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Research

A Validated Rp-Hplc Method For The Simultaneous Estimation Of Ciprofloxacin And Fluocinolone In Bulk Form And Pharmaceutical Dosage Forms

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Abstract

A precise, simple, accurate and selective method was developed and validate for simultaneous estimation of Ciprofloxacin and Fluocinolone in API form and Pharmaceutical Dosage Form. Reversed phase high performance liquid chromatographic (RP-HPLC) method was developed for routine quantification of Ciprofloxacin and Fluocinolone in the API form as well as in combined pharmaceutical dosage form. Chromatographic separation was achieved on a Phenomenex Gemini C18 (4.6mm×250mm) 5µm particle size utilizing mobile phase of filtered and degassed mixture of Methanol and Phosphate buffer (pH-3.8) (40:60% v/v) at a flow rate of 1.0mL/min with UV detection at 225nm. The method has been validated for linearity, accuracy and precision. In RP-HPLC method, the calibration graphs were linear in the concentration range of 10-30 µg/ml for Ciprofloxacin and 30-90µg/ml for Fluocinolone with percentage recoveries are within the limits. The results obtained by RP-HPLC methods are rapid, accurate and precise. Therefore proposed method can be used for routine analysis of Ciprofloxacin and Fluocinolone in the API form as well as in combined pharmaceutical dosage form.

Keywords: Ciprofloxacin and Fluocinolone, RP-HPLC, Validation, ICH Guidelines.

INTRODUCTION

Analysis may be defined as the science and art of determining the composition of materials in terms of the elements or compounds contained in them. In fact, analytical chemistry is the science of chemical identification and determination of the composition (atomic, molecular) of substances, materials and their chemical structure.

Chemical compounds and metallic ions are the basic building blocks of all biological structures and processes which are the basis of life. Some of these naturally occurring compounds and ions (endogenous species) are present only in very small amounts in specific regions of the body, while others such as peptides, proteins, carbohydrates, lipids and nucleic acids are found in all parts of the body. The main object of analytical chemistry is to develop scientifically substantiated methods that allow the qualitative and quantitative evaluation of materials with certain accuracy. Analytical chemistry derives its principles from various branches of science like chemistry, physics, microbiology, nuclear science and electronics. This method provides information about the relative amount of one or more of these components. [1]

Every country has legislation on bulk drugs and their pharmaceutical formulations that sets standards and obligatory quality indices for them. These regulations are presented in separate articles relating to individual drugs and are published in the form of book

called "Pharmacopoeia" (e.g. IP, USP, and BP). Quantitative chemical analysis is an important tool to assure that the raw material used and the intermediate products meet the required specifications. Every year number of drugs is introduced into the market. Also quality is important in every product or service, but it is vital in medicines as it involves life.

There is a time lag from the date of introduction of a drug into the market to the date of its inclusion in pharmacopoeias. This happens because of the possible uncertainties in the continuous and wider usage of these drugs, report of new toxicities and development of patient resistance and introduction of better drugs by the competitors. Under these conditions standard and analytical procedures for these drugs may not be available in Pharmacopoeias. In instrumental analysis, a physical property of the substance is measured to determine its chemical composition. Pharmaceutical analysis comprises those procedures necessary to determine the identity, strength, quality and purity of substances of therapeutic importance. [2]

Pharmaceutical analysis deals not only with medicaments (drugs and their formulations) but also with their precursors i.e. with the raw material on which degree of purity and quality of medicament depends. The quality of the drug is determined after establishing its authenticity by testing its purity and the quality of pure substance in the drug and its formulations.

Quality control is a concept which strives to produce a perfect product by series of measures designed to prevent and eliminate errors at different stages of production. The decision to release or reject a product is based on one or more type of control action. With the growth of pharmaceutical industry during last several years, there has been rapid progress in the field of pharmaceutical analysis involving complex instrumentation. Providing simple analytical procedure for complex formulation is a matter of most importance. So, it becomes necessary to develop new analytical methods for such drugs. In brief the reasons for the development of newer methods of drugs analysis are:

1. The drug or drug combination may not be official in any pharmacopoeias.
2. A proper analytical procedure for the drug may not be available in the literature due to Patent regulations.
3. Analytical methods for a drug in combination with other drugs may not be available.
4. Analytical methods for the quantitation of the drug in biological fluids may not be available.

The existing analytical procedures may require expensive reagents and solvents. It may also involve cumbersome extraction and separation procedures and these may not be reliable.

Based on type of analysis

Qualitative analysis*:* Which is used to identify the compound,detect the presence of impurities to find out the number of components. This is done by using retention time values.

Quantitative analysis: This is done to determine the quantity of individual or several components of mixture. This is done by comparing the peak area of the standard and sample.

INSTRUMENTATION OF HPLC

The basic liquid chromatograph consists of six basic units. The mobile phase supply system, the pump and programmer, the sample valve, the column, the detector and finally a means of presenting and processing the results.

Mobile phase (solvent) reservoirs and solvent degassing

The mobile phase supply system consists of number of reservoirs (200 mL to 1,000 mL in capacity). They are usually constructed of glass or stainless steel materials which are chemically resistant to mobile phase**.**

Mobile phase

Mobile phases in HPLC are usually mixtures of two or more individual solvents. The usual approach is to choose what appears to be the most appropriate column, and then to design a mobile phase that will optimize the retention and selectivity of the system. The two most critical parameters for nonionic mobile phases are strength and selectivity.

Mobile phase preparation

Mobile phases must be prepared from high purity solvents, including water that must be highly purified. Mobile phases must be filtered through ≤1 μm pore size filters and be degassed before use.

Degassing of solvents

Many solvents and solvent mixtures (particularly aqueous mixtures) contain significant amounts of dissolved nitrogen and oxygen from the air. These gasses can form bubbles in the chromatographic system that cause both serious detector noise and loss of column efficiency. These dissolved gases in solvent can be removed by the process of degassing. Every solvent must be degassed before introduction into pump as it alter the resolution of column and interfere with monitoring of the column effluent.

Degassing is done in many ways:

- By warming the solvents
- By stirring vigorously with a magnetic stirrer
- By subjecting tovaccum filtration
- By ultra sonication (using ultrasonicator)

By bubbling He gas through the solvent reservoir.

Pumping systems

The pumping system is one of the most important features of an HPLC system. There is a high resistance to solvent flow due to the narrow columns packed with small particles and high pressures are therefore required to achieve satisfactory flow rate.
The main requirements of pumping systems are:

1. Generation of pressures up to 6000 psi.
2. Pulse free output
3. Flow rates ranging from 0.01 to 10 mL/min
4. Flow control and flow reproducibility of ± 0.5%
5. Corrosion resistant components (seals of Teflon and stainless steel)
6. Should be easy to dismantle and repair.

There are three basic types of pumps in common use.

1. Reciprocating pumps.
2. Displacement pumps or syringe pumps.
3. Pneumatic pumps or constant pressure pumps.

Sample introduction system

Injection ports are of two basic types,

1. The sample is injected directly into the column.
2. The sample is deposited before the column inlet and then swept by a valving action into the column by the mobile phase.

Injectors should provide the possibility of injecting the liquid sample within the range of 0.1 to 100 mL of volume with high reproducibility and under high pressure (up to the 4000psi).They should also produce minimum band broadening and minimize possible flow disturbances. The most useful and widely used sampling device for modern LC is the micro sampling injector valve. With these sampling valves, samples can be introduced reproducibly into pressurized columns without significant interruption of flow, even at elevated temperatures. High-performance valves provide extra column band-broadening characteristics comparable or superior to that of syringe injection.

Columns

Typical analytical columns are 10, 15 and 25 cm in length and are fitted with extremely small diameter (3, 5 or 10 μm) particles. The internal diameter of the columns is usually 4 or 4.6 mm; this is considered the best compromise among sample capacity, mobile phase consumption, speed and resolution. Preparative columns are of larger diameter. Packing of the column tubing with the small diameter particles requires high skill and specialized equipment. For this reason, it is generally recommended that the most experienced chromatographers purchase prepacked columns, since it is difficult to match the high performance of professionally packed LC columns without a large investment in time and equipment. The column can be classified based on the material bonded to the silica packed surface such as C_4, C_8, C_{18}, phenyl, chiral, cyanomicrobore columns (1mm to 100cm), U shaped and coiled columns are available. Guard columns are used before the analytical columns to increase the life of analytical columns by retaining non eluted components and particulate matter.

Column Thermostats

Control of column temperature is important in liquid chromatography. The effect of temperature on retention times and reproducibility is quite significant, especially when using the reverse phase models.

Detectors

Optical detectors are most frequently used. These detectors pass a beam of light through the flowing column effluent as it passes through a low volume (~ 10 mL) flow cell. The most commonly used detector in LC is the ultraviolet absorption detector. A variable wavelength detector of this type, capable of monitoring from 190 to 460-600 nm, will be found suitable for the detection of the majority samples. Other types of Detectors:

1. UV detector
2. Refractive index detector
3. Flourimetric detector
4. Conductivity detector
5. Amperometric detector
6. Photodiode array detector (PDA detector).

Data handling

Data handling in chromatography now ranges from a simple pen recorder to complicated computer integration and computerized data handling systems. Several manufacturers today offered microprocessor controlled chromatographs. Thus the solvent delivery system, injector, oven, detector, fraction collector and data reduction can be carried under the control of a central microprocessor with the capability to program sequential parameters.

MATERIALS AND METHODS

Ciprofloxacin (Pure)-Sura labs, Fluocinolone (Pure)-Sura labs, Water and Methanol for HPLC-LICHROSOLV (MERCK), Acetonitrile for HPLC- Merck

HPLC METHOD DEVELOPMENT
TRAILS

Preparation of standard solution: Accurately weigh and transfer 10 mg of Ciprofloxacin and Fluocinolone working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol. Further pipette 0.2ml of Ciprofloxacin and 0.6ml of Fluocinolone from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

***Procedure*:** Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Mobile Phase Optimization: Initially the mobile phase tried was Methanol: WaterandACN: Water with varying proportions. Finally, the mobile phase was optimized to Methanol and Phosphate buffer in proportion 40:60 v/v respectively.

Optimization of Column: The method was performed with various C18columns like Symmetry, X terra and ODS column. Phenomenex Gemini C18 (4.6×250mm) 5µm was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

OPTIMIZED CHROMATOGRAPHIC CONDITIONS

Instrument used	: Waters Alliance 2695 HPLC with PDA Detector 996 model.
Temperature	: 35ºC
Column	: Phenomenex Gemini C18 (4.6×250mm) 5µm particle size
Mobile phase	: Methanol and Phosphate buffer(pH-3.8) (40:60% v/v)
Flow rate	: 1ml/min
Wavelength	: 225nm
Injection volume	: 20µl
Run time	: 6minutes

VALIDATION
PREPARATION OF MOBILE PHASE

Preparation of mobile phase

Accurately measured 400ml of Methanol (40%) of and 600ml of HPLC Water (60%) were mixed and degassed in adigital ultrasonicater for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation

The Mobile phase was used as the diluent.

RESULTS AND DISCUSSION

Optimized Chromatogram (Standard)

Mobile phase ratio	: Methanol and Phosphate buffer(pH-3.8) (40:60% v/v)
Column	: Phenomenex Gemini C18 (4.6×250mm) 5µm particle size
Column temperature	: 35ºC
Wavelength	: 225nm
Flow rate	: 1ml/min
Injection volume	: 20µl
Run time	: 6minutes

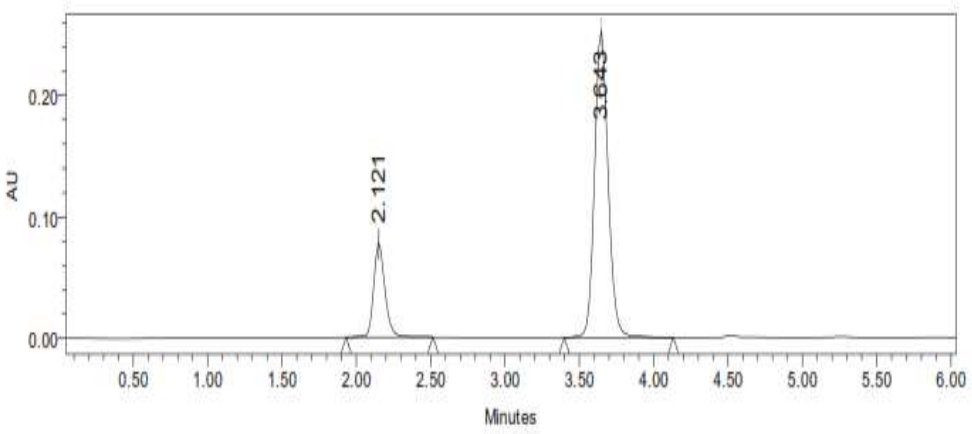

Fig 1: Optimized Chromatogram (Standard)

Table 1: Optimized Chromatogram (Standard)

S.no	Name	RT	Area	Height	USPTailing	USPPlate Count	Resolution
1	Ciprofloxacin	2.121	513567	78659	1.2	4536	
2	Fluocinolone	3.643	1625892	265321	1.1	7985	9.8

From the above chromatogram it was observed that the Ciprofloxacin and Fluocinolone peaks are well separated and they shows proper retention time, resolution, peak tail and plate count. So it's optimized trial.

Optimized Chromatogram

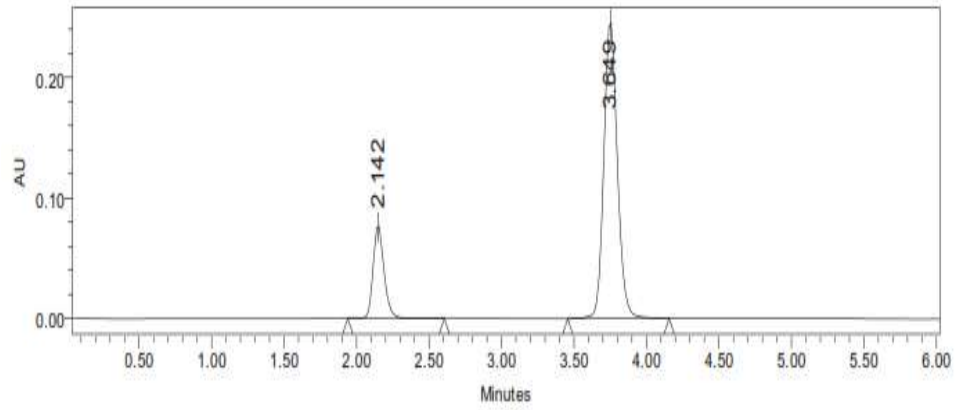

Fig 2: Optimized Chromatogram (Sample)

Table 2: Optimized Chromatogram (Sample)

S.no	Name	Rt	Area	Height	USPTailing	USPPlate Count	Resolution
1	Ciprofloxacin	2.142	512659	78956	1.2	4652	
2	Fluocinolone	3.649	1615985	263587	1.1	7982	10.3

- Resolution between two drugs must be not less than 2.
- Theoretical plates must be not less than 2000.
- Tailing factor must be not less than 0.9 and not more than 2.
- It was found from above data that all the system suitability parameters for developed method were within the limit.

System suitability

Table 3: Results of system suitability for Ciprofloxacin

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Ciprofloxacin	2.152	513652	78542	4698	1.2

2	Ciprofloxacin	2.157	513524	78654	4785	1.2
3	Ciprofloxacin	2.141	513425	78541	4682	1.2
4	Ciprofloxacin	2.133	513647	78454	4854	1.2
5	Ciprofloxacin	2.166	514824	78655	4872	1.2
Mean			513814.4			
Std.Dev.			572.2004			
%RSD			0.111363			

- *%RSD of five different sample solutions should not more than 2.*
- *The %RSD obtained is within the limit, hence the method is suitable.*

Table 4: Results of system suitability for Fluocinolone

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing	Resolution
1	Fluocinolone	3.674	1635285	265421	7985	1.1	10.1
2	Fluocinolone	3.631	1635241	265484	7898	1.1	10.1
3	Fluocinolone	3.625	1652547	253498	7954	1.1	10.1
4	Fluocinolone	3.692	1658458	265241	7965	1.1	10.1
5	Fluocinolone	3.629	1652894	265348	7985	1.1	10.1
Mean			1646885				
Std.Dev.			10865.58				
%RSD			0.659766				

- *%RSD of five different sample solutions should not more than 2.*
- *The %RSD obtained is within the limit, hence the method is suitable.*

Assay (Standard)

Table 5: Peak results for assay standard of Ciprofloxacin

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Ciprofloxacin	2.152	513538	78074	1.2	4562	1
2	Ciprofloxacin	2.198	513975	79001	1.2	4620	2
3	Ciprofloxacin	2.179	513283	78048	1.2	4652	3

Table 6: Peak results for assay standard of Fluocinolone

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Fluocinolone	3.646	1625632	265325	1.1	7949	1
2	Fluocinolone	3.604	1635458	265423	1.1	7919	2
3	Fluocinolone	3.610	1635241	265874	1.1	7926	3

Assay (Sample)

Table 7: Peak results for Assay sample of Ciprofloxacin

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Ciprofloxacin	3.651	513265	78548	1.2	4582	1
2	Ciprofloxacin	2.150	513254	78547	1.2	4658	2
3	Ciprofloxacin	2.187	513876	78498	1.2	4597	3

Table 8: Peak results for Assay sample of Fluocinolone

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Fluocinolone	3.646	1625284	78569	1.1	7985	1
2	Fluocinolone	3.651	1624613	78547	1.1	7898	2
3	Fluocinolone	3.601	1625874	78462	1.1	7854	3

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample Purity}}{\text{Weight of sample 100}} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

The % purity of Ciprofloxacinand Fluocinolonein pharmaceutical dosage form was found to be 99.8%

LINEARITY

CHROMATOGRAPHIC DATA FOR LINEARITY STUDY OF CIPROFLOXACIN

Concentration µg/ml	Average Peak Area
10	245899
15	365687
20	481526
25	589854
30	705882

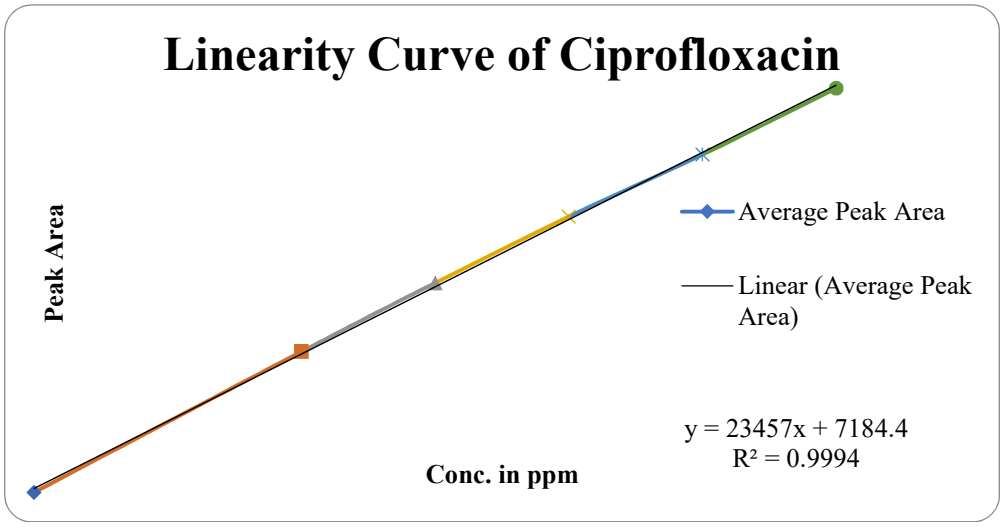

Fig 3: Calibration Graph of Ciprofloxacin

CHROMATOGRAPHIC DATA FOR LINEARITY STUDY OF FLUOCINOLONE

Concentration µg/ml	Average Peak Area
30	863094
45	1249397
60	1678592
75	2050412
90	2468444

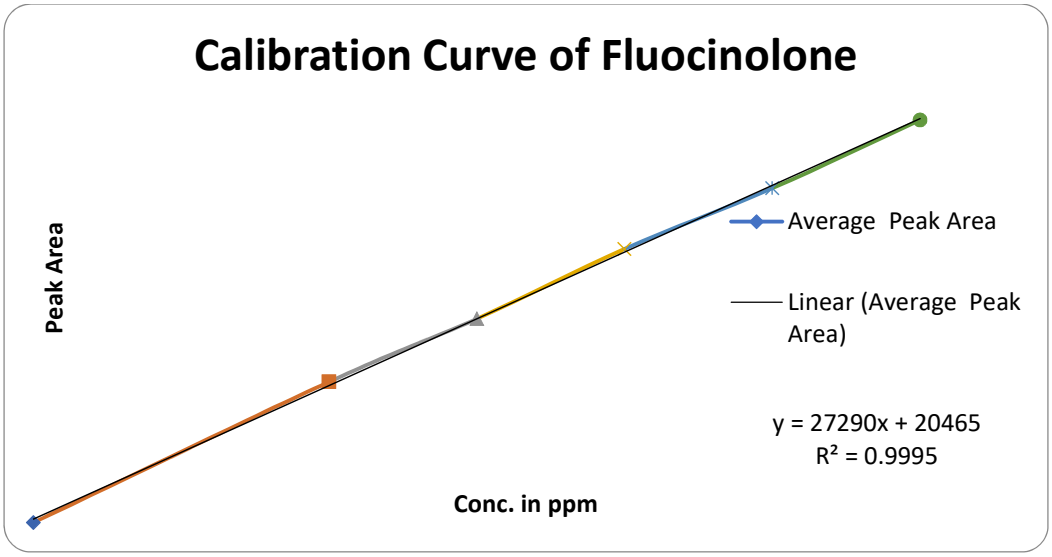

Fig 4: Calibration Curve of Fluocinolone

REPEATABILITY

Table 9: Results of repeatability for Ciprofloxacin

S. No	Peak name	Retention time	Area(µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Ciprofloxacin	2.157	513568	78546	1.2	4528
2	Ciprofloxacin	2.159	513685	78541	1.2	4572
3	Ciprofloxacin	2.186	513659	79852	1.2	4598
4	Ciprofloxacin	2.160	513254	78498	1.3	4529
5	Ciprofloxacin	2.170	513647	77898	1.2	4572
Mean			513562.6			
Std.dev			177.9475			
%RSD			0.03465			

- *%RSD for sample should be NMT 2.*
- *The %RSD for the standard solution is below 1, which is within the limits hence method is precise.*

Table 10: Results of repeatability for Fluocinolone

S. No	Peak name	Retention time	Area(µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Fluocinolone	3.603	1635625	265325	1.1	7985
2	Fluocinolone	3.608	1658744	264588	1.1	7859
3	Fluocinolone	3.600	1652985	265985	1.2	7845
4	Fluocinolone	3.696	1645898	264898	1.1	7969
5	Fluocinolone	3.629	1652364	268489	1.1	7846
Mean			1649123			
Std.dev			8811.631			
%RSD			0.534322			

Intermediate precision
Day 1

Table 11: Results of Intermediate precision for Ciprofloxacin

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Ciprofloxacin	2.198	514658	78698	4658	1.2

2	Ciprofloxacin	2.196	514354	78599	4598	1.2
3	Ciprofloxacin	2.160	513985	79854	4652	1.2
4	Ciprofloxacin	2.160	514875	79879	4561	1.2
5	Ciprofloxacin	2.160	514658	79865	4659	1.2
6	Ciprofloxacin	2.186	516452	79854	4589	1.2
Mean			514830.3			
Std.Dev.			852.3705			
%RSD			0.165563			

- *%RSD of five different sample solutions should not more than 2.*

Table 12: Results of Intermediate precision for Fluocinolone

S.No	Peak Name	Rt	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing	Resolution
1	Fluocinolone	3.623	1645875	266589	7985	1.1	10.1
2	Fluocinolone	3.611	1658554	265898	8001	1.1	10.1
3	Fluocinolone	3.696	1649854	265415	7985	1.1	10.1
4	Fluocinolone	3.696	1659842	265154	7956	1.1	10.1
5	Fluocinolone	3.696	1645985	266598	7985	1.1	10.1
6	Fluocinolone	3.642	1659852	265341	8002	1.1	10.1
Mean			1653327				
Std.Dev.			6838.733				
%RSD			0.413635				

- *%RSD of five different sample solutions should not more than 2.*

Table 13: Results of Intermediate precision Day 2 for Ciprofloxacin

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USPlate count	USP Tailing
1	Ciprofloxacin	2.198	514658	78572	4672	1.2
2	Ciprofloxacin	2.196	514895	78516	4639	1.2
3	Ciprofloxacin	2.178	514658	78572	4783	1.2
4	Ciprofloxacin	2.142	514784	78372	4623	1.2
5	Ciprofloxacin	2.177	515268	78592	4639	1.2
6	Ciprofloxacin	2.177	514598	78526	4737	1.2
Mean			514810.2			
Std.Dev.			248.5224			
%RSD			0.048275			

- *%RSD of five different sample solutions should not more than 2.*

Table 14: Results of Intermediate precision Day 2 for Fluocinolone

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing	Resoltion
1	Fluocinolone	3.611	1638732	264384	7985	1.1	10.1
2	Fluocinolone	3.623	1637438	265827	7946	1.1	10.1
3	Fluocinolone	3.684	1638474	266382	7943	1.1	10.1
4	Fluocinolone	3.697	1634273	269183	7964	1.1	10.1
5	Fluocinolone	3.684	1636372	261931	7968	1.1	10.1
6	Fluocinolone	3.684	1639283	264356	7982	1.1	10.1
Mean			1637429				
Std.Dev.			1860.366				
%RSD			0.113615				

- *%RSD of five different sample solutions should not more than 2.*

ACCURACY

Table 15: The accuracy results for Ciprofloxacin

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery

50%	245954	10	10.179	101.79%	101.36%
100%	483747	20	20.316	101.58%	
150%	715961	30	30.	100.72%	

- *The percentage recovery was found to be within the limit (98-102%).*

Table 16: The accuracy results for Fluocinolone

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	842287	30	30.114	100.38%	100.26%
100%	1659744	60	60.068	100.113%	
150%	2483885	90	90.268	100.297%	

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

Robustness

CIPROFLOXACIN

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	513567	2.121	4536	1.2
Less Flow rate of 0.9 mL/min	523652	2.210	4462.3	0.9
More Flow rate of 1.1 mL/min	502146	2.184	4325.1	1.0
Less organic phase	521574	2.200	4632.4	0.9
More Organic phase	502416	2.172	4190.8	0.8

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

FLUOCINOLONE

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	1625892	3.643	4536	1.1
Less Flow rate of 0.9 mL/min	1758455	4.498	4426.4	0.9
More Flow rate of 1.1 mL/min	1742514	3.505	4421.5	0.8
Less organic phase	1726451	4.504	4355.1	0.9
More organic phase	1725466	3.512	4426.6	0.9

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Ciprofloxacin and Fluocinolone in bulk drug and pharmaceutical dosage forms. Ciprofloxacin was found to be practically insoluble in water, very slightly soluble in dehydrated alcohol and in dichloromethane, and soluble in dilute acetic acid slightly soluble in DMF and ethanol and Fluocinolonewas found to be soluble in acetone, slightly soluble in ethanol, dioxane but insoluble in water and petroleum ether, soluble in alcohol, acetone and methanol; slightly soluble in chloroform. Methanol and Phosphate buffer (pH-3.8) (40:60% v/v) was chosen as the mobile phase. The solvent system used in this method was economical. The %RSD values were within 2 and the method was found to be precise. The results expressed inTablesfor RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods. This method can be used for the routine determination of Ciprofloxacin and Fluocinolone in bulk drug and in Pharmaceutical dosage forms.

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