mL of Fluorometholone (stock B solution) and for Sulfacetamide sodium 10 mL of stock A' was pipetted out into a 100mL volumetric flask and the volume was made upto the mark with mobile phase to obtain a concentration of 100 µg/mL (stock B' solution).

Preparation of sample solution

From the formulation, a quantity containing 100 mg of fluorometholone was measured accurately and transferred to to 100 mL of volumetric flask, volume was made up to mark with solvent to get 1000 µg/mL of fluorometholone (Stock A). The contents were sonicated for 15 min and the final volume was made up to the mark.

From Stock A 10 mL aliquot was taken and dissolved to 100mL with solvent to get A concentration of 100 µg/mL fluorometholone which also contains 50 µg/mL of Sulfacetamide sodium (Stock B).

Appropriate aliquots were prepared from the above sample stock "B" solution to get a concentration of 10, 20, 30, 40 and 50 µg/mL of FLUO which also contains 5, 10, 15, 20 and 25 µg/mL of SULF.From the above concentrations six replicates of 30 µg/mL of FLUO and 15 µg/mL of SULF were prepared and analyzed at the selected analytical wavelength of 254 nm and the results were statistically validated.

RESULTS AND DISCUSSIONS

The developed method for determination of Fluorometholone and Sulfacetamide Sodium was further validated by using following parameters:

Linearity

Linearity was established by least square regression analysis of the calibration curve. The constructed calibration curves were linear over the concentration range of 10-50 µg/mL for FLUO and 5-25 µg/mL for SULF respectively. Peak areas of FLUO and SULF were plotted with their respective concentrations and linear regression analysis was performed on the resultant curves. (fig 6 &7) The regression equation was found to be y = 30038x - 12620 (r^2 = 0.998) for FLUO and y = 84272x - 28196 (r^2 = 0.998) for SULF.

LOD and LOQ

The limit of detection (LOD) is defined as the lowest concentration of an analyte that an analytical process can reliably differentiate from back-ground levels. The limit of quantification (LOQ) is defined as the lowest concentration of the standard curve that can be measured with an acceptable accuracy, precision. In this study, LOD and LOQ were determined based on the standard deviation of the response and the slope of the corresponding curve using the following equations. LOD = 3.3 SD/Slope and LOQ = 10 SD/Slope. Where, SD is the standard deviation of the absorbance of the sample and the slope of the related calibrations curve. The LOD and LOQ of FLUO and SULF were found to be 0.0510 µg/mL and 0.1547 µg/mL, 0.0031 µg/mL and 0.0095 µg/mL respectively.

Accuracy

Accuracy studies were done as percent recovery, it was performed by adding constant amount of the standard drug to the sample taken from formulations at levels of 80%, 100% and 120% of the test concentration. The results are tabulated in (table 2).

Precision

The Intraday and Inter day precisions of the proposed method were determined by estimating the corresponding responses three times on the same day and on 3 different days over a period of one week for 3 different concentration and 3 replicates of FLUO and SULF and reported in terms of relative standard deviation (RSD). Statistical validation of data for Intraday and Inter day precision methods as shown in (table 3) and (table 4).

Robustness

The evaluation of robustness should be considered during the development phase and

depends upon the type of procedure under study. It should show the reliability of analysis with respect to deliberate variations in method parameters. The solution containing 30µg/ml of Fluorometholone and 15µg/ml of Sulfacetamide Sodium was injected into sample injector of HPLC three times under different parameters like deliberate variations in flow rate (table 5) and wavelengths (table 6).

Ruggedness

The evaluation of ruggedness should be considered during the development phase and depends upon the type of procedure under study. It should show the reliability of analysis with respect to deliberate variations in analyst or instrument. The solution containing 30µg/ml of Fluorometholone and 15µg/ml of Sulfacetamide Sodium was injected into sample injector of HPLC two times by different analysts. (table 7).

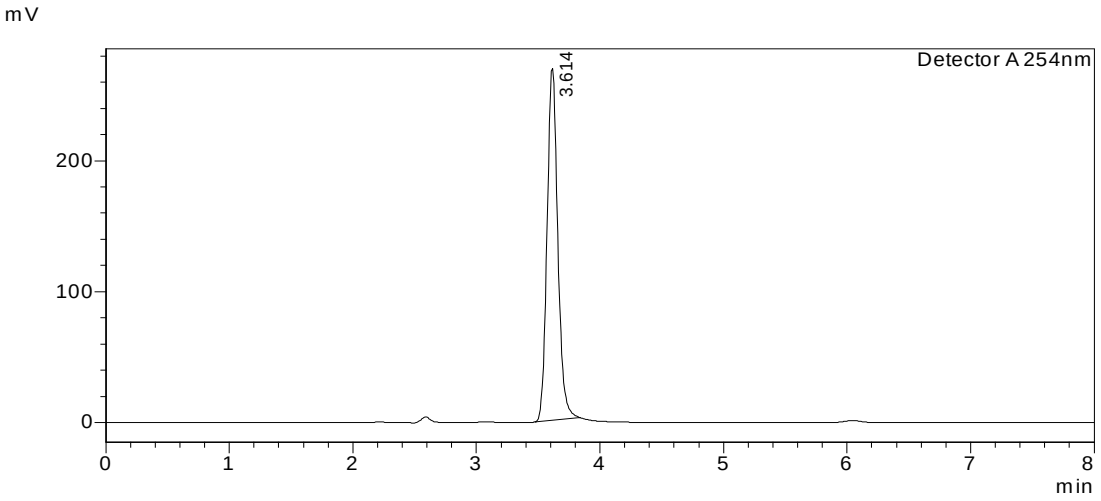

Fig. 3: Chromatogram of FLU at 254 nm

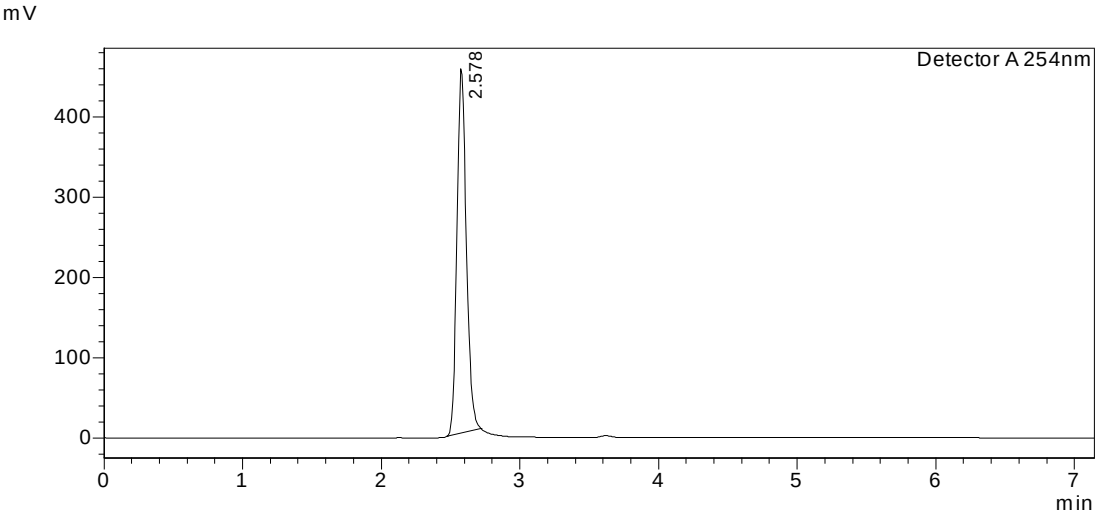

Fig. 4: Chromatogram of SULF at 254 nm

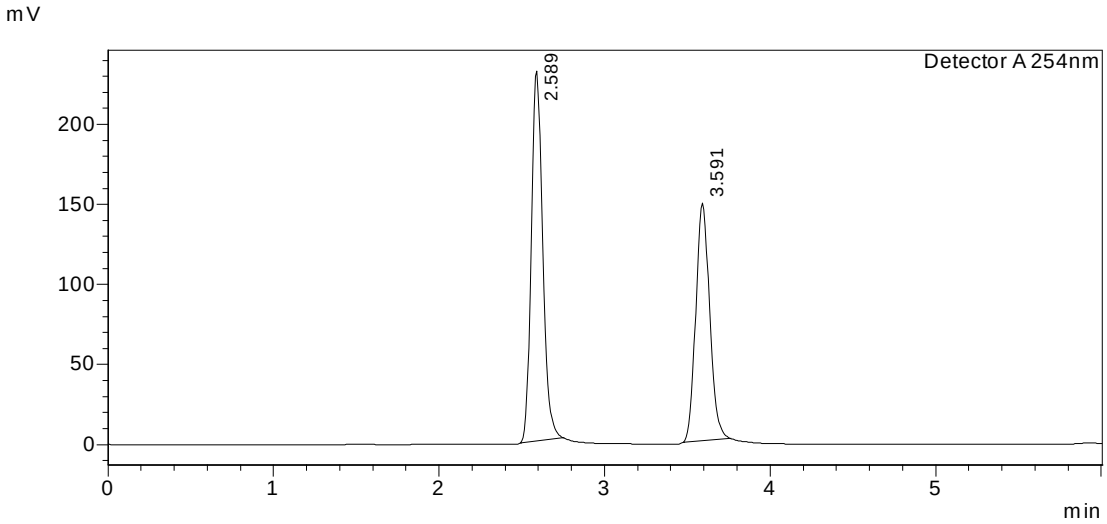

Fig. 5: Chromatogram of FLUO and SULF at 254 nm

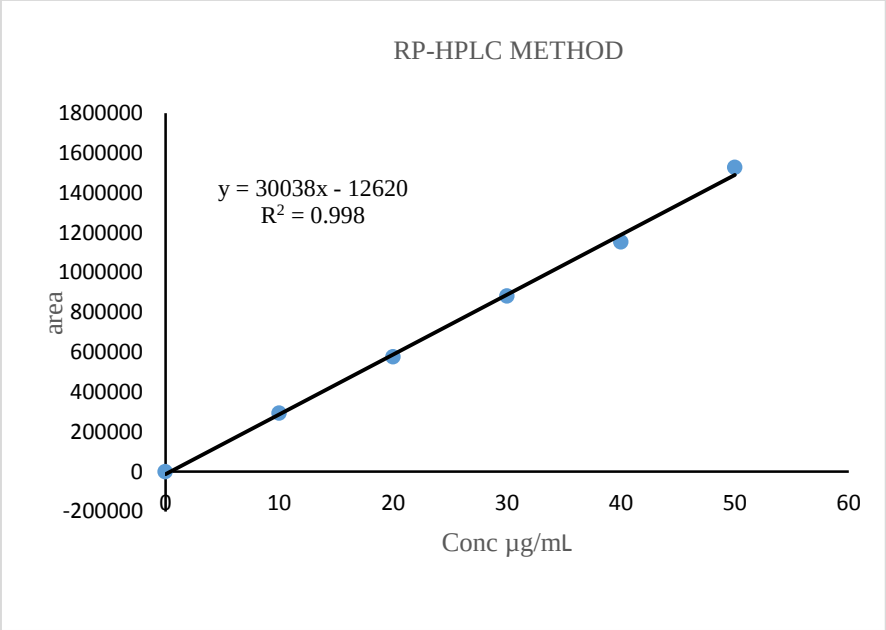

Fig. 6: Calibration curve for FLUO at 254 nm by RP-HPLC Method.

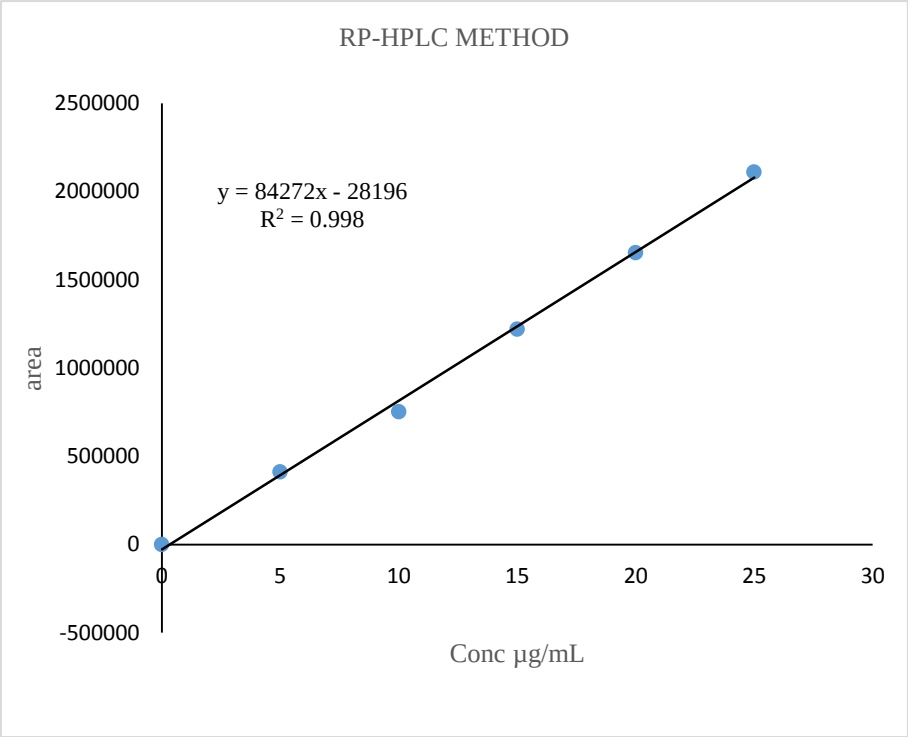

Fig. 7: Calibration curve for SULF at 254 nm by RP-HPLC Method.

Table 1: Summary of Validation Parameters by Developed Methods

Parameters	FLUO	SULFA
Linearity Range µg/mL	10-50	5-25
Slope	30038	84272
Intercept	12620	28196
Regression Coefficient (r^2)	0.998	0.998
Limit of Detection (µg/mL)	0.05105	0.003147
Limit of Quantification µg/mL	0.15470	0.009538
Retention time (min)	3.614	2.578
Tailing factor	1.142	1.182
Resolution factor	6.533	
Theoretical plate	7285	5501

Table 2: Statistical Validation Data for Accuracy Determination

Level of % recovery	Mean		Standard Deviation		Co-efficient of variation		Standard error	
	FLUO	SULF	FLUO	SULF	FLUO	SULF	FLUO	SULF
80%	100.03	100.3	0.6599	0.7128	0.6597	0.7101	0.2785	0.3923
100%	99.86	100.4	0.1626	0.5894	0.1628	0.5868	0.2726	0.3925
120%	100.04	100.1	0.3557	0.4239	0.3555	0.4234	0.2734	0.3926

*n = 3

Table 3: Statistical Validation Data for Intra-day Precision.

Components	Mean	Std. deviation	Co-efficient of variation	Standard error
FLUO	100.25	0.9075	0.9052	0.2785
SULF	100.29	1.2042	1.2007	0.3923

*n = 5

Table 4: Statistical Validation Data for Inter-day Precision.

Components	Mean	Std. deviation	Co-efficient of variation	Standard error
FLUO	100.288	0.9614	0.9586	0.2785
SULF	100.518	1.2488	1.2423	0.3923

n^* = 3

Table 5: Robustness result for variations in Flow Rate (mL/min).

Method Parameter	Level	Retention Time		Tailing factor	
Flow rate (mL/min)		**FLUO**	**SULF**	**FLUO**	**SULF**
0.9	-1	3.630	2.598	1.149	1.203
1.0	0	3.614	2.578	1.142	1.182
1.1	+1	3.591	2.545	1.156	1.197

Table 6: Robustness result for variations in Wavelength (nm).

Method Parameter	Level	Retention Time		Tailing factor	
Wavelength(nm)		**FLUO**	**SULF**	**FLUO**	**SULF**
252	-2	3.644	2.591	1.154	1.162
254	0	3.614	2.578	1.142	1.182
256	+2	3.598	2.542	1.181	1.193

Table 7: Ruggedness result for variations in Analyst.

Method Parameter	Retention Time		Tailing Factor	
Analysts	**FLUO**	**SULF**	**FLUO**	**SULF**
Analyst 01	3.614	2.558	1.142	1.182
Analyst 02	3.598	2.542	1.154	1.162

CONCLUSION

A simple, accurate, sensitive and precise HPLC method with UV detection for the simultaneous estimation of Fluorometholone and Sulfacetamide Sodium was developed and can be used for routine analysis. Above method was validated as per ICH guidelines.

Acknowledgement

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REFERENCE

[1]. Sethi PD. HPLC 'High Performance Liquid Chromatography', Quantitative Analysis of Pharmaceutical Formulation, CBS Publishers and Distributors, New Delhi, 1, 2001, 3-72, 116-120.

[2]. http://en.wikipedia.org/wiki/Fluorometholone.

[3]. https://pubchem.ncbi.nlm.nih.gov/Fluorometholone
[4]. www.everydayhealth.com/drugs/fluorometholone.
[5]. www.pdr.net/drug-summary/FML-Ophthalmic.
[6]. www.chemspider.com. Sulfacetamide sodium.
[7]. http://en.wikipedia.org./wiki/Sulfacetamide sodium.
[8]. https://pubchem.ncbi.nlm.nih.gov/Sulfacetamide sodium.
[9]. www.webmd.com/drugs/Sulfacetamide sodium.
[10]. Ramzia I El-bagary, Marwa A F, Monal A E and Enas H T. Stability -indicating RP-HPLC methods for the determination of Fluorometholone in its mixture with sodium cromoglycate and tetrahydrozile HCl. J of Chromatogr Sci, 54(6), 2016, 923-33.
[11]. Narendra A and Annapurna M M. Development and validation of Stability Indicating HPLC Method for the determination of Fluorometholone in Eye drops formulations. Acta Scientific Pharmaceutical Sciences, 2(11), 2018, 07-14.
[12]. Melvin H. Penner et al., Assay of sulfacetamide Sodium in ophthalmics by High Performance Liquid Chromatography. J of Ph Sci. 64(6), 1975, 1017-1019.