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STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT AND VALIDATION OF SIMULTANEOUS ESTIMATION OF CIPROFLOXACIN AND FLUOCINOLONE ACETONIDE IN BULK AND PHARMACEUTICAL DOSAGE FORM

Ponna Venkatesh*, R.V. Valli Kumari, S. Marakatham, Meruva Sathish Kumar

Malla Reddy Institute of Pharmaceutical Sciences, Maisammaguda, Dullapally, Hyderabad.

*Corresponding author E-mail: **ponnavenkatesh836@gmail.com**

ARTICLE INFO

Key Words

Ciprofloxacin, Fluocinolone, RP-HPLC

ABSTRACT

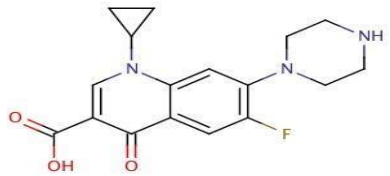
A simple, Accurate, precise method was developed for the simultaneous estimation of the Ciprofloxacin and Fluocinolone in ear drops. Chromatogram was run through Std Kromosil C18 250 x 4.6 mm, 5µ. Mobile phase containing Buffer 0.1% OPA (2.2ph): Acetonitrile taken in the ratio 55:45 was pumped through column at a flow rate of 0.9ml/min. Buffer used in this method was 0.1% OPA. Temperature was maintained at 30°C. Optimized wavelength selected was 246 nm. Retention time of Ciprofloxacin and Fluocinolone were found to be 2.290min and 2.901min. %RSD of the Ciprofloxacin and Fluocinolone were and found to be 0.2 and 0.2 respectively. %Recovery was obtained as 99.51% and 99.46% for Ciprofloxacin and Fluocinolone respectively. LOD, LOQ values obtained from regression equations of Ciprofloxacin and Fluocinolone were 0.12, 0.47 and 0.05, 0.14 respectively. Regression equation of Ciprofloxacin is y = 25741x + 1481., and y = 50539x + 785.5 of Fluocinolone. Retention times were decreased and run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

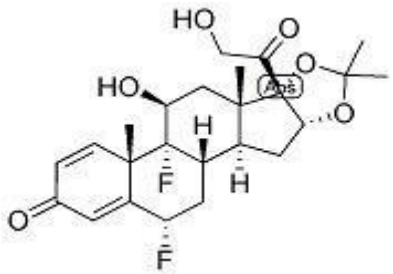
INTRODUCTION

Ciprofloxacin (1 - cyclopropyl – 6 – fluoro – 4 – oxo – 7 - (piperazin – 1 - yl) - 1, 4– dihydroquinoline - 3 - carboxylic acid). Ciprofloxacin is a broad-spectrum antimicrobial carboxy fluoroquinoline. The germicidal action of ciprofloxacin results from inhibition of the enzymes topoisomerase II (DNA gyrase) and topoisomerase IV, that area unit needed for microorganism deoxyribonucleic acid replication, transcription, repair, strand supercoiling repair, and recombination. Ciprofloxacin is an antibiotic used to treat a number of bacterial infections. This includes bone and joint infections, intra-abdominal infections, certain type of infectious diarrhea, respiratory tract infections, skin infections, typhoid fever, and urinary tract infections, among others. For some infections it is used in addition to other antibiotics. It can be taken by mouth, in eye drops, or intravenously. Fluocinolone acetonide (1S, 2S, 4R, 8S,

9S, 11S, 12R, 13S, 19S) - 12, 19 – difluoro – 11 – hydroxy – 8 - (2 – hydroxyl acetyl) - 6, 6, 9, 13 – tetramethyl - 5, 7 – dioxapentacyclo [10.8.0.0$^{2,\ 9.0^{4},\ 8.0^{13},\ 18}$] icosa - 14, 17 – dien – 16 - one). A glucocorticoid derivative used topically inside the remedy of various pores and skin issues. It is also hired as a cream, gel, lotion, or ointment. It is used to treat a variety of skin conditions (e.g., eczema, dermatitis, allergies, rash). Fluocinolone reduces the swelling, itching, and redness that can occur in these types of conditions. The main aim of the present study is to develop an accurate, precise, sensitive, selective, reproducible and rapid analytical technique for cost effective estimation of Betamethasone, Gentamicin and Miconazole in combination. The objectives are to develop analytical method. Selecting the HPLC separation mode. Selecting/ optimizing the mobile phase. Selecting column for analysis. Selecting the appropriate detector systems. Selecting appropriate gradient/ isocratic medium.

Fig 1: Ciprofloxacin & Fig 2: Fluocinolone Acetonide





MATERIALS AND METHODS

Materials and Instruments: The following materials used were either AR/LR grade or the best possible Pharma grade available as supplied by the manufacturer or supplier without further purification or investigation. Drug Samples Were obtained from Spectrum pharma research solutions pvt. Ltd

METHODS:

Diluent: primarily based up at the solubility of the medicine, diluent become decided on, Acetonitrile and Water taken in the ratio of 50:50.

Preparation of Standard stock solutions: Accurately weighed 15 mg of Ciprofloxacin, 1.25mg of Flucinolone and transferred to 50ml and 50ml individual volumetric flasks and 3/4 thof diluents was added to theseflaskand sonicated for 10 minutes. Flask were made up with diluents and labeled as Standard stock solution. 1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (30µg/ml Ciprofloxacin of and 2.5µg/ml ofFluocinolone)

Preparation of Sample stock solutions: A volume of ear drops (OTOVEL) equivalent to0.75 mg and 0.0625 mg of CIP and FLU respectively transferred into a 10ml volumetric flask,5mlofdiluentswasaddedandsonicatedfor25min,furtherthe volume wasmadeup with diluent and filtered by HPLC filters (75µg/ml of Ciprofloxacin and 6.25µg/ml of flucinolone).

Preparation of Sample working solutions (100% solution): 4ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (30µg/ml of Ciprofloxacin and 2.5µg/ml of Flucinolone)

Preparation of buffer: 0.1% OPA Buffer: 1ml of Conc Ortho Phosphoric acid was diluted to 1000ml with water.

RESULTS AND DISCUSSION

System suitability: All the system suitability variables were within the range and satisfactory as per ICH guidelines. According to ICH guidelines plate count should be more than 2000, tailing factor should be less than 2 and resolution must be more than 2. All the system suitable parameters were passed and were within the limits.

Specificity: Retention times of Ciprofloxacin and Fluocinolone were 2.290min and 2.901 min respectively. We did not found and interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific.

Accuracy:

Preparation of Standard stock solutions: Exactly weighed 15 mg of Ciprofloxacin, 1.25mg of flucinolone and transferred to 50ml and 50ml independent volumetric flasks and 3/4th of diluents become brought to those flask and sonicated for 10 mins. Flask were made up with diluents and categorised as widespread inventory answer.

Preparation of 50% Spiked Solution: 0.5ml of test stock arrangement was taken into a 10ml volumetric cup, to that 1.0ml from every standard stock arrangement was pipetted out, and made up to the stamp with diluent.

Preparation of 100% Spiked Solution: 1ml of test stock arrangement was taken into a 10ml volumetric cup, to that 1.0ml from every standard stock arrangement was pipetted out, and made up to the stamp with diluent.

Preparation of 150% Spiked Solution: 1.5ml of test stock arrangement was taken into a 10ml volumetric cup, to that 1.0ml from every standard stock arrangement was pipetted out, and made up to the stamp with diluent.

Precision:

Preparation of Standard stock solutions: Exactly weighed 15mg of Ciprofloxacin, 1.25mg of Flucinolone and transferred to 50ml and 50ml independent volumetric flasks and 75%of diluents was added to this flaskand sonicated for 10 minutes. Flask were made up with diluents and named as Standard stock arrangement. 1ml from each stock arrangement was pipetted out and taken into a 10ml volumetric flagon and made up with diluent. (100µg/ml Ciprofloxacin of and 50µg/ml of Fluocinolone)

Linearity:

Preparation of Standard stock solutions: Accurately weighed 15 mg of Ciprofloxacin, 1.25mg of Flucinolone and transferred to 50ml and 50ml individual volumetric flasks and 3/4th of diluents was added to these flask and sonicated for 10 minutes. Flask were made up with diluents and labeled as Standard stock solution. 1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (30µg/ml Ciprofloxacin of and 2.5µg/ml of Fluocinolone)

25% Standard solution: 0.25ml each from two standard stock solutions was pipette out and made up to 10ml.

50% Standard solution: 0.5ml each from two standard stock solutions was pipette out and made up to 10ml.

75% Standard solution: 0.75ml each from two standard stock solutions was pipette out and made up to 10ml.

Table: 1. System suitability parameters for Ciprofloxacin and Fluocinolone

S no	Ciprofloxacin			Fluocinolone			
Inj	RT(min)	USP Plate Count	Tailing	RT(min)	USP Plate Count	Tailing	Resolution
1	2.285	3418	1.41	2.889	4674	1.38	3.7
2	2.290	3480	1.47	2.901	5036	1.31	3.7
3	2.300	3493	1.39	2.907	4839	1.42	3.7
4	2.306	3463	1.49	2.909	4691	1.44	3.6
5	2.306	3548	1.44	2.916	5126	1.35	3.7
6	2.309	3663	1.44	2.916	5142	1.33	3.8

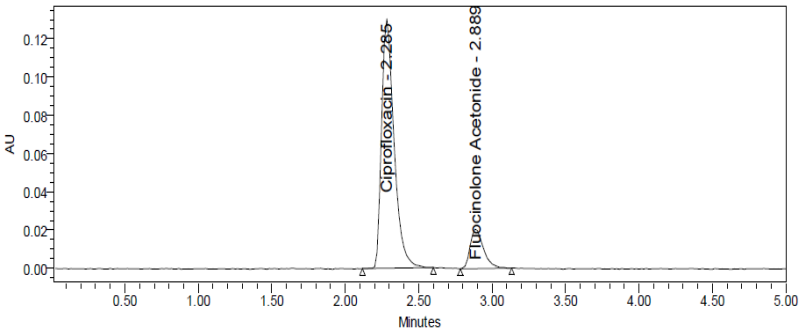


Fig 3. System suitability Chromatogram

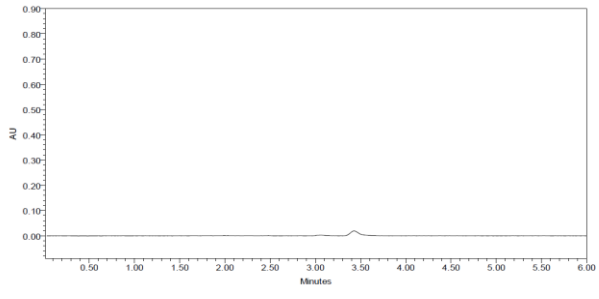


Fig 4. Chromatogram of Blank

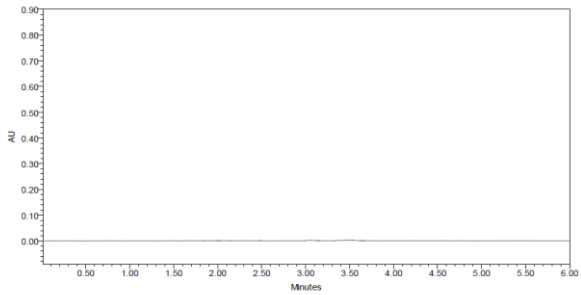


Fig 5. Chromatogram of Placebo

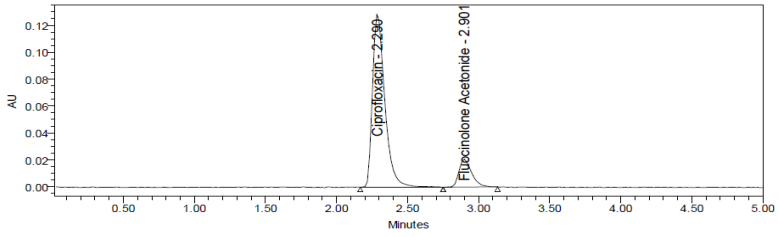


Fig 6. Optimized Chromatogram

Table 2. Accuracy table of ciprofloxacin

%Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	15	14.97	99.81	99.51%
	15	14.96	99.74	
	15	14.92	99.46	
100%	30	29.77	99.25	
	30	29.85	99.49	
	30	29.87	99.55	
150%	45	44.81	99.58	
	45	44.92	99.82	
	45	44.51	98.91	

Table 3. Accuracy table of flucinolone

%Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	1.25	1.24	99.26	99.46%
	1.25	1.25	99.71	
	1.25	1.25	99.91	
100%	2.5	2.48	99.05	
	2.5	2.49	99.52	
	2.5	2.49	99.62	
150%	3.75	3.73	99.48	
	3.75	3.72	99.08	
	3.75	3.73	99.54	

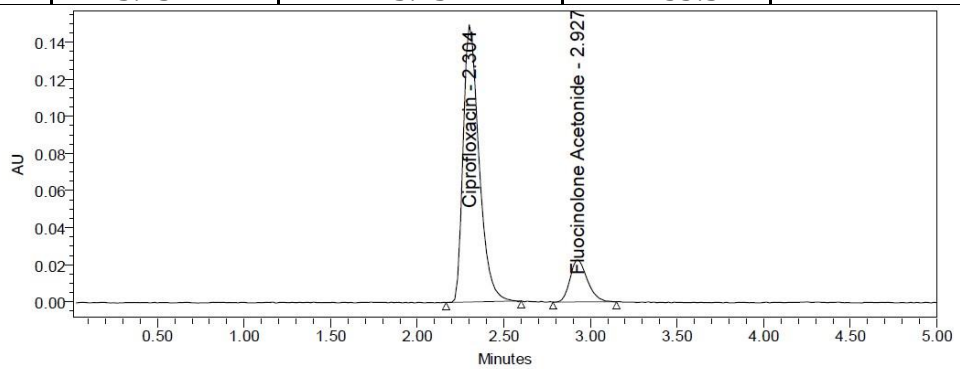


Fig 7. Accuracy 50% Chromatogram of Ciprofloxacin and Fluocinolone

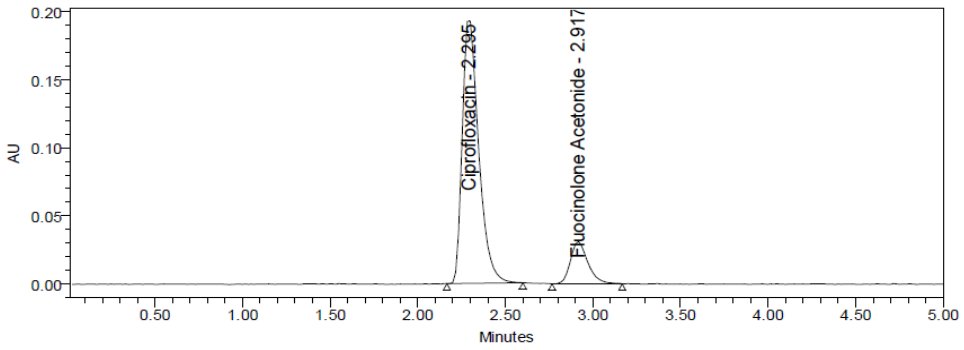


Fig 8. Accuracy 100% Chromatogram of Ciprofloxacin and Fluocinolone

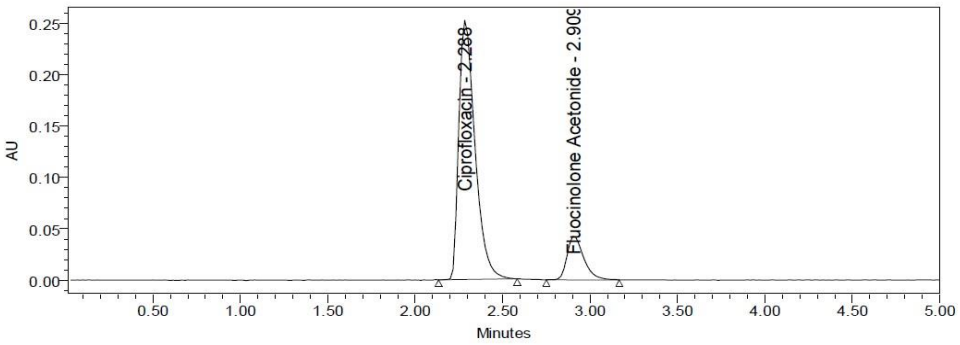


Fig 9. Accuracy 150% Chromatogram of Ciprofloxacin and Fluocinolone

Table 4. System precision table of Ciprofloxacin and fluocinolone

S. No	Area of Ciprofloxacin	Area of fluocinolone
1.	767458	130367
2.	776154	130241
3.	781720	130572
4.	777095	130576
5.	772878	131365
6.	773670	131038
Mean	774829	130693
S.D	4771.9	426.5
%RSD	0.6	0.3

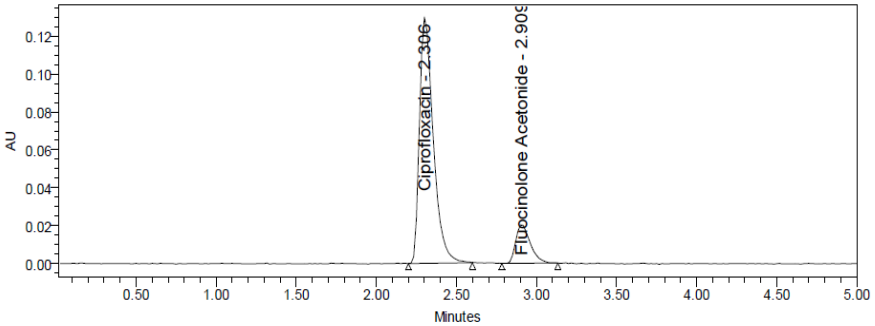


Fig 10. System precision chromatogram

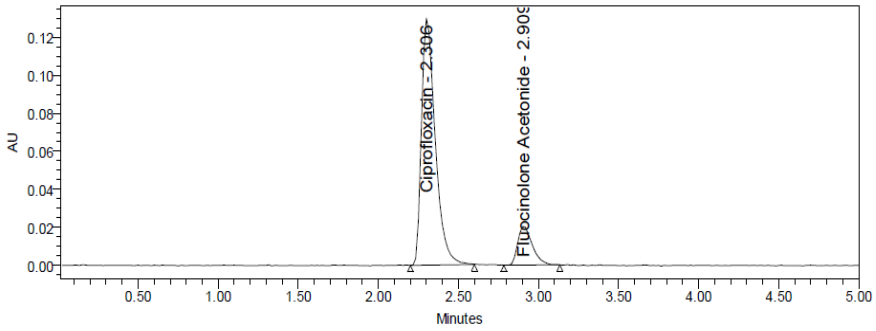


Fig 11. Intermediate precision Chromatogram

Table 6. Linearity table for Ciprofloxacin and Fluocinolone.

Ciprofloxacin		Fluocinolone	
Conc (µg/mL)	**Peak area**	**Conc (µg/mL)**	**Peak area**
0	0	0	0
7.5	191827	0.625	33546
15	387372	1.25	63627
22.5	592805	1.875	93999
30	764606	2.5	129428
37.5	971974	3.125	158949
45	1156053	3.75	189275

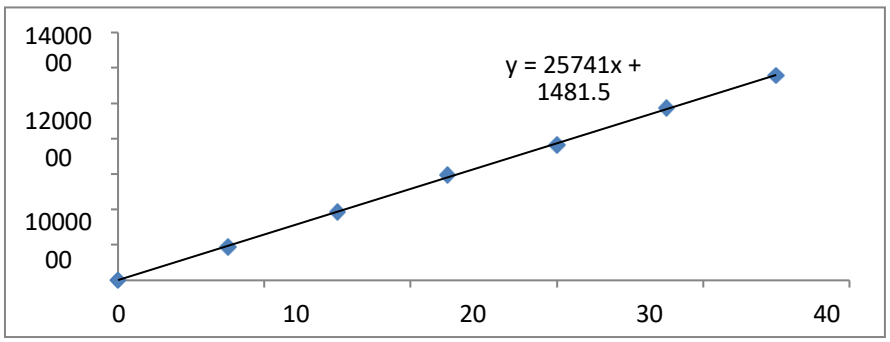


Fig 12. Calibration curve of Ciprofloxacin

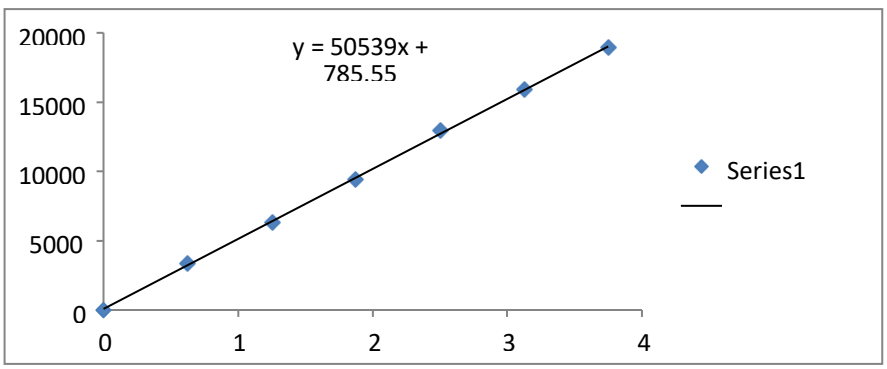


Fig 13. Calibration curve of Fluocinolone

Table 7. Robustness data for Ciprofloxacin and Fluocinolone.

S.no	Condition	%RSD of Ciprofloxacin	%RSD of Fluocinolone
1	Flow rate (-) 0.8ml/min	0.3	0.4
2	Flow rate (+) 1.1ml/min	0.5	0.4
3	Mobile phase (-) 50B:50A	0.5	0.6
4	Mobile phase (+) 60B:40A	0.7	0.8
5	Temperature (-) 25°C	0.4	0.3
6	Temperature (+) 35°C	1.0	1.2

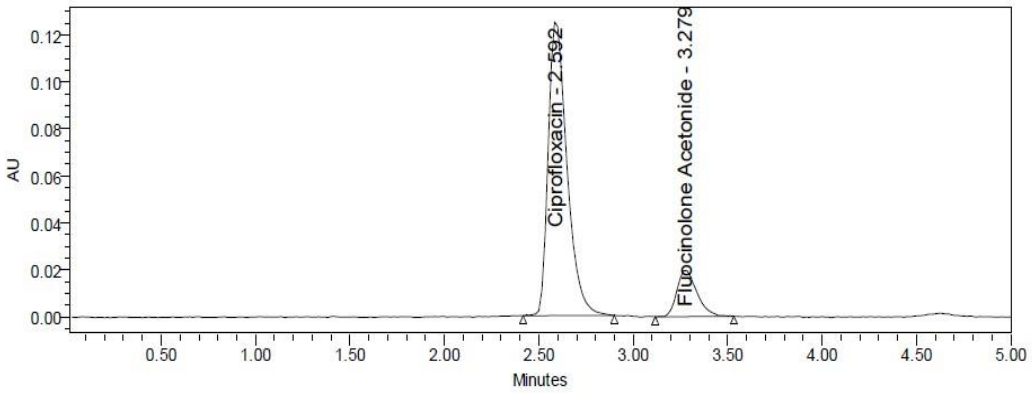


Fig 14. Flow minus Chromatogram of Ciprofloxacin and Fluocinolone

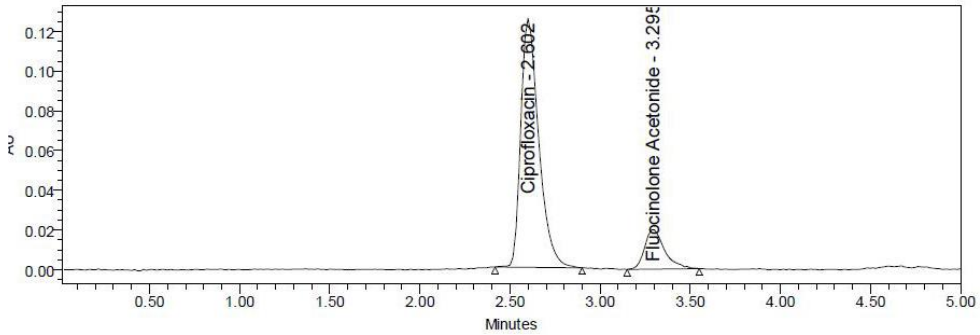


Fig 15. Flow plus Chromatogram of Ciprofloxacin and Fluocinolone

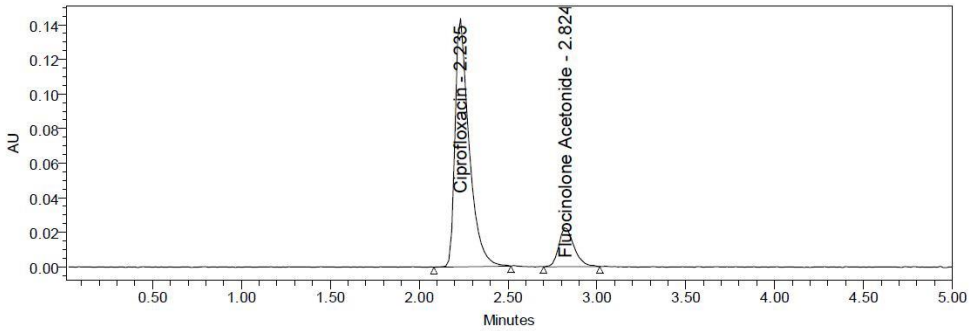


Fig 16. Mobile phase minus Chromatogram of Ciprofloxacin and Fluocinolone

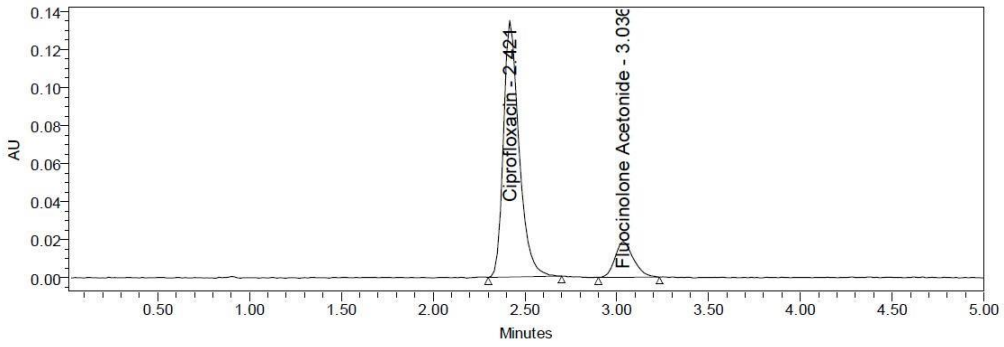


Fig 17. Mobile phase Plus Chromatogram of Ciprofloxacin and Fluocinolone

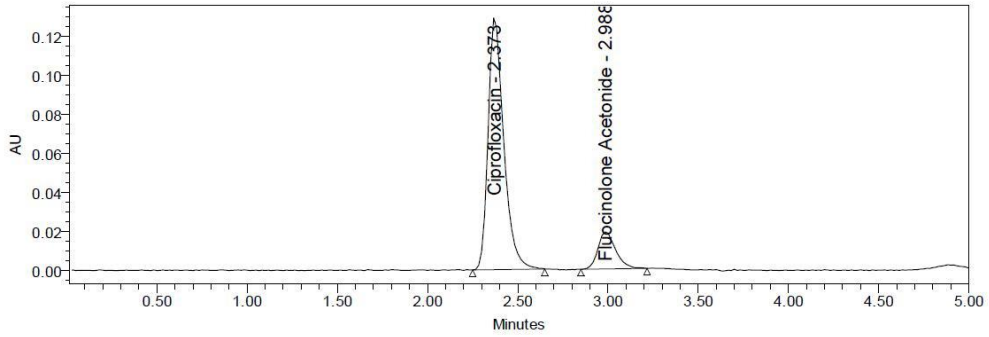


Fig 18. Temperature minus Chromatogram of Ciprofloxacin and Fluocinolone

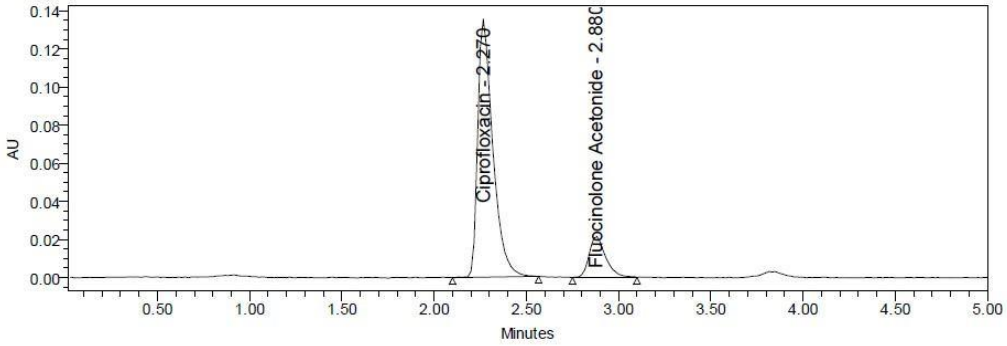


Fig 19. Temperature plus Chromatogram of Ciprofloxacin and Fluocinolone

Table 8. Sensitivity table of Ciprofloxacin and Fluocinolone

Molecule	LOD(µg/mL)	LOQ(µg/mL)
Ciprofloxacin	0.12	0.37
Fluocinolone	0.05	0.14

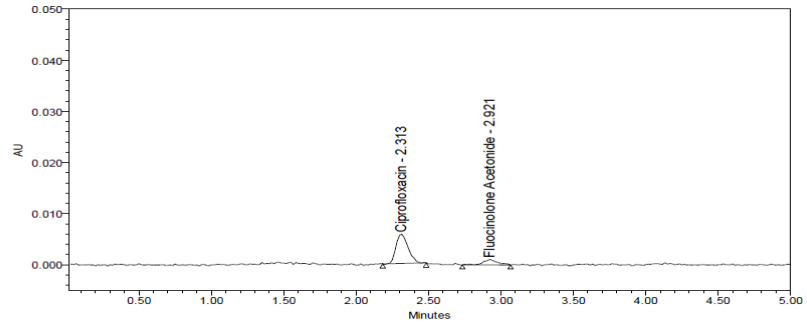


Fig 15. LOD Chromatogram of Standard

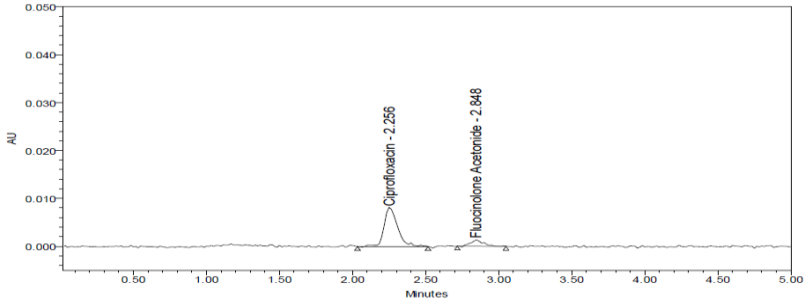


Fig 16. LOQ Chromatogram of of Standard

Table 9. Assay Data of Ciprofloxacin

S.no	Standard Area	Sample area	% Assay
1	767458	773813	99.67
2	776154	770138	99.20
3	781720	771719	99.40
4	777095	772912	99.55
5	772878	771957	99.43
6	773670	768714	99.01
Avg	774829	771542	99.38
Stdev	4772.0	1855.1	0.2
%RSD	0.6	0.2	0.2

Table 10. Assay Data of Fluocinolone

S. no	Standard Area	Sample area	% Assay
1	130367	129905	99.20
2	130241	129997	99.27
3	130572	130088	99.34
4	130576	130269	99.48
5	131365	130625	99.75
6	131038	129913	99.20
Avg	130693	130133	99.37
Stdev	426.5	276.2	0.2
%RSD	0.3	0.2	0.2

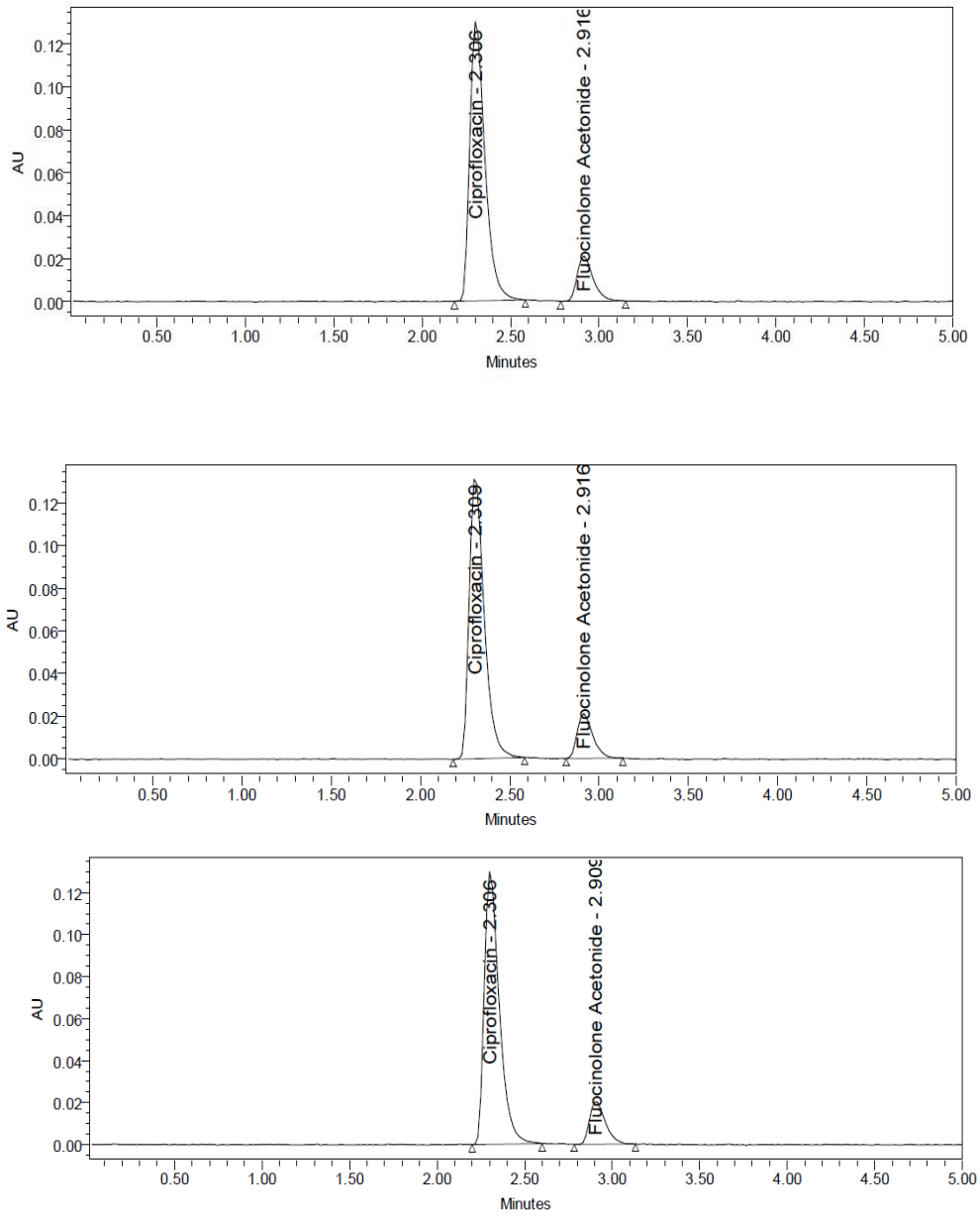
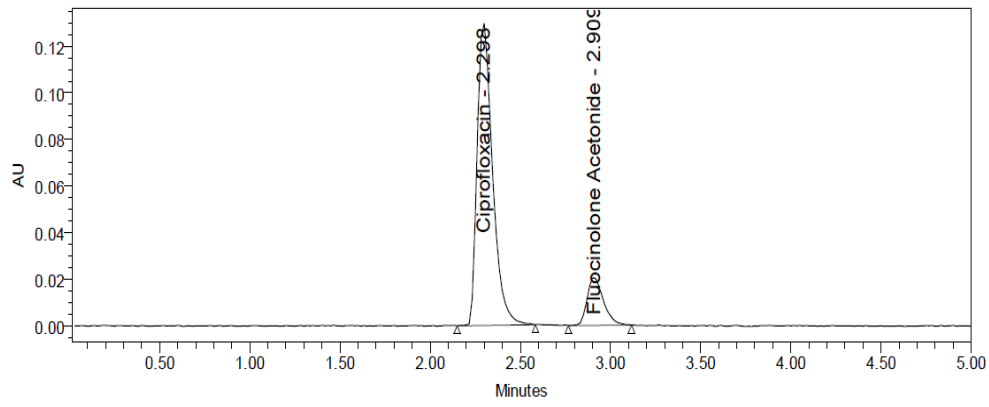


Fig 17. Chromatogram of working standard solution



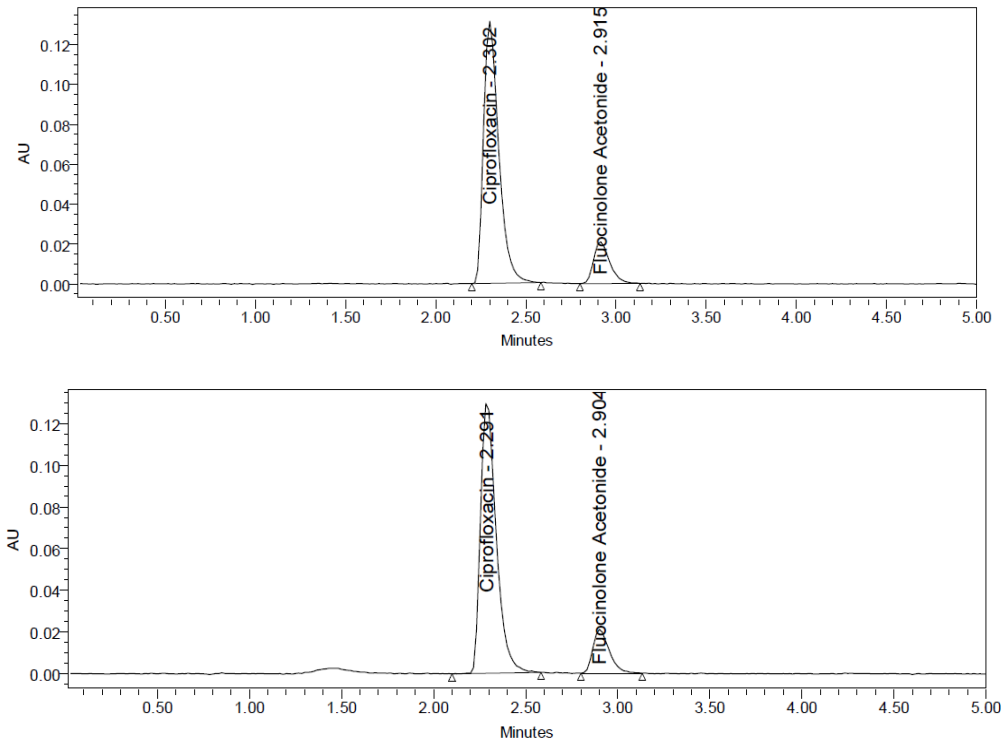


Fig 18. Chromatogram of working sample solution

100% Standard solution: 1.0ml each from two standard stock solutions was pipette out and made up to 10ml.

125% Standard solution: 1.25ml each from two standard stock solutions was pipette out and made up to 10ml.

150% Standard solution: 1.5ml each from two standard stock solutions was pipette out and made up to 10ml.

Robustness: Robustness conditions like flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus, Mobile phase plus, Temperature minus (25°C) and temperature plus (35°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit

LOD sample Preparation: 0.25ml each from two standard stock solutions was pipetted out and transferred to two separate 10ml volumetric flasks and made up with diluents. From the above solutions 0.1ml each of ciprofloxacin, Fluocinolone, solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluents

LOQ sample Preparation: 0.25ml each from two standard stock solutions was pipetted out and transferred to two separate 10ml volumetric flask and made up with diluent. From the above solutions 0.3ml each of Ciprofloxacin, fluocinolone, and solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluent.

Assay: Arbor Pharmaceuticals Ltd. (OTOVEL), bearing the label claim ciprofloxacin 0.3% and fluocinolone acetonide 0.025%. Assay was performed with the above formulation. Average % Assay for Ciprofloxacin and Fluocinolone obtained was 99.38% and 99.37% respectively

CONCLUSION

A simple, Accurate, precise method was developed for the simultaneous estimation of the Ciprofloxacin and Fluocinolone in ear drops. Retention time of Ciprofloxacin and Fluocinolone were found to be 2.290in and 8.901min. %RSD of the Ciprofloxacin and Fluocinolone were and found to be 0.2 and 0.2 respectively. %Recovery was obtained as 99.61% and 99.46% for Ciprofloxacin and Fluocinolone respectively. LOD, LOQ values obtained from regression equations of Ciprofloxacin and Fluocinolone were 0.12, 0.37 and 0.05, 0.14 respectively. Regression equation of Ciprofloxacin is y = 25741x + 1481, and y = 50539x + 785.5 of Fluocinolone. Retention times were decreased and that run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries

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