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DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC METHOD FOR ESTIMATION FLUOCINOLONE ACETONIDE IN BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A rapid, simple, selective, sensitive, precise and specific UV Spectrophotometric method has been developed for the determination of Fluocinolone acetonide in bulk and(200-400nm) in 1cm quartz cell in a double beam UV Spectrophotometer. The spectrophotometric detection was carried out at an absorption maximum of 235 nm using Methanol as solvent. The detector response for the Fluocinolone acetonide was linear over the selected concentration range 1.25-6.25µg /ml with a correlation coefficient of 0.999and equation for the regression curve was found to be *y=0.1596x + 0.0175*. The accuracy was between 99-100%. The precision (%RSD) among six samples preparation was 0.121%.The LOD and LOQ was 0.1125 and 0.309µg /ml respectively. Statistical analysis proved that the methods are repeatable and specific for the determination of the said drug. These methods can be adopted in the routine assay analysis of Fluocinolone acetonidein bulk and pharmaceutical dosage form.

KEYWORDS

Fluocinolone acetonide, UV Spectrophotometer, Methanol and Method Validation.

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INTRODUCTION

Fluocinolone acetonide (6α, 9α-difluoro-11β, 21-dihydroxy-16α, 17α-isopropylidenedioxypregna –1, 4 - diene-3, 20– dione) is adrenocortical steroid used topically for treatment of variety of skin disorders and inflammatory eye, ear and nose disease. It has high anti-inflammatory activity[1,2].

Fluocinolone Acetonide is the acetonide salt form of fluocinolone, a synthetic fluorinated corticosteroid with antiinflammatory, antipruritic

and vasoconstrictive properties. Fluocinolone is a glucocorticoid receptor agonist that binds to cytoplasmic glucocorticoid receptors and subsequently translocates to the nucleus where it initiates the transcription of glucocorticoid-responsive genes such as lipocortins. Lipocortins inhibit phospholipase A2, thereby blocking the release of arachidonic acid from membrane phospholipids and preventing the synthesis of prostaglandins and leukotrienes, both are potent mediators of inflammation. Fluocinolone exerts its vasoconstrictive effect through inhibition of nitric oxide synthase, thereby blocking nitric oxide production and effectively diminishing the effect of nitric oxide on vascular smooth muscles leading to reduced blood flow[1,3].

Fluocinolone acetonide is aglucocorticoids (6α, 9α-difluoro-11β, 21-dihydroxy-16α, 17α-isopropylidenedioxypregna –1, 4 - diene-3, 20–dione)[3].

Fluocinolone acetonideit is a white crystalline powder which is soluble in Acetone, Methanol, slightly soluble in Chloroform[3].

The present work is a simple, sensitive, accurate and precise Spectrophometric Method for the estimation of Fluocinolone acetonidein bulk and its Pharmaceutical Dosage Forms with the help of Methanol.

MATERIAL AND METHODS

Instruments

For weighing, a calibrated weighing balance (Shimadzu) of 1mg sensitivity was used. A Systronic UV-visible double beam spectrophotometer- 2201 was used. All the glass wares and were made of borosilicate and were calibrated.

Chemicals

API- Fluocinolone acetonide pure drug was gifted by Aadhaar life sciences Pvt Ltd. Solapur.

Fluocinolone acetonide topical solution 0.01%w/v strength were purchased from the local pharmacy in Solapur under commercially available brand name FLUCORT (Glenmark Pharmaceutical Ltd.), Methanol LR was used in this study.

UV Spectroscopic Method

Solvent Selection

Fluocinolone acetonide is soluble in Methanol so, methanol is used as the solvent.

Preparation of Standard Stock Solution

The standard stock solution Fluocinolone acetonide (FLU) was prepared by transferring accurately weighed 10 mg of Fluocinolone acetonide into 10 ml volumetric flask containing Methanol dissolved properly. Then volume was made up to the mark by using Methanol to give a concentration of 1000µg/ml. From this, 1ml of the solution was transferred to a 10ml volumetric flask and make up the volume with Methanol to give a concentration of 100µg/ml. From this, 2.5ml of the solution was transferred to a 10ml volumetric flask and make up the volume with Methanol to give a concentration of 25µg/ml which is a standard stock solution. And it is further diluted with Methanol to get concentration range of 1.25-6.25µg/ml.

Determination of Absorption Maxima

The standard stock solution of 10µg/ml was scanned in the range of 200-400nm to determine the wavelength of Maximum Absorption. The drug showed Absorption maxima at 235nm.

Preparation of Calibration Curve

For the preparation of calibration curve, the concentration of1.25-6.25µg/ml were prepared by pipetting out 0.5, 1, 1.5, 2, 2.5ml of the 25µg/ml solution into 10 ml volumetric flasks and made up the volume with Methanol.

The absorbance of each solution was measured at 235 nm against Methanol as blank. Calibration curve of the Fluocinolone acetonide was plotted by taking the absorbance obtained on the y-axis and concentration of the solution on the x-axis. The curve showed linearity in the range of 1.25-6.25µg/ml with correlation coefficient 0.999.

Quantitative Analysis of Topical Solution

The whole content of the topical solution was transferred in to the 50ml volumetric flask and volume was made with the Methanol. The solution was sonicated for 10 minutes. An appropriate dilution was prepared from this solution to get sample concentration (10µg/ml) in the range of linearity for spectroscopic method. The absorbance

of sample solution was observed in multipoint calibration curve as a quantitative mode at 235nm to get the concentration.

Method Validation

The developed method was validated as per ICH guidelines for the following parameters:

Linearity

0.5, 1, 1.5, 2, 2.5ml of standard FLU solution was transferred into a series of 10 ml volumetric flasks. The volume was made up to the mark with Methanol to obtain the concentration of 1.25, 2.5, 3.75, 5, 6.25µg/ml. Then absorption of these solutions was recorded and the graph was plotted of absorption against concentration. The correlation coefficient (r^2) of least square linear regression of FLU was calculated.

Range

The Range of the analytical method was decided from the interval between upper and lower level of calibration curve by plotting curve.

Accuracy

Recovery study was carried out by the standard addition method by adding a known amount of FLU to the pre-analyzed sample at three different concentration levels that is 80%, 100%, 120% of assay concentration and percent recovery were calculated. 1 ml of topical solution was transferred to 4 different 10 ml volumetric flasks separately and 0.8, 1, 1.2ml from 100µg/ml standard solution was added respectively and the volume was made up to the mark with Methanol. Absorbances were noted for these samples. Standard deviation and %RSD was calculated. Accuracy is reported as % recovery, which was calculated from the expression as equation given below:

% Recovery = Observed value / True value×100

Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scattering) between a series of measurements obtained from multiple sampling of the same sample under the prescribed conditions. The precision of the method was determined in terms of repeatability and intra-day and inter-day precisions. Intra-day and inter-day precision (Intermediate Precision)

Intraday precision was determined by analyzing the drugs at concentrations (5µg/ml) and each concentration for three times, on the same day. Inter-day precision was determined similarly, but the analysis being carried out daily, for two consecutive days.

Repeatability

Repeatability of the method was determined by analyzing six samples of same concentrations of the drug (5µg/ml). Absorbance of each was measured.

Robustness

The robustness of the developed method is its capacity to remain unaffected by small changes in altered conditions. To determine the robustness of the method, the wavelength of analysis was deliberate and the assay was evaluated. The effect of detection wavelength was studied at ±5 nm.

Ruggedness

Ruggedness was determined by carrying out analysis by two different analysts and the respective absorbance was noted and the results were indicated as % RSD.

Limit of Detection

Detection limit was determined based on the standard deviation of absorbance of same concentration that is a standard solution of FLU (5µg/ml) and LOD calculated by LOD = 3.3(SD/S) Where, SD- standard deviation; S= slope of the curve

Limit of Quantification

Quantification limit was determined based on the standard deviation of peak area of same concentration that is standard solution FLU (5µg/ml) prepared six times and LOQ calculated by LOD = 10(SD/S) Where, SD= standard deviation; S= slope of Curve.

RESULTS AND DISCUSSION

Determination of wavelength of maximum absorption the wavelength of maximum absorption was found to be 235nm.

Linearity

The linearity of this method was determined at ranges from 1.25-6.25 µg/ml for Fluocinolone acetonide. The regression equation was found to be $Y=0.1596x + 0.0175$, $R^2=0.999$.

The linearity for Fluocinolone acetonide was found to be linear in the range of 1.25-6.25µg/ml with R^2= 0.999 and the straight line equation as *y= 0.1596x + 0.0175*.

Accuracy

The accuracy of the analytical method for Fluocinolone acetonide was determined at 80%, 100% and 120% levels of standard solution. Absorbance was measured at 235 nm and results were expressed in terms of % recoveries.

Precision

The precision (measurement of intra-day, inter-day, repeatability) results showed good reproducibility with the relative standard deviation (% RSD) below 2.0 %. This indicated that method was highly precise.

Ruggedness (5µg)

Ruggedness was determined by carrying out analysis by two different analysts and the respective absorbance was noted and the results were indicated as % RSD.

Preliminary Analysis of Fluocinolone acetonide

Preliminary analysis of Fluocinolone acetonide such as description, solubility was performed.

UV-spctrophotometry for Fluocinolone acetonide

Fluocinolone acetonide being UV absorbing has been successfully employed for its quantitative determination by UV Spectrophotometric method. Being soluble in Methanol, stock solutions and working standards were prepared in Methanol.

The maximum wavelength of absorption of drug was determined by taking scan of the drug solution in the UV region (200-400 nm). The correlation of the standard curve for the drug was 0.999. The accuracy was from 99-100%at 235nm. The proposed method showed absorption maxima at235nm and obeyed Beer's law in the concentration range of 1.25-6.25µg/ml. The limit of detection (LOD) was found to be 0.1125µg/ml and limit of quantification (LOQ) to be 0.3409µg/ml respectively. All statistical data prove validity of the proposed method, which can be applied for routine analysis of Fluocinolone acetonide.

Assay of Topical Solution

Amount of drug present in solution was calculated using equation at 235 nm, and *y=0.1596x + 0.0175* and amount of Fluocinolone acetonide were found to be 102% of label claim respectively. This method can be employed for routine analysis of Fluocinolone acetonide.

Summary and conclusion

Summary of UV Spectrophotometeric Method of Fluocinolone acetonide.

Table No.1: Results obtained in the determination of FLU in dosage form

S.No	formulation	Label claim	Amount taken	Amount found	Assay%
1	FLU(0.01% w/v)	10 µg/ml	10 µg/ml	10.25µg/ml	102%

Table No.2: Linearity table

S.No	Conc.	Absorbance
1	1.25	0.215
2	2.5	0.425
3	3.75	0.61
4	5	0.81
5	6.25	1.02

Table No.3: Table for accuracy

S.No	Level of % Recovery	Amount present (µg/ml)	Amount of standard drug added (µg/ml)	Amount recovered (µg/ml)	% Recovery
1	0	10	0	0	100%
2	80	10	8	17.89	99%
3	100	10	10	19.90	99.5%
4	120	10	12	21.90	99.54%

Intra-day Precision

Table No.4: Intra-day precision

S.No	Concentration (µg/ml)	Absorbance	SD	% RSD
1	5	0.81		
2	5	0.811		
3	5	0.812	0.000983	0.121
4	5	0.812		
5	5	0.81		
6	5	0.810		
		$\bar{y}$ = 0.810833		

Inter-day Precision

Table No.5: Inter-day precision study

S.No	Concentration (µg/ml)	Absorbance	SD	%RSD
1	5	0.810		
2	5	0.812		
3	5	0.812	0.001095	0.135
4	5	0.812		
5	5	0.810		
6	5	0.810		
		$\bar{y}$ = 0.811		

Repeatability

Table No.6: Repeatability study

S.No	Concentration (µg/ml)	Absorbance	SD	%RSD
1	5	0.80		
2	5	0.80		
3	5	0.801	0.005441	0.676
4	5	0.801		
5	5	0.811		
6	5	0.811		
		$\bar{y}$ = 0.804		

Limit of Detection

Table No.7: For Limit of Detection

LOD (µg/ml)	0.1125 µg/ml

Limit of Quantification

Table No.8: For Limit of Quantification

LOQ (µg/ml)	0.3409 µg/ml

Robustness (5µg)

Table No.9: Robustness study

S.No	Wavelength (nm)	Absorbance	SD	%RSD
1	235	0.81		
2	236	0.81	0.005774	0.7158
3	237	0.80		
		$\bar{y}$ =0.806667		

Table No.10: For Ruggedness

S.No	Analyst-1		
	Concentration (µg/ml)	Absorbance	Statistically analysis
1	5	0.81	Mean = 0.806667
2	5	0.81	SD = 0.005774
3	5	0.80	% RSD = 0.7158
Analyst-2			
4	5	0.810	Mean = 0.811333
5	5	0.812	SD=0.001155
6	5	0.812	%RSD=0.1423

Table No.11: For Summary

S.No	Parameters	Values
1	Beer's Law limit (µg/ml)	1.25-6.25
2	Absorption maxima (nm)	235
3	Standard regression equation	0.1596x + 0.0175
4	Correlation coefficient (R^2)	0.999
5	Accuracy	99-100%
6	Precision (%RSD) Repeatability	0.121
7	LOD (µg/ml)	0.1125
8	LOQ (µg/ml)	0.3409
9	Robustness (%RSD)	0.7158
10	Ruggedness	0.7158 and 0.1423
11	Assay (%)	102%

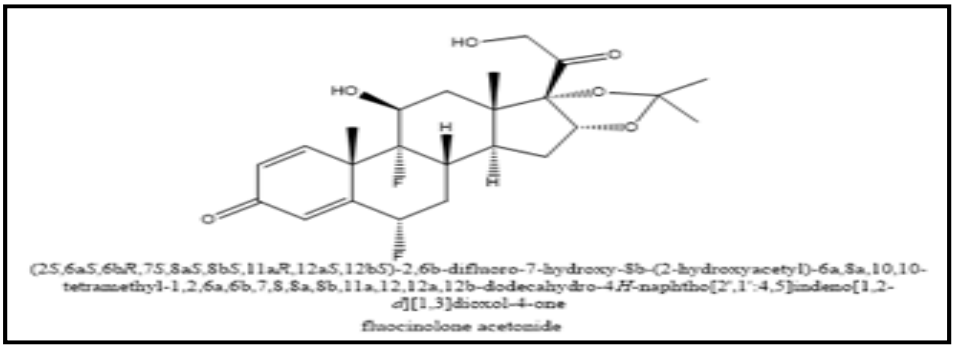

Figure No.1: Chemical structure of Fluocinolone acetonide

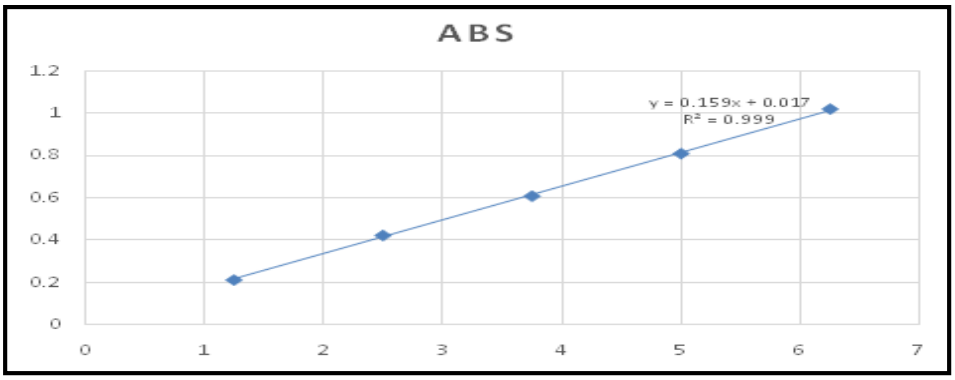

Figure No.2: Calibration curve of Fluocinolone acetonide (FLU)

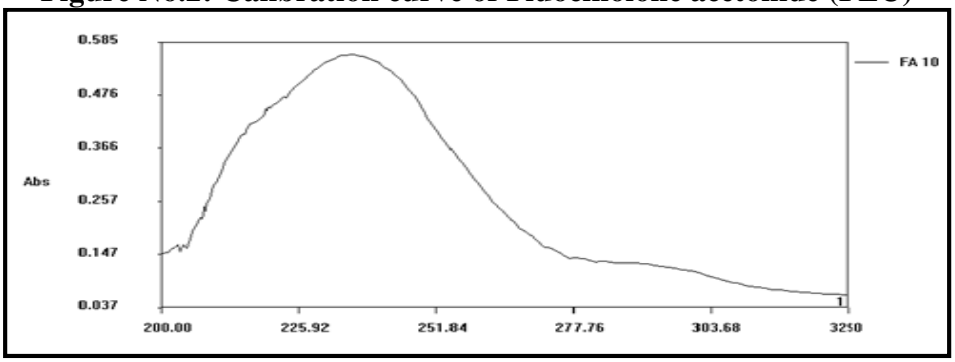

Figure No.3: Wavelength of maximum absorption of Fluocinolone acetonide

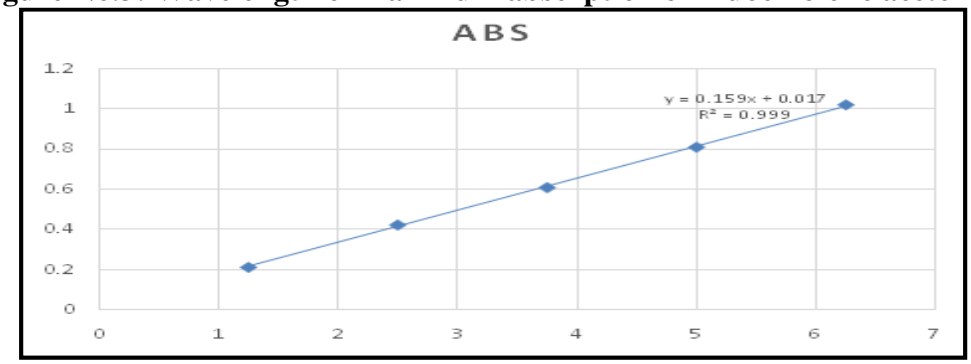

Figure No.4: Linearity graph of Fluocinolone acetonide

CONCLUSION

The UV-Spectrophotometric method was developed and it is found to be simple, accurate, precise, highly sensitive, reproducible and inexpensive. The proposed method was found suitable for determination of Fluocinolone acetonide in bulk and its dosage form without any interference from the excipients. This method can be effectively applied for the routine analysis of Fluocinolone acetonide in bulk. Its advantages are the low cost of reagents, speed and simplicity of sample treatment, satisfactory precision and accuracy.

ABBREVIATIONS

UV- Ultra Violet
API- Active Pharmaceutical Ingredient
FLU- Fluocinolone acetonide

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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