

1
2
3
4
5
6
7
8
9

Analytical Method Development and Validation of Beclomethasone, Clotrimazole, Ofloxacin, Lidocaine by RP-HPLC in Combination Dosage Form

ABSTRACT

A rapid and precise method (in accordance with ICH guidelines) is required for the quantitative simultaneous determination of drugs in a combined pharmaceutical dosage form. The pursuit of desired quality is a continual challenge for pharmaceutical industries, necessitating a meticulous approach known as validation. Sensitive and specific RP-HPLC method involving UV detection was carried for determination and quantification of various drug in combination dosage form. The aim is "Analytical Method development and Validation of Beclomethasone, Clotrimazole, Ofloxacin, Lidocaine by RP-HPLC In Combination Dosage Form" as per ICH guidelines. The developed method was validated for various parameters as per ICH guidelines like system suitability, specificity, linearity, system precision, method precision, accuracy, ruggedness and robustness. The separation method was carried out by using a mobile phase A consisting of Potassium dihydrogen phosphate buffer (0.05M): Methanol 45:55 v/v pH 3.5 ± 0.1 and Mobile phase B comprises Acetonitrile in the ratio of 50:50 v/v. The detection was carried out by using UV detector at 300nm. The column was Agilent C 18 (250X4.6mm) 5µ. The flow rate was selected as 1.5 ml/min. The retention time of Ofloxacin, Lidocaine, Beclomethasone, and Clotrimazole, was found to be 5.67, 9.44, 18.29, and 21.85 respectively

Keywords: Beclomethasone, Clotrimazole, Lidocaine, Ofloxacin, and % RSD

1. INTRODUCTION

"Lidocaine, Clotrimazole, Beclomethasone, Ofloxacin are a combination of four medicines. Lidocaine is a local anesthetic which works by blocking pain signals from the nerves to brain to decrease pain sensation in the ear" [1-2]. Clotrimazole is an antifungal which stops the growth of fungi [3-4] while Ofloxacin is an antibiotic that prevents bacterial cells from dividing and repairing, This treats your ear infection [5-6]. "Beclomethasone is a steroid. It blocks the production of certain chemical messengers (prostaglandins) that make the ear red, swollen and itchy" [7- 8].

"Ear Drop is a medicine used in the treatment of various types of ear infections. It prevents the growth of microorganisms that cause infection in the ears" [9-10]. "It also relieves the symptoms of inflammation such as pain, itchiness, and irritation" [6]. The combination dosage form ear drops containing beclomethasone, clotrimazole, ofloxacin, and lignocaine offers a potent therapeutic option for treating various ear infections and inflammations.¹³⁻¹⁴ Beclomethasone, a corticosteroid, provides anti-inflammatory action; clotrimazole, an antifungal agent, combats fungal infections; ofloxacin, a broad-spectrum antibiotic, targets bacterial infections; and lignocaine, a local anesthetic, alleviates pain and discomfort [15-16]. To ensure the efficacy, safety, and quality of these ear drops, it is imperative to develop a robust and reliable analytical method for their quantification [7]. Reverse-phase high-performance liquid chromatography (RP-HPLC) stands as a preferred technique due to its versatility, sensitivity, and specificity in analyzing multi-component formulations [8]. The development and validation of an RP-HPLC method for the simultaneous determination of beclomethasone, clotrimazole, ofloxacin, and lignocaine in ear drops pose unique challenges, including the need for optimal chromatographic conditions to achieve baseline separation of all compounds, as well as ensuring specificity in the

35 presence of excipients or potential impurities [9]. This study aims to address these challenges by systematically
36 developing and validating an RP-HPLC method tailored to the specific requirements of the combination ear drop
37 formulation [9,10]. Key parameters such as specificity, linearity, accuracy, precision, limit of detection (LOD), limit of
38 quantification (LOQ), robustness, and system suitability will be thoroughly evaluated according to international guidelines,
39 including those established by the International Council for Harmonisation of Technical Requirements for Pharmaceuticals
40 for Human Use (ICH) [10].

41 "Beclomethasone dipropionate is a second-generation synthetic corticosteroid and diester of beclomethasone,
42 which is structurally similar to dexamethasone. Beclomethasone dipropionate is used in various inflammatory conditions"
43 (Sweetman, 2009) [8]. "Ofloxacin is a synthetic fluoroquinolone antibacterial agent that inhibits the supercoiling activity of
44 bacterial DNA gyrase, halting DNA replication. Ofloxacin is an antibacterial agent used for the treatment of bacterial
45 infections in many parts of the body, including the respiratory tract, kidney, skin, soft tissue, and urinary tract" [9].
46 "Clotrimazole is a broad-spectrum antimycotic or antifungal agent. Clotrimazole's antimycotic properties were discovered
47 in the late 1960s. Clotrimazole falls under the imidazole category of azole antifungals, possessing broad-spectrum
48 antimycotic activity" [8]. "Lidocaine is a local anesthetic agent commonly used for local and topical anesthesia, but it also
49 has antiarrhythmic and analgesic uses and can be used as an adjunct to tracheal intubation. It is a tertiary amine and is a
50 class Ib antiarrhythmic agent" [10]. "The validated RP-HPLC method will serve as a valuable tool for routine quality control
51 analysis, enabling accurate and reliable quantification of the active pharmaceutical ingredients in the ear drops.
52 Furthermore, it will contribute to ensuring compliance with regulatory standards and safeguarding patient safety and
53 therapeutic efficacy" (Snyder et al., 2010) [10].

54 55 **2. MATERIAL AND METHODS**

56 57 **2.1 Instrumentation and Analytical Conditions**

58 The HPLC analyses were carried out on an Agilent 1200 system (Santa Clara, CA), composed of a
59 quaternary pump, autosampler, and DAD. Agilent HP ChemStation Software was used for data acquisition and analysis.
60 Separations were performed on Agilent C18 column (250 × 4.6 mm id, 5 µm particle size) from Merck at 30°C. UV
61 detection was at 290 nm for Beclomethasone and 300 nm for clotrimazole, ofloxacin, and lignocaine. Mobile phase A was
62 a mixture of Buffer Potassium dihydrogen phosphate :Methanol ; [45:55 v/v] and mobile phase B was acetonitrile.
63 Gradient elution was carried out as follows: 100% mobile phase A was first held for 5 min, then mobile phase B was
64 raised up to 52% in 7 min, mobile phase B was held at this level until 12 min, and at 12.01 min, mobile phase A was
65 switched back to 100% until 17 min (re-equilibration). The flow rate was 1.5 mL/min, and the injection volume was 20 µL.

66 **2.2 Preparation of standard stock solution:**

67 **2.2.1 Beclomethasone:**

68 Weigh and transfer accurately 1 mg of Beclomethasone working standard into a 100ml clean and dry volumetric flask and
69 make up with 100ml of diluent and sonicate to dissolve.

70 **2.2.2 Clotrimazole:**

71 Weigh and transfer accurately 40 mg of Clotrimazole working standard into a 100ml clean and dry volumetric flask and
72 make up with 100ml of diluent and sonicate to dissolve.

73 **2.2.3 Ofloxacin:**

74 Weigh and transfer accurately 12 mg of Ofloxacin working standard into a 100ml clean and dry volumetric flask and make
75 up with 100ml of diluent and sonicate to dissolve.

76 **2.2.4 Lidocaine:**

77 Weigh and transfer accurately 80 mg of Lidocaine working standard into a 100ml clean and dry volumetric flask and make
78 up with 100ml of diluent and sonicate to dissolve.

79 **2.3 Sample Solution**

80 Prepare the sample test solution equivalent to the Lidocaine 800 ppm, ofloxacin 120 ppm, Clotrimazole 400 ppm,
81 Beclomethasone 10 ppm, in 100ml volumetric flask, added 100ml of diluents phase sonicated for 30 minutes. Make up the
82 volume with diluents. Mixed well and filtered through 0.45µ nylon filter paper discarded first few ml of the filtrate.

83 2.4 EXPERIMENT

84 2.4.1 Optimization of mobile phase:

85 Separation of both the drugs was tried using the following combination of mobile phases. The table 1 gives the details of
86 the same.
87

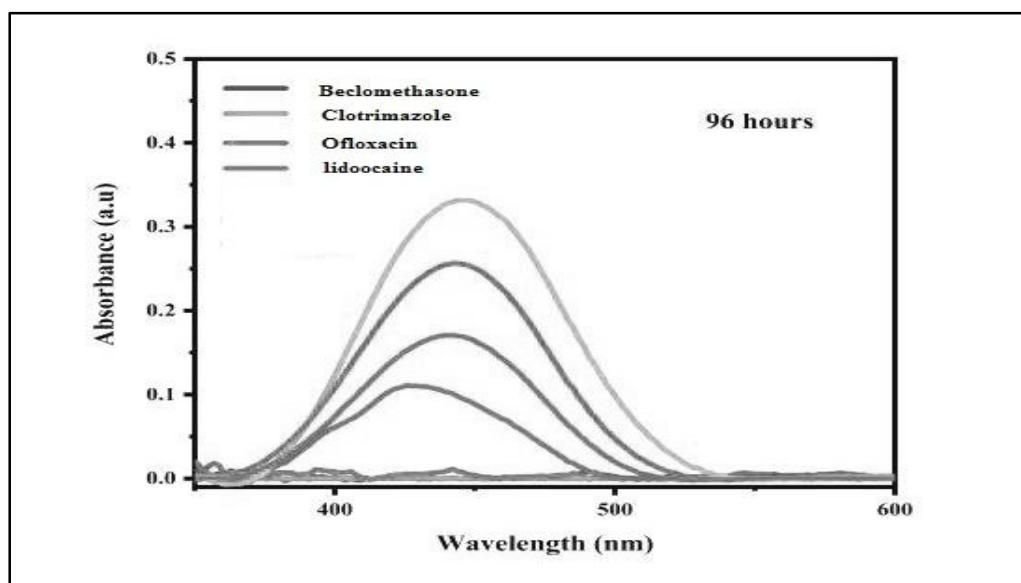
88 **Table 1 Optimization of Mobile Phase**

Sr No.	Mobile phase	Ratio (v/v)	Elution of Peak
1	Buffer: Acetonitrile	30 : 70	Not Proper Separation
2	Buffer: Acetonitrile	50 : 50	Not Proper Separation
3	Methanol: Acetonitrile	45 : 55	Separation of Peak
4	Buffer: Methanol	20:80	Separation of Peak
5	[Buffer :Methanol; 45:55 v/v] : Acetonitrile	50:50	Good Separation

89
90 Out of 5 trials the **5th trial was selected for further studies** because when compared to other trails. 4th trial was found
91 less in retention time due to the ratio of organic solvent in mobile phase.
92

93 2.4.2 Selection of Wavelength

94 Solution of Beclomethasone, Clotrimazole, Ofloxacin and Lidocaine were scanned in the UV region and spectrum
95 was recorded (200-400nm). The solvent used was[Buffer : Methanol : 45:55 v/v] : Acetonitrile Adjust pH with potassium
96 dihydrogen phosphate buffer in ratio of 50:50. It was seen that 300nm as shown in figure 1 All compounds have very good
97 absorbance, which can be used for the estimation of compounds by HPLC.



98
99 **Figure 1 Overlay UV spectrum of Beclomethasone, Clotrimazole, Ofloxacin and Lidocaine showing selection of**
100 **wavelength detection.**
101

102 **2.4.3 Optimized Conditions**

103 The following optimized parameters were used as a final method for the simultaneous estimation of Beclomethasone,
104 Clotrimazole, Ofloxacin and Lidocaine.

105 **2.4.3.1 Instrument**

106 Column Agilent C18 (250x4.6mm) 5μ
107 Column Oven Temperature 30° C
108 Wave length 300 nm
109 Flow rate 1.5 ml/min
110 Injection Volume 20 μl
111 Runtime 30 minutes
112 Mode of Operation Reverse phase

114 **2.4.3.2 Mobile Phase**

115 Solvent A (Potassium dihydrogen phosphate Buffer&Methanol 45:55 v/v)
116 Solvent B (Acetonitrile-50 v/v)
117 Solvent ratio 50 v/v of Solvent A: B

118 **2.4.4 Method Validation**

120 The method was validated according to International Conference on Harmonization Guidance for Industry Q2 (R1)
121 Validation of Analytical Procedures: Text and Methodology (13).

123 **3. RESULTS AND DISCUSSION**

125 **3.1 SYSTEM SUITABILITY**

126 System suitability of the method was performed by calculating the parameters namely, resolution and number of
127 theoretical plates on the 6 replicate injection of standard solution into HPLC system and calculates. The %RSD for 6
128 replicate injections should not more than 2.0%.The system suitability parameters and % RSD for peak areas for 6
129 replicate injections of standard solution was found to be within limits presented in table 2.

130 **Table 2 Result System suitability**

Inj No.	OFLOXACIN		LIDOCAINE		BECLOMETHASONE		CLOTRIMAZOLE	
	RT	Area Response	RT	Area Response	RT	Area Response	RT	Area Response
1	5.67	12517517	9..44	14893246	18.29	1014107	21.85	278518
2	5.64	12474563	9..46	14896344	18.31	996936	21.89	276745
3	5.62	12524563	9..43	14517517	18.27	1002914	21.91	278976
4	5.68	12465631	9..45	14861819	18.36	1021217	21.86	278570

5	5.63	12615668	9..49	14813093	18.25	1002291	21.83	271971
6	5.68	12535678	9.52	14897540	18.26	1007492	21.88	275611
%RSD	0.99		1.02		0.89		0.96	

3.2 SPECIFICITY

The specificity of the RP-HPLC method was demonstrated by the distinct retention times (shown in table 3) for each active pharmaceutical ingredient: Beclomethasone at 18.29 minutes, Clotrimazole at 21.85 minutes, Lidocaine at 9.44 minutes, and Ofloxacin at 5.67 minutes. There was no interference from the diluent or mobile phase at 300 nm, confirming the method's specificity for the quantification of these compounds. see figure 2.

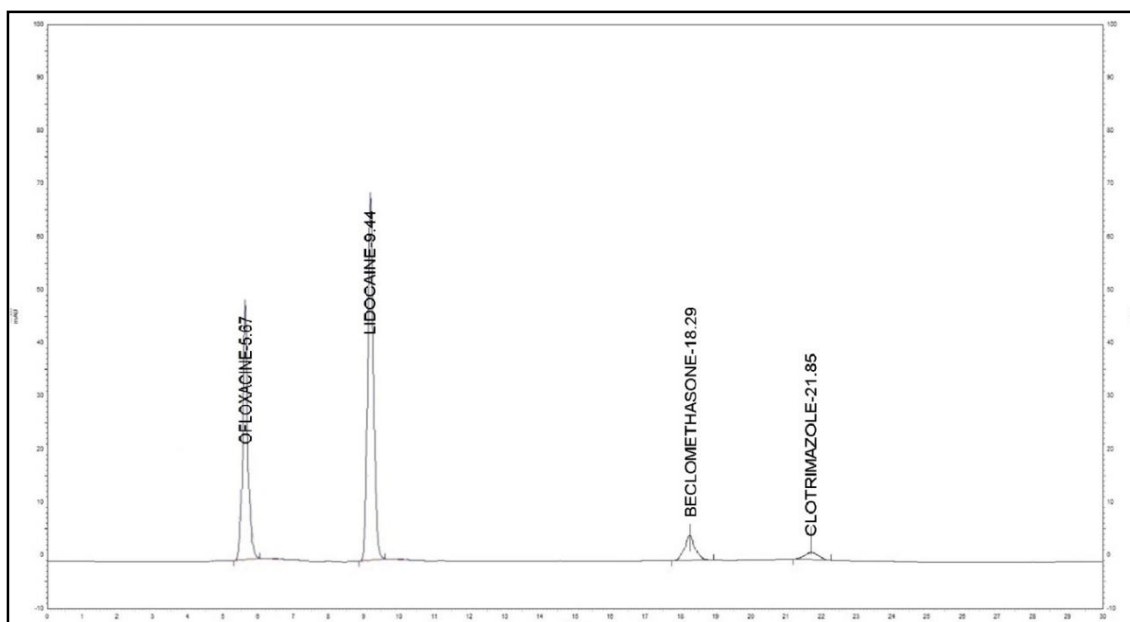


Figure 2 Chromatograms of sample solution at 300 nm of Beclomethasone, Clotrimazole, Ofloxacin and Lidocaine

3.3 LINEARITY

The linearity of the RP-HPLC method was assessed by preparing a series of standard solutions at varying concentrations. Appropriate aliquots of the four-drug combination (1.25, 2.5, 5, 10, and 20 ml) were pipette from the stock solution into a series of 100 ml volumetric flasks and diluted to volume with the makeup phase. (figure 4 Each concentration was then injected (20 ul) into the HPLC system under the optimized chromatographic conditions. The concentration range for each drug, along with the corresponding area of response and correlation coefficient, is provided in Table 4.

These results demonstrate that the method exhibits excellent linearity across the tested concentration ranges for all four compounds. The correlation coefficients for Beclomethasone, Clotrimazole, and Ofloxacin were exactly 1.0, indicating a perfect linear relationship between concentration and response. For Lidocaine, the correlation coefficient was slightly above 1.0, which may indicate an extremely high degree of linearity within the tested range

158

159

160

16

16

16

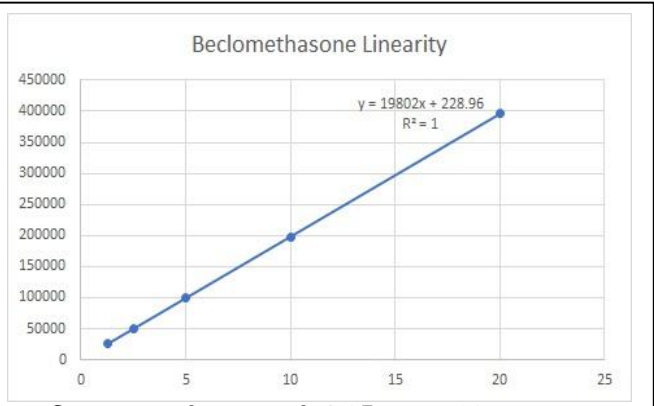
16

16

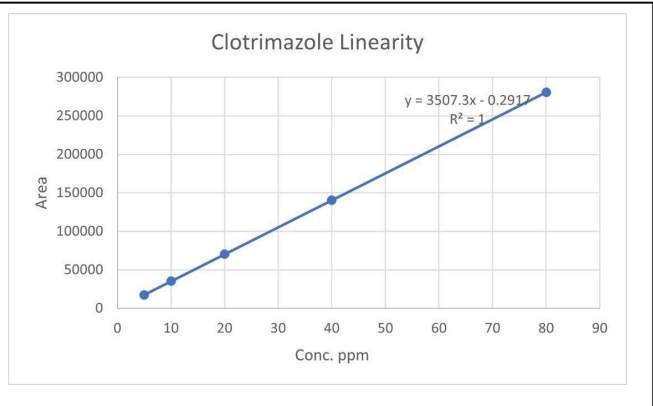
16

16

16



Concentration range is 1.25ppm to 20ppm



Concentration range for is 5ppm to 80ppm

169

17

17

17

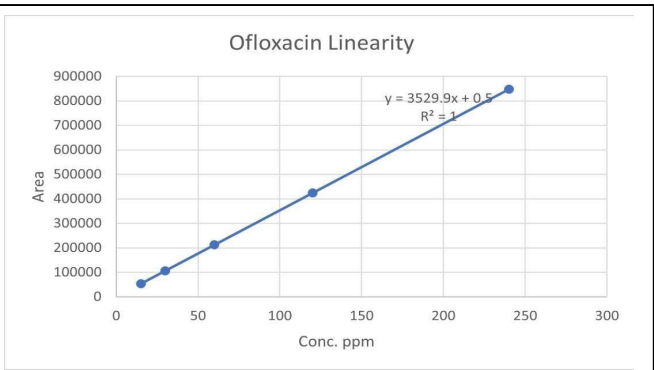
17

17

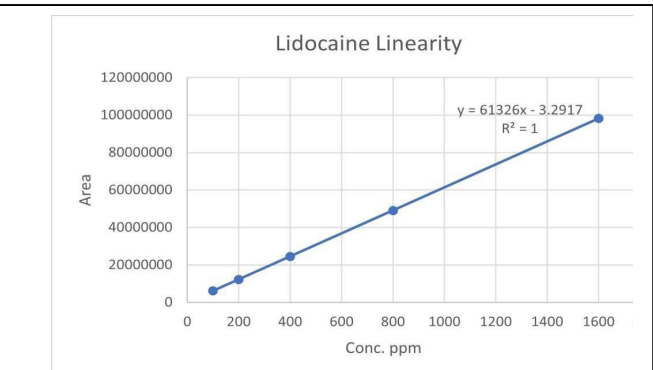
17

17

177



Concentration range for is 15ppm to 240ppm



Concentration range for is 100ppm to 1600ppm

178

179

180

Figure 3 ;The linearity graphs for Beclomethasone, Clotrimazole, Ofloxacin, and Lidocaine These figures illustrate the direct proportionality between the concentration of the analytes and their corresponding responses, validating the method's suitability for quantitative analysis.

181

182

Table 3 Overview of the linearity data and Statistics.

Drug	Linearity level	Concentration (PPM)	Area response	Correlation coefficient
Beclomethasone	1	1.25	25131	1.0
	2	2.5	49543	
	3	5	99086	
	4	10	198172	
	5	20	395102	

Clotrimazole	1	05	17539	1.0
	2	10	35072	
	3	20	70145	
	4	40	140290	
	5	80	280580	
Ofloxacin	1	15	52951	1.0
	2	30	105894	
	3	60	211792	
	4	120	423583	
	5	240	847169	
Lidocaine	1	100	6132563	1.01
	2	200	12265100	
	3	400	24530262	
	4	800	49060504	
	5	1600	98120991	

183

184

3.4 ROBUSTNESS

185

186

187

188

189

190

The robustness of the method was determined by carrying out experiment at different flow rate and variation in mobile phase composition. Result of the Robustness at different variable parameter given in table 5. Results for actual mobile phase composition (50:50 Acetonitrile: Methanol & phosphate buffer) has been considered from accuracy standard.

Table 4 Result of the Robustness at different variable parameter

Sr. No	Name	Flow Rate (ml/min)	System Suitability Result		Change in composition Mobile Phase	System Suitability Result	
			Plate Count	SpTailing		Plate Count	SpTailing
1	Ofloxacin	0.5	4236	1.48	2 % less	4239	1.43
		1	4239	1.48	Actual	4242	1.43
		1.5	4963	1.56	2% More	4966	1.51
2	Lidocaine	0.5	5156	1.57	2 % less	5159	1.52
		1	5158	1.57	Actual	5161	1.52
		1.5	5363	1.66	2% More	5367	1.61
3	Beclomethasone	0.5	3369	1.31	2 % less	3371	1.26

		1	2983	1.27	Actual	2987	1.22
		1.5	3102	1.29	2% More	3104	1.24
4	Clotrimazole	0.5	2156	1.20	2 % less	2159	1.19
		1	2346	1.19	Actual	2349	1.17
		1.5	2349	1.19	2% More	2353	1.19

The % RSD of retention time and asymmetry were within limits for variation (+ 2 %) in composition of mobile phase. Hence the method was found to be robust.

3.5 PRECISION

Standard solutions containing (0.1, 0.2, 0.4 µg/mL) of Beclomethasone, (0.4, 0.8, 1.6 µg/mL) of Clotrimazole, (1.2, 2.4, 3.6µg/mL) of Ofloxacin and (8, 16, 32 µg/mL) of Lidocaine were analyzed three times on the same day for the determination of intra-day precision and on three different days for the determination of inter-day precision. % RSD values for intra-day and inter-day precision are represented in table 7.

Table 5 Repeatability Data For Ofloxacin, Lidocaine, Beclomethasone, Clotrimazole

Ofloxacin			Lidocaine			Beclomethasone			Clotrimazole		
Conc (ug/ml)	PeakArea	% RSD	Conc (ug/ml)	PeakArea	% RSD	Conc (ug/ml)	Peak Area	% RSD	Conc (ug/ml)	Peak Area	% RSD
60	12517517	0.99	400	14893246	1.02	5	1014107	0.89	20	278518	0.96
	12474563			14896344			996936			276745	
	12524563			14517517			1002914			278976	
	12465631			14861819			1021217			278570	
	12615668			14813093			1002291			271971	
	12535678			14897540			1007492			275611	

Table 6 Day And Inter-Day Precision For Ofloxacin, Lidocaine, Beclomethasone, Clotrimazole

Beclomethasone		Clotrimazole		Ofloxacin		Lidocaine	
Conc (ug/ml)	Mean=S.D	Conc (ug/ml)	MeanS.D	Conc (ug/ml)	Mean S.D	Conc (ug/ml)	Mean S.D
Intra-Day Precision							
0.1	1014110 =15.9	0.4	136985=1.92	1.2	6258745=3.92	8	7416623=16.21
0.2	2031562 =37.3	0.8	278518=8.56	2.4	12517517=3.71	16	14833246=22.23

0.4	3953565=47.21	1.6	560053=32.91	3.6	24936245=3.77	32	28555657=35.76
Inter-Day Precision							
0.1	1014113 =26.9	0.4	136981=1.92	8.2	6258753=3.02	8	7416623=26.41
0.2	2031568 =48.3	0.8	278526=8.56	14.3	12517524=9.24	16	14833246=62.31
0.4	3953559=68.21	1.6	560062=32.91	28.9	24936249=11.78	32	28555657=95.69

3.6 ACCURACY

Accuracy was calculated at three different levels in terms of % recovery by spiking known amount of standard solution (80%, 100% and 120%) to the solution of a synthetic laboratory mixture of Beclomethasone, Clotrimazole, Ofloxacin and Lidocaine. % mean recovery of each drug is given in table 8, 9, 10 and 11 respectively.

Table 7 Accuracy in Terms of % Recovery for Beclomethasone

Conc. Level(%)	Sample amount (ug/mL)	Amount of Standard Added (ug/mL)	Beclomethasone		
			Amount Recovered (ug/mL)	% Recovery	% Mean Recovery
80%	5	4	4.02	100.5	100.5
	5	4	4.06	101.5	
	5	4	3.98	99.5	
100%	5	5	4.99	99.8	100.6
	5	5	4.98	99.6	
	5	5	5.12	102.4	
120%	5	6	6.01	100.16	100.55
	5	6	6.13	101.83	
	5	6	5.98	99.66	

Table 8 Accuracy in Terms of % Recovery for Clotrimazole

Conc. Level (%)	Sample amount (ug/mL)	Amount of Standard Added (ug/mL)	Clotrimazole		
			Amount Recovered (ug/mL)	% Recovery	% Mean Recovery

80%	20	16	16.24	101.21	100.70
	20	16	15.94	99.62	
	20	16	16.26	101.29	
100%	20	20	20.19	100.74	99.67
	20	20	19.78	99.12	
	20	20	19.79	99.17	
120%	20	24	23.57	98.57	98.84
	20	24	23.88	99.60	
	20	24	23.51	98.36	

212

213

Table 9 Accuracy in Terms of % Recovery for Ofloxacin

Conc. Level (%)	Sample amount (ug/mL)	Amount of Standard Added (ug/mL)	Ofloxacin		
			Amount Recovered (ug/mL)	% Recovery	% Mean Recovery
80%	60	48	48.12	100.25	100.24
	60	48	47.87	99.72	
	60	48	48.36	100.75	
100%	60	60	60.23	100.38	100.02
	60	60	60.01	100.01	
	60	60	59.81	99.68	
120%	60	72	72.11	100.15	99.96
	60	72	72.09	100.12	
	60	72	71.73	99.62	

214

215

216

217

218

Table 10 Accuracy in Terms of % Recovery for Lidocaine

		Amount of	Lidocaine
--	--	-----------	-----------

Conc. Level (%)	Sample amount (ug/mL)	Standard Added (ug/mL)	Amount Recovered (ug/mL)	% Recovery	% Mean Recovery
80%	400	320	320.56	100.17	100.02
	400	320	319.81	99.94	
	400	320	319.88	99.96	
100%	400	400	400.04	100.01	100.08
	400	400	401.00	100.25	
	400	400	399.98	99.99	
120%	400	480	480.96	100.20	100.05
	400	480	480.01	100.00	
	400	480	479.82	99.96	

219
220
221
222
223
224
225
226
227
228
229
230
231
232

4. CONCLUSION

UV Detector and Agilent C18 (250x4.6mm) 5 μ column, injection of 20 μ l is injected and eluted with the mobile phase of acetonitrile and Potassium Dihydrogen phosphate buffer with pH 3.5 & Methanol (45:55 v/v) in the ratio 50:50 which was pumped at a flow rate of 1.5ml at 300nm. The peak of within limits was found well separated within 30 min. The developed method was validated for various parameters as per ICH guidelines like system suitability, specificity, linearity, system precision, method precision, accuracy, ruggedness and robustness.

In conclusion, the developed RP-HPLC method proved to be reliable, accurate, and sensitive for the quantitative analysis of beclomethasone, clotrimazole, ofloxacin, and lignocaine in combination ear drops. This validated method can be effectively utilized for routine quality control analysis and batch release of pharmaceutical formulations, ensuring product safety and efficacy.

233
234
235
236
237
238

ACKNOWLEDGEMENTS

The authors would like to acknowledge the Faculty of Pharmaceutical Chemistry and Analysis at Gondwana University Gadchiroli for facilitating the accomplishment of the current work. The research did not receive any funding. The research was performed at Maharashtra Institute of Pharmacy, Betala, Bramhapuri.

239 **COMPETING INTERESTS**

240
241 The authors declare that there are no conflicts of interest regarding the publication of this paper

242
243 **AUTHORS' CONTRIBUTIONS**

244
245 All persons who meet authorship criteria are listed as authors, and all authors certify that they have participated
246 sufficiently in the work to take public responsibility for the content, including participation in the concept, design, analysis,
247 writing, or revision of the manuscript. Furthermore, each author certifies that this material or similar material has not been
248 and will not be submitted to or published in any other publication.

249 Disclaimer (Artificial intelligence)

250 Option 1:

251 Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.)
252 and text-to-image generators have been used during the writing or editing of this manuscript.

253 Option 2:

254 Author(s) hereby declare that generative AI technologies such as Large Language Models, etc. have been used during the
255 writing or editing of manuscripts. This explanation will include the name, version, model, and source of the generative AI
256 technology and as well as all input prompts provided to the generative AI technology

257 Details of the AI usage are given below:

258 1.

259 2.

260 3.

261
262
263 **REFERENCES**

264
265 [1] Sobel, J. D., Faro, S., Force, R. W., Foxman, B., Ledger, W. J., Nyirjesy, P. R., ... Wiesenfeld, H. C. (2003). Vulvovaginal
266 Candidiasis: Epidemiologic, Diagnostic, and Therapeutic Considerations. *American Journal of Obstetrics and Gynecology*, 188(6
267 Suppl), S85–S94. [PubMed](<https://pubmed.ncbi.nlm.nih.gov/12824949/>)

268
269
270
271 [2] Roland, P. S., & Marple, B. F. (2005). Disorders of the External Auditory Canal. *American Journal of Rhinology*, 19(5),
272 517–526. [PubMed](<https://pubmed.ncbi.nlm.nih.gov/16370204/>)

273
274
275 [3] Richardson, M. D., & Warnock, D. W. (1997). *Fungal Infection: Diagnosis and Management* (3rd ed.). Oxford: Blackwell
276 Science.

277
278 [4] Covino, B. G., & Vassallo, H. G. (1976). *Local Anesthetics: Mechanisms of Action and Clinical Use*. New York: Grune &
279 Stratton.

280
281 [5] Snyder, L. R., Kirkland, J. J., & Dolan, J. W. (2010). *Introduction to Modern Liquid Chromatography* (3rd ed.). Hoboken,
282 NJ: John Wiley & Sons.

283
284

285 [6] GÖrÖg S. Recent advances in the analysis of steroid hormones and related drugs. Analytical sciences.
286 2004;20(5):767-82.

287
288 [7] Gumułka P, Żandarek J, Dąbrowska M, Starek M. UPLC technique in pharmacy—an important tool of
289 the modern analyst. Processes. 2022 Nov 24;10(12):2498.

290
291 [8] Masareddy R, Sandure P, Patil A, Gaude Y, Patil A. In situ gastric floating gel of atazanavir sulphate for
292 sustained release: formulation, optimization and evaluation. Therapeutic Delivery. 2023 Oct 1;14(10):619-33.

293
294 [9] Kalshetti S, Rao KP. Development and validation of HPLC method for the quantification of clotrimazole
295 in a gelatin film formulation. Future Journal of Pharmaceutical Sciences. 2021 Dec;7:1-9.

296
297 [10] Gilpin RK, Gilpin CS. Pharmaceuticals and related drugs. Analytical chemistry. 2009 Jun
298 15;81(12):4679-94.

301 302 **ABBREVIATIONS**

303 μ L - Microliter (volume)

304 mL - Milliliter (volume)

305 nm - Nanometer (wavelength)

306 μ g - Microgram (mass)

307 mg - Milligram (mass)

308 g - Gram (mass)

309 % - Percentage (concentration or proportion)

310 min - Minute (time)

311 18. °C - Degrees Celsius (temperature)

312 19. AU - Absorbance Units (absorbance measurement)

313 HPLC - High-Performance Liquid Chromatography

314 UV - Ultraviolet (Detector)

315 MS - Mass Spectrometry (Detector)

316 RT - Retention Time

317 RP-HPLC - Reverse Phase High-Performance Liquid Chromatography

318 LOD - Limit of Detection

319 LOQ - Limit of Quantitation

320 API - Active Pharmaceutical Ingredient

321 C18 - Octadecylsilane (common type of stationary phase))

322 %R - Percentage Recovery

323 ICH Q2(R1) - International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human
324 Use, Guideline Q2(R1): Validation of Analytical Procedures: Text and Methodology

325 %RSD - Percent Relative Standard Deviation

326 NMT -Not More Than

327