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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF CIPROFLOXACIN HYDROCHLORIDE AND FLUOCINOLONE ACETONIDE IN THEIR SYNTHETIC MIXTURE

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ABSTRACT

The present research work aims to develop a simple, precise, accurate, rapid, reproducible and economical method for the estimation of Fluocinolone acetonide and Ciprofloxacin hydrochloride in their synthetic mixture by RP-HPLC method. An absorbance maximum for this combination was found to be at 225 nm using methanol as a solvent. Development And Validation Of RP-HPLC method for estimation of Fluocinolone acetonide and Ciprofloxacin hydrochloride in their combined marketed dosage form was performed on a Hypersil BDS (250 x 4.6mm C18) column with mobile phase containing, buffer (pH 6.5-KH_2PO_4):Methanol in ratio of 50:50. The flow rate was 1 ml/min and the eluent was monitored at 225 nm. Detection was carried in UV-2000 detector. The selected chromatographic conditions were found effectively to separate Fluocinolone acetonide and Ciprofloxacin hydrochloride at 3.33 and 4.77 min respectively.

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INTRODUCTION

Ciprofloxacin hydrochloride – 1 - cyc1opropyl – 6 – fluoro -1, 4 – dihydro – 4 – oxo – 7 - Cl-piperazinyl) – 3 - quinoline carboxylic acid hydrochloride monohydrate is Antibacterial agent and Fluocinolone acetonide - 6α,9α-difluoro-11β,21-dihydroxy-16α,17α-isopropylidenedioxypregna –1, 4 - diene-3, 20– dione is Adrenocortical steroid, Anti inflammatory agent. Structure of CIP and FLU is shown in Fig.1 and Fig.2. They are used in otitis. This combination is used synergistically by preventing the growth of bacteria and increasing cellular permeability and inhibiting the growth of microorganism, As well as reduces the inflammation. As per literature survey methods like RP-HPLC, UV spectrophotometric methods have been reported for ciprofloxacin hydrochloride and Fluocinolone acetonide individually and in combination with other drugs. But there is no any method have been reported for RP-HPLC method for simultaneous estimation of both the drugs in their synthetic mixture or marketed pharmaceutical dosage form. With the advent of International Conference on Harmonization (ICH) guidelines, the requirement of establishment of stability -indicating assay method (SIAM) has become more clearly mandated. However, no method has been reported to develop analytical method for estimation in this combination for otic solution, Thus the objectives of this work is to develop and validate a new sensitive RP-HPLC method for simultaneous determination of Ciprofloxacin hydrochloride and Fluocinolone acetonide in synthetic mixture.

MATERIALS AND METHODS

Standard ciprofloxacin hydrochloride was obtained a gift sample from makcur laboratories ltd., zak, dascroi, Ahmedabad and Fluocinolone acetonide was obtained as gift sample from Pharma Supply Agencies, Navrangpura, Ahmedabad; Thermoseparation (gradient) chromatograph with UV 2000 detector was used with Data Ace Software. Methanol and KH_2PO_4- HPLC grade, Water - HPLC grade, Merck India Ltd., Mumbai, was used. A synthetic mixture was prepared in laboratoty.

Selection of Detection wavelength:

Solution of 25 ppm and 350 ppm of Ciprofloxacin Hydrochloride(CIP) and Fluocinolone Acetonide (FLU) were prepared Respectively and scanned over the range 200-400 nm and the spectra were recorded. Wavelength 225 nm (at which both the drugs showed good absorbance) was selected as detection wavelength (figure 3).

Selection of Mobile phase

After trials of various mobile phase compositions, buffer (0.05M KH_2PO_4 having pH=6.5) : methanol 50:50 is selected for the estimation. Chromatogram in optimized mobile phase is shown in Fig. 4.

Preparation of standard and stock solution

Standard stock solution of FLU:

25mg in100ml with methanol, Further take 1ml in 10ml with methanol (25µg/ml) (stock solution), further Take 1ml from FLU stock solution in 10ml with methanol (FLU-2.5µg/ml)

Standard stock solution of CIP:

35mg in 100ml with methanol (350µg/ml) (stock solution). Further Take 1ml from CIP stock solution in 10ml with methanol (CIP -35µg/ml)

Optimized Chromatographic Conditions.

Column	Hypersil BDS (250 x 4.6mm C18)
Mobile Phase	buffer (0.05M KH_2PO_4 having pH 6.5):Methanol - 50:50
Flow rate	1 ml/min
Detection	225nm
Column Temperature	Room temperature
Retention Time	FLU (3.330min), CIP(4.773min)
Run Time	10min
Injection volume (loop)	20 µl

Calibration of standards

Calibration curve of Fluocinolone Acetonide (FLU) and Ciprofloxacin Hydrochloride (CIP) in the range of 1.25-3.75 µg/ml and 17.5-52.5 µg/ml was prepared respectively by pipette out different volumes from each stock solution and dilute up to the marks with mobile phase.

METHOD VALIDATION

Linearity

Calibration curve of Fluocinolone Acetonide (FLU) and Ciprofloxacin Hydrochloride (CIP) in the range of 1.25-3.75 µg/ml and 17.5-52.5 µg/ml respectively was found linear. The calibration curve was linear and regression analysis was obtained. Linearity plots were shown in Fig. 5 and Fig. 6. Results for linearity are shown in table 1.

Accuracy (Recovery study)

Accuracy of an analysis is determined by calculating systemic error involved. Recovery of FLU & CIP were calculated by standard addition method at three different concentration levels of drug. Accuracy was determined at three different level 80 %, 100% and 120 % of the target concentration 1, 1.25, and 1.5µg/ml of FLU and 14, 17.5 and 21 µg/ml of CIP in triplicate and calculating % recovery. The mean % recovery was found to be 99.69 % - 101.23 % for FLU and 99.06 %- 100.32% CIP respectively Results are shown in table 2.

Precision:

Repeatability was assessed by analyzing six injection of a homogeneous sample of 2.5 µg/ml of FLU and 35 µg/ml of CIP. Intra-day precision was performed using three different concentration 1.25 µg/ml, 2.5 µg/ml, 3.75 µg/ml for FLU and 17.5 µg/ml, 35 µg/ml, 52.5 µg/ml for CIP in triplicate at three different time interval in a day. Inter-day precision was performed using three different concentration 1.25 µg/ml, 2.5 µg/ml, 3.75 µg/ml for FLU and 17.5 µg/ml, 35 µg/ml, 52.5 µg/ml for CIP in triplicate for three consecutive days. (Table 3).

LOD and LOQ

LOD and LOQ of the drug were calculated from signal-to-noise ratio (i.e. 3.3 for LOD and 10 for LOQ) taking the 1.25, 1.875, 2.5, 3.125 and 3.75 µg/ml of FLU and 17.5, 26.25, 35, 43.75 and 52.5 µg/ml of CIP. The results were shown in table 4.

Robustness

Small variation in the flow rate (± 0.2 ml/min.), organic phase ratio (±2%), by using 2.5 µg/ml of FLU and 35 µg/ml of CIP were made. The results were shown in table 5.

System suitability

It is defined as tests to measure the method that can generate result of acceptable accuracy and precision. The system suitability was carried out after the method development and validation have been completed. For this, parameters like Plate number (N), Resolution (R), tailing factor, Capacity factor, HETP, Peak symmetry of samples were measured. The results were shown in table 6.

Assay of Synthetic Mixture

Take synthetic mixture equivalent to 35mg of ciprofloxacin and 2.5mg of fluocinolone in to a 100ml volumetric flask. Add 60 ml methanol. Shake for 15 minutes and sonicate for 10 minutes. Make up volume with methanol. Filter this solution with Whatman filter paper no-1. (FLU-25 µg/ml, CIP-350 µg/ml).(Working sample preparation) Take 1ml from sample stock solution into a 10ml and make up with mobile phase. (FLU-2.5 µg/ml, CIP-35 µg/ml) The resulting solution was filtered using 0.45 µm filter. 20 µl of the test solution was injected and chromatogram was recorded under optimized chromatographic condition and peak area was measured. The assay procedure was made in triplicate and % drug was calculated. Results are shown in table 7 and figure 6.

RESULT AND DISCUSSION

The present work aimed development and validation of RP-HPLC method for simultaneous estimation of FLU and CIP. Method was developed in mobile phase containing buffer (0.05M KH_2PO_4 having pH 6.5): Methanol - 50:50 Detection was carried out at 225 nm. Method was validated as per ICH guidelines. Linearity and regression data were shown in table 1 and Fig.4, 5. % recovery for FLU and CIP were within the range (98% - 102%). Results were shown in table 2. Hence it is found that the developed method is accurate. %RSD values were <2 for repeatability, intra-day and inter-day precision. Results were shown in table 3. So, the developed method was found to be precise. LOD and LOQ values were shown in table 4. LOD & LOQ confirms the method to be sensitive. Small changes were carried out in mobile phase and flow rate for robustness study, in that % RSD of area was found to be <2. Results were shown in table 5.So, the developed method was found to be robust. IT has been considered as reasonable and acceptable for validation of chromatographic assay. Summary Results were shown in table 8.

Table 1. Statistical analysis data of calibration curve.

Concentration (µ/ml)FLU	Area FLU	Concentration (µ/ml)CIP	Area CIP
1.25	59.493	17.5	2522.02
1.875	89.778	26.25	3804.576
2.5	120.375	35	5097.347
3.125	151.674	43.75	6370.105
3.75	181.695	52.5	7631.646
SD	0.334272344	SD	10.68707635
Slope	49	Slope	146.1
Correlation co-efficient	0.999982136	Correlation co-efficient	0.999989519

Table 2: Accuracy study.

Recovery Study For Fluocinolone acetonide.

Level of recovery (%)	Sr no.	Amount recovered	%recovery	Avg.	SD	%RSD
80%	1	0.993870	99.38709	100.61495	1.14757	1.14055
	2	1.016603	101.6603			
	3	1.007973	100.7973			
100%	1	1.246966	99.75729	100.37443	0.64620	0.64379
	2	1.263078	101.0462			
	3	1.253997	100.3197			
120%	1	1.513979	100.9319	100.40495	0.51122	0.50916
	2	1.498667	99.91117			
	3	1.505575	100.3717			

Recovery Study For Ciprofloxacin Hydrochloride.

Level of recovery (%)	Sr no.	Amount recovered	%recovery	Avg.	SD	%RSD
80%	1	13.83097	98.79264			
	2	14.07523	100.53742	99.85763	0.93399	0.93532
	3	14.03399	100.24282			
100%	1	17.34148	99.09421			
	2	17.46627	99.80728	99.53187	0.38322	0.38502
	3	17.44647	99.69412			
120%	1	21.04699	100.22379			
	2	20.81286	99.10888	99.69753	0.56006	0.56176
	3	20.94958	99.75993			

Table 3: Precision Repeatability study.

Fluocinolone acetonide (2.5 µg/ml)		Ciprofloxacin hydrochloride (35 µg/ml)	
At 100%		At 100%	
Std.	**Area**	**Std.**	**Area**
1	119.757	1	5071.761
2	119.874	2	5076.918
3	119.994	3	5082.139
4	120.231	4	5092.26
5	120.375	5	5085.75
6	120.115	6	5087.097
Avg.	120.0576667	Avg.	5082.654
SD	0.229006259	SD	7.394932
%RSD	0.190746885	%RSD	0.145494

Intraday and Interday Precision study for FLU.

Intraday precision of Fluocinolone acetonide						
%	**µg/ml**	**No Of Runs**	**Area**	**Avg. Area**	**SD**	**%RSD**
50%	1.25	1	59.014	59.131	0.117	0.197865756
		2	59.131			
		3	59.248			
100%	2.5	1	119.412	119.649	0.237	0.198079382
		2	119.649			
		3	119.886			
150%	3.75	1	180.264	180.623	0.357016	0.197658552
		2	180.627			
		3	180.623			
Interday precision of Fluocinolone acetonide						
50%	1.25	1	59.191	59.15167	0.067263	0.113713
		2	59.19			
		3	59.074			
100%	2.5	1	119.769	119.8073	0.17763	0.148263
		2	120.001			
		3	119.652			
150%	3.75	1	180.804	180.862	0.273649	0.151303
		2	181.16			
		3	180.622			

Intraday and Interday Precision study for CIP.

Intraday precision of Ciprofloxacin Hydrochloride						
%	**µg/ml**	**No Of Runs**	**Area**	**Avg. Area**	**SD**	**%RSD**
50%	17.5	1	2501.85	2503.188667	8.143938994	0.325342
		2	2495.797			596
		3	2511.919			
100%	35	1	5056.507	5062.647667	5.422000492	0.107098
		2	5066.775			12
		3	5064.661			
150%	52.5	1	7570.547	7581.849333	9.921507765	0.130858
		2	7585.879			677
		3	7589.122			
Interday precision of Ciprofloxacin Hydrochloride						
50%	17.5	1	2509.414	2505.507	6.758464	0.269744
		2	2509.404			
		3	2497.703			
100%	35	1	5071.786	5070.112	3.001348	0.059197
		2	5071.903			
		3	5066.647			
150%	52.5	1	7593.472	7590.046	3.949741	0.052038
		2	7590.941			
		3	7585.726			

Table 4: LOD and LOQ of FLU and CIP.

Parameters	Fluocinolone acetonide	Ciprofloxacin hydrochloride
LOD	0.02251 µg/ml	0.24139µg/ml
LOQ	0.06821 µg/ml	0.73149µg/ml

Table 5: Robustness study.

Robustness study for FLU.

For Fluocinolone Acetonide (for 2.5µg/ml)						
parameters	**Changed condition**	**No. of runs**	**Area**	**Avg. area**	**SD**	**%RSD**
Flow Rate	+0.2 ml/min	1	116.529	117.2477	0.723548	0.617111
		2	117.238			
		3	117.976			
	-0.2 ml/min	1	123.942	124.6673	0.729536	0.585186
		2	124.659			
		3	125.401			
Mobile Phase	+2%	1	116.646	117.4443	0.783921	0.667483
		2	117.474			
		3	118.213			
	-2%	1	122.638	123.3933	0.777365	0.629989
		2	123.351			
		3	124.191			
pH	+0.2 pH	1	114.258	114.9827	0.720536	0.626648
		2	114.991			
		3	115.699			
	-0.2 pH	1	122.761	123.4753	0.715001	0.579064
		2	123.474			
		3	124.191			

Robustness study for CIP.

For Ciprofloxacin Hydrochloride (for 35µg/ml)						
parameters	**Changed condition**	**No. of runs**	**Area**	**Avg. area**	**SD**	**%RSD**
Flow rate	+0.2 ml/min	1	4934.896	4957.723	33.07482	0.667137
		2	4942.619			
		3	4995.653			
	-0.2 ml/min	1	5250.056	5275.444	32.18099	0.610015
		2	5264.639			
		3	5311.637			
Mobile phase	+2%	1	4939.788	4965.503	35.23756	0.709647
		2	4951.053			
		3	5005.669			
	-2%	1	5193.964	5219.064	36.21754	0.693947
		2	5202.645			
		3	5260.583			
pH	+0.2 pH	1	4838.134	4860.254	19.36798	0.398497
		2	4868.462			
		3	4874.167			
	-0.2 pH	1	5199.181	5223.611	21.97531	0.420692
		2	5229.885			
		3	5241.767			

Table 6. System suitability data for the developed method.

System Suitability Parameter	FLU	CIP
Retention time (min)	3.320 minute	4.759 minute
Resolution	7.710	
Asymmetric	1.350	1.407
Theoretical Plates	8135.5	7118.66

Table 7. Assay of synthetic mixture.

Drug	Sr. No	Label Claim	Result	%Assay	Avg. %Assay	SD	%RSD
FLU	1		0.02557	102.31403			
	2		0.02562	102.51507	102.51619	0.20271	0.19773
	3	0.025	0.02567	102.71945			
CIP	1		0.33934	98.45360804			
	2		0.34001	98.38841263	98.37069234	0.09305	0.094591
	3	0.35	0.33987	98.27005636			

Table 8: Summary of validation parameters.

Parameters	Fluocinolone acetonide (FLU)	Ciprofloxacin hydrochloride (CIP)
Linear Range	1.25-3.75 µg/ ml	17.5-52.5 µg/ml
Regression Coefficient	0.999	0.999
Recovery %	99.69 % - 101.23 %	99.06 %- 100.32%
Repeatability (RSD, n=6)	0.19074	0.14549
Precision (RSD)		
Intra - day (n=3)	0.19786–0.19765%	0.32534–0.13085%
Inter - day (n=3)	0.1137–0.1513%	0.26974–0.05203%
Limit of Detection (µg/ml)	0.02251 µg/ml	0.24139 µg/ml
Limit of Quantitation (µg/ml)	0.06821 µg/ml	0.73149 µg/ml
Robustness	Robust	Robust

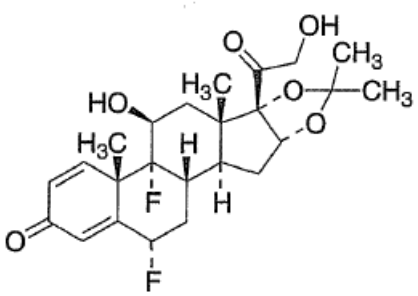

Fig 1. Structure of Fluocinolone acetonide.

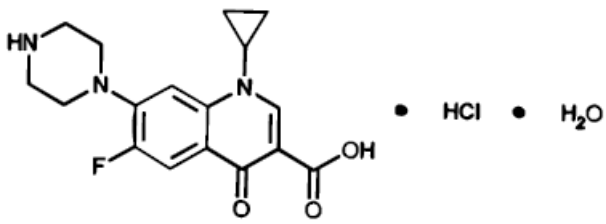

Fig 2. Structure of Ciprofloxacin hydrochloride.

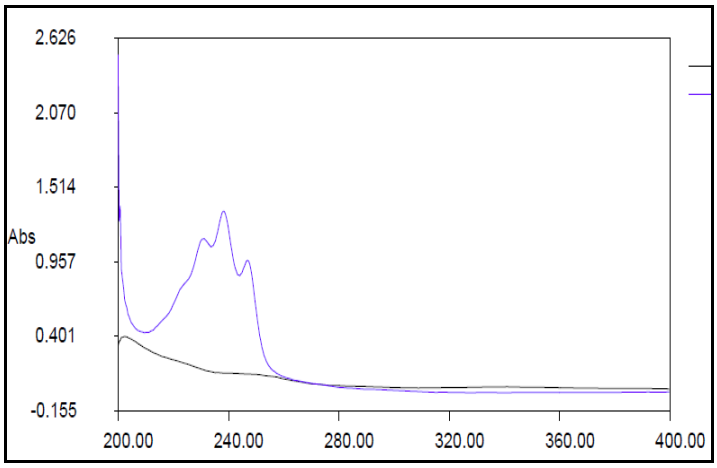

Figure 3: Selection of analytical wavelength:

CIP – blue line

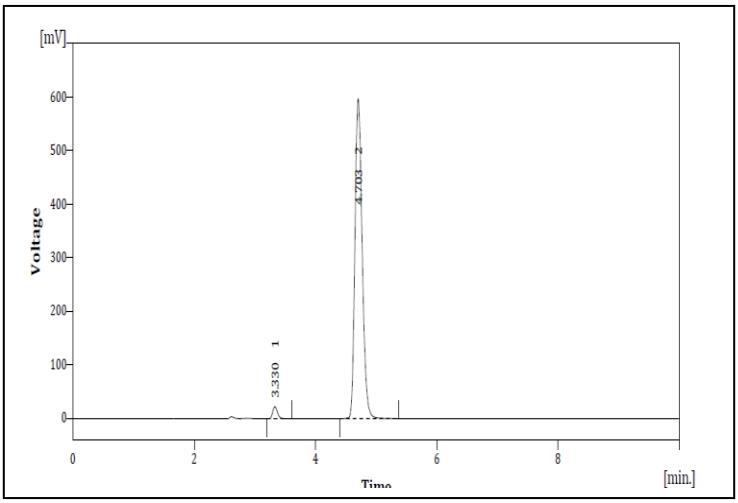

Figure 4: Chromatogram in optimized mobile phase.

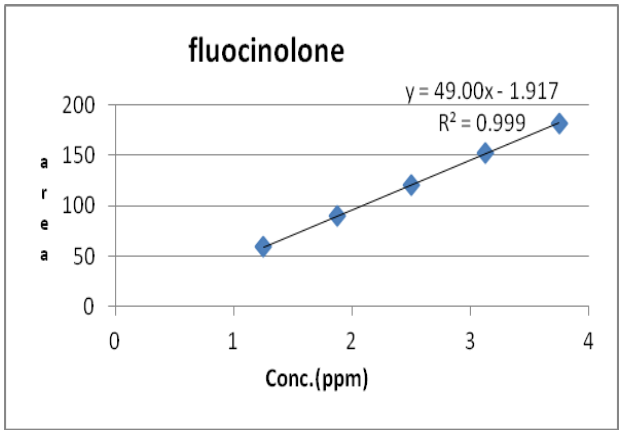

Fig 5. Calibration curve of FLU.

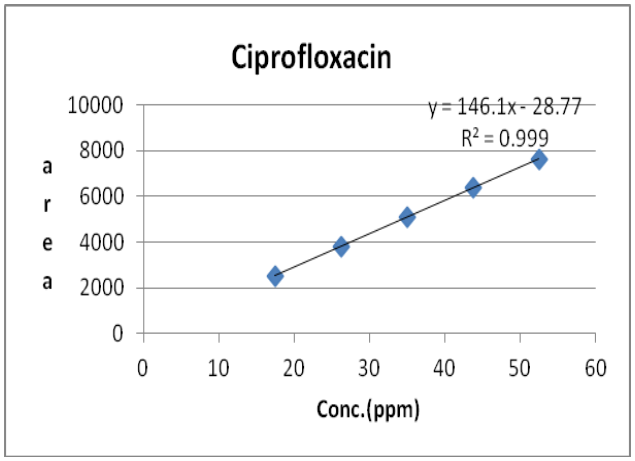

Fig 6. Calibration curve of CIP.

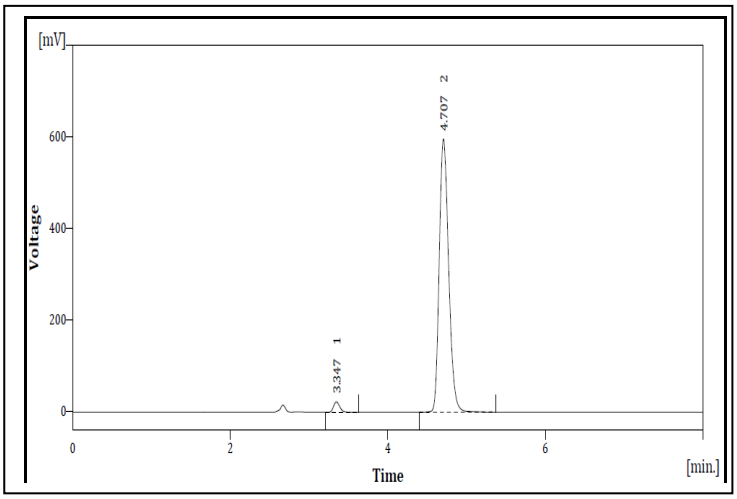

Fig7. Chromatogram of Fluocinolone acetonide (FLU) and Ciprofloxacin hydrochloride (CIP) in synthetic mixture

CONCLUSION

RP-HPLC method for simultaneous estimation of FLU and CIP was developed and validated as per ICH guidelines. The developed method was found to be accurate and precise with % RSD <2%. So, the developed method is simple, accurate, precise, sensitive and robust. Also this method can be used for routine drug analysis in Quality control department.

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Conflict of Interest:
None

Ethical Permission:
None

ABBREVIATIONS

FLU	-	Fluocinolone acetonide
CIP	-	Ciprofloxacin hydrochloride
µg	-	Microgram

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