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(RESEARCH ARTICLE)

A novel RP-HPLC method for the simultaneous quantification of azithromycin and dexamethasone in marketed ophthalmic drops

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Abstract

A straightforward, precise, accurate, specific, and sensitive RP-HPLC technique has been established for the quantification of Azithromycin Dihydrate and Dexamethasone Sodium Phosphate in their commercial formulation. The wavelength maxima for Azithromycin and Dexamethasone were considered to be 215 nm for estimation. In RP-HPLC separations were carried out on a Zorbax SB C18 column (250mm X 4.6mm, 5 µm) and mobile phase consisting of Acetonitrile: Monobasic Potassium Phosphate (KH_2PO_4) buffer in the proportion of 75:25 v/v and pH of the buffer was adjusted to 5.5 with OPA. The estimation was carried out at 215 nm using UV detector keeping the flow rate of 1.2 ml/min and injection volume of 50 µl. The current method demonstrates good linearity over the range of 20 - 120 µg/ml and 2 - 12 µg/ml for Azithromycin and Dexamethasone respectively. No interference of excipients was found during estimation. The recommended techniques were verified and determined to be specific, accurate, and exact. The approaches were effectively used for the concurrent quantification of both medicines in pharmaceutical formulations, making them suitable for regular quality control examination.

Keywords: Azithromycin Dihydrate (AZI); Dexamethasone Sodium Phosphate (DEX); RP-HPLC method; ICH Q2 (R1) guidelines.

1. Introduction

Conjunctivitis, referred to as pink eye, is an inflammation or infection of the conjunctiva, the delicate, transparent membrane that envelops the sclera and the inner eyelids. It may be induced by viruses, germs, allergies, or irritants. Viral and bacterial conjunctivitis are highly transmissible, often disseminating via direct contact with contaminated surfaces or respiratory droplets. Symptoms include erythema, pruritus, excessive lacrimation, discharge, and a gritty feeling in the ocular region. [1-3] Although the majority of cases cure without issues, untreated bacterial infections may result in severe outcomes, including corneal ulcers, scarring, and compromised vision. Allergic conjunctivitis, although non-contagious, may lead to persistent discomfort if the underlying allergens are not addressed. There is no specific treatment available for conjunctivitis, but Antibiotic eye drops or ointments are effective in treating viral and bacterial conjunctivitis. Antibiotic like Trifluridine, Acyclovir, Ganciclovir, Tobramycin, Ofloxacin, Ciprofloxacin, Gentamicin, Azithromycin and Doxycycline used to treat viral and bacterial conjunctivitis. While, Prednisolone, Dexamethasone, Valacyclovir and Famciclovir used for Heraptic and Chlamydial conjunctivitis. [4-10]

Azithromycin (AZI) is an antibiotic useful for the treatment of bacterial infections (more active against gram negative bacteria). It is an Azide subclass of macrolide antibiotic which inhibit protein synthesis. The IUPAC name of AZI is (2R,3S,4R,5R,8R,10R,11R,12S,13S,14R)-2-ethyl-3,4,10trihydroxyl 3,5,6,8,10,12,14-heptamethyl-15-oxo-11-{[3,4,6-trideoxy 3- (dimethylamino) -β-D-xylo - hexopyranosyl] oxy}-1-oxa-6-azacyclopentadec-13-yl 2, 6- dideoxy-3-C-

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methyl-3-O-methyl-α-L-ribo-hexopyranoside. AZI is somewhat more potent against certain bacterial species than erythromycin, but its widespread popularity arises primarily from its slow elimination from the body, which allows many infections to be treated with 3-5 days of once-daily administration compared to 3-4 times a day for up to two weeks for erythromycin. [1-8] Dexamethasone (DEX) is a type of steroid medication. It has an anti-inflammatory and immunosuppressant effects. It is 25 times more potent than cortisol in its glucocorticoid effect, while having minimal mineralocorticoid effect. The chemical name of DEX is (8S,9R,10S,11S,13S,14S,16R,17R)-9 Fluoro-11,17-dihydroxy-17(2hydroxyacetyl)-10,13,16 trimethyl 6,7,8,9,10,11,12,13,14,15,16,17dodecahydro-3H-cyclo penta [a]phenanthren-3-one 9α-fluoro-11β,17α, 21- trihydroxy -16α-methyl-1,4-pregnadiene-3, 20-dione. DEX is used for the treatment of many conditions including: rheumatologic problems, a number of skin diseases, severe allergies, asthma, chronic obstructive lung disease, cerebral edema, in addition to other medications in tuberculosis and a number of other infectious diseases among others. [10-13] It is pregnancy category C in the United States and Class A in Australia meaning that it has been frequently used in pregnancy and not been found to cause problems for the baby. Figure 1 represents the chemical structure for Azithromycin (AZI) and Dexamethasone (DEX) respectively.

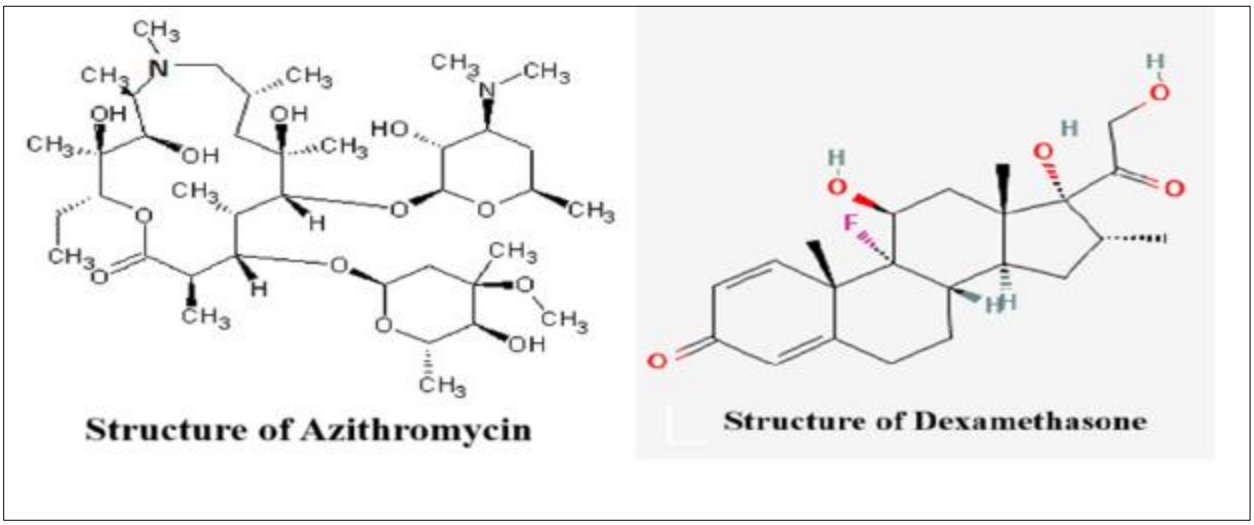

Figure 1 Chemical structure for Azithromycin (AZI) and Dexamethasone (DEX)

Through literature survey concludes various analytical method reported for qualitative or quantative estimation of AZI and DEX bulk drug and drug product as well in combination product [1-19]. Chandrakanth Bandapally et. al [2], developed and validated RP-HPLC method for estimation of AZI and DEX, while Akshata et. al performed HPLC validation method for DEX in bulk and tablet form [3]. Okaru AO et. al, developed robust HPLC method for AZI estimation in Bulk Samples, Tablets and Suspensions [4] and Chen Q evaluated topical AZI for treatment of conjunctivitis [5]. In this research, validated analytical method [20] developed for simultaneous estimation of AZI and DEX in marketed formulation.

2. Materials and method

2.1. Reagents and chemicals

The AZI and DEX sample was generously supplied by Mediwin Pharmaceuticals for this testing. HPLC-grade methanol and acetonitrile were procured from Merck. The sodium hydroxide pellets and hydrochloric acid were of analytical grade procured from Loba Chemicals Ltd. The experiment used just Milli-Q water. The Zaha-DX Eye Drop brand was acquired from the local market.

2.2. Preparation of Standard Solution

2.2.1. Preparation of Standard stock solution of AZI (1000 µg/ml)

Accurately weighed 50 mg AZI was transferred into 50 ml volumetric flask. Sufficient mobile phase was added to dissolve the drug and the volume was made up to mark with mobile phase to obtained stock solution (1000 µg/ml) and labeled as Standard Solution1(SS1).

2.2.2. Preparation of Standard stock and diluted solutions of DEX (100 µg/ml)

Accurately weighed 50 mg DEX was transferred into 50 ml volumetric flask. Sufficient mobile phase was added to dissolve the drug and the volume was made up to mark with mobile phase to obtained stock solution (1000 µg/ml). An aliquot of solution was transferred suitable volumetric flask and diluted with mobile phase to be obtained working standard solution (100 µg/ml) and labeled as Standard Solution 2(SS2).

2.2.3. Preparation of combined standard solution of AZI and DEX

AZI 1000 µg/ml and DEX 100 µg/ml were accurately transferred to a suitable volumetric flask to obtain final concentration 40 µg/ml of AZI and 4 µg/ml of DEX. The solution was labelled as Standard Solution 3(SS3).

2.3. Preparation of Sample Solution

One ml of sample solution was transferred into 10 ml volumetric flask and the volume was made up to mark with mobile phase. (The solution contains 1000 µg/ml of AZI and 100 µg/ml of DEX). An aliquot of 0.4 ml from this solution was transferred in 10 ml volumetric flask and diluted up to mark with mobile phase. (The final solution contains 40 µg/ml of AZI and 4 µg/ml of DEX). The solution was filtered through 0.45 µm PVDF syringe filter and first 2 ml of filtrate were discarded.

2.4. Optimization of chromatographic conditions

A trial-and-error process was used to optimize the chromatographic conditions. Numerous trials were taken with different ranges of buffers in composition with suitable solvents to achieve proper elution of AZI and DEX. On a conclusion basis, the mobile phase in combination with ACN: KH_2PO_4 buffer pH 5.5 (adjusted with Orthophosphoric Acid) with a ratio of 75:25, 1.2 mL/minute flow rate with Zorbax SB C18 column (250mm X 4.6mm, 5 µm) was chosen for better elution pattern. The injection volume was 50 µL and 215 nm wavelength was selected for the symmetry of the analyte peak.

2.5. Validation of the analytical method

The analytical technique was validated using ICH recommendations Q2 (R1) for specificity, linearity, accuracy, precision, limit of detection, limit of quantitation, and robustness [20].

2.5.1. Specificity

The analytical method's specificity was used to weed out any potential interference from blank samples at the retention time of the AZI and DEX analyte peak.

2.5.2. Linearity

The linearity of AZI and DEX was plotted by a series of dilutions from Stock Standard Solution (SS1 and SS2) with concentrations of AZI in the range of 20-120µg/ml (20, 40, 60, 80, 100 and 120 µg/ml) and of DEX in the range of 2-12µg/ml (2, 4, 6, 8, 10 and 12 µg/ml). The plot of peak area against concentration was plotted Correlation coefficient and regression line equations for AZI and DEX were calculated.

2.5.3. Accuracy

Accuracy was determined by calculating the % recovery of AZI and DEX from marketed formulation by the standard addition method in which, known amounts of standards powders of AZI and DEX at 50%, 100% and 150% levels were added to the pre-analysed samples. The recovered amounts of AZI and DEX were calculated at each level and % recovery was reported.

2.5.4. Precision

The proposed strategy's intra-day and inter-day precision was determined by measuring the corresponding responses 3 times on the same day for 3 different concentration of AZI (40, 60 and 80 µg/ml) and DEX (4, 6 and 8 µg/ml) each. The results were reported in terms of relative standard deviation. The repeatability of the proposed method was determined by measuring the corresponding responses 6 times for 100% test concentration of AZI (40 µg/ml) and DEX (4 µg/ml) each.

2.5.5. Limit of Detection and Limit of Quantification

The limit of detection (LOD) and the limit of quantification (LOQ) were calculated using the standard deviation of response (SD) and slope (S) of the calibration curve.

LOD=3.3*SD/S

LOQ=10*SD/S

2.5.6. Robustness

By altering the approach's flow rate, wavelength and mobile phase ratio parameters, the robustness analysis was carried out. Robustness was assessed by adjusting the mobile phase ratio (± 2% absolute), wavelength (±2 nm) and flow rate (± 0.1ml/min). The alterations were observed and contrasted with standard circumstances.

2.6. Estimation of AZI and DEX in market formulation by the proposed method

The solution was prepared according to the Blank, followed by the injection of standard and sample preparations, and the assay was determined. The ultimate solution was introduced into the HPLC apparatus. The apex regions of AZI and DEX were extracted from the chromatogram and used for the quantification of AZI and DEX in the commercial formulation.

3. Result and discussion

3.1. Selection of Chromatographic Conditions

Multiple experiments were conducted with diverse mobile phase ratios at changing flow rates with different USP column types, including C8 and C18, to get optimal symmetry of the analyte peaks, AZI and DEX. Numerous experiments for mobile phase selection were conducted by evaluating the pKa value and solubility of the chemical. The pH modification of the buffer, in conjunction with the solvent composition in the mobile phase, is crucial for optimizing the separation of the analyte peak from the degradation peak. Finally monobasic phosphate buffer (KH_2PO_4) pH 5.5 (adjusted with Orthophosphoric Acid) with Acetonitrile in ratio of 25:75 (v/v) with Zorbax SB C18 column (250mm X 4.6mm, 5 µm) at 1.2 mL per minute flow shows better separation of analyte peak. The injection volume was adjusted to 50µL and the analyte peak was detected at 215 nm. The analyte peak elutes within the time frame of 15 minutes. The chromatograms of the blank and standard were shown in Figure 2 and 3.

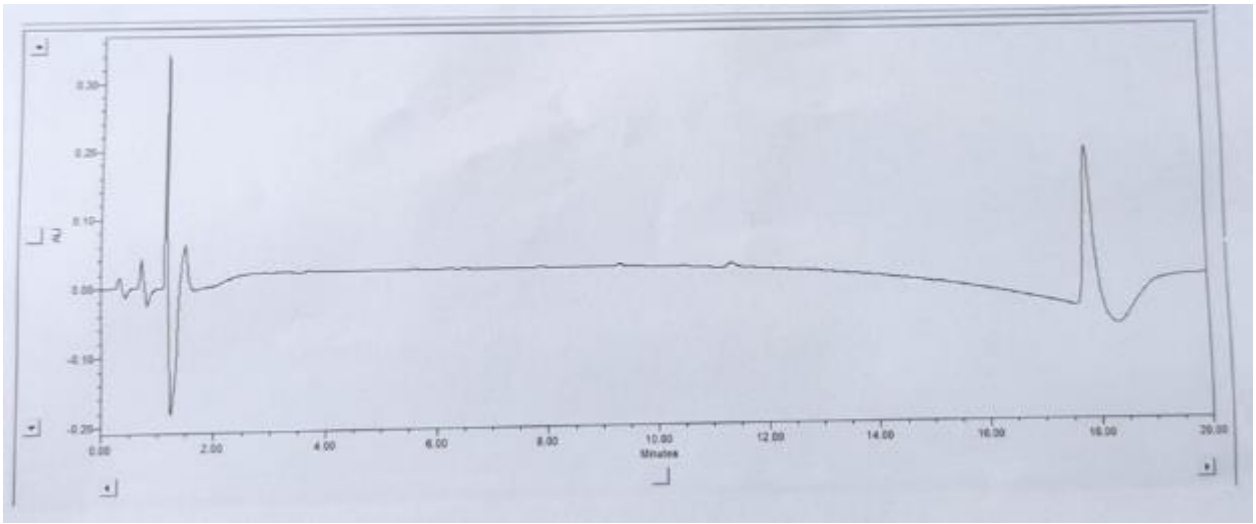

Figure 2 Chromatogram of AZI and DEX Blank Solution

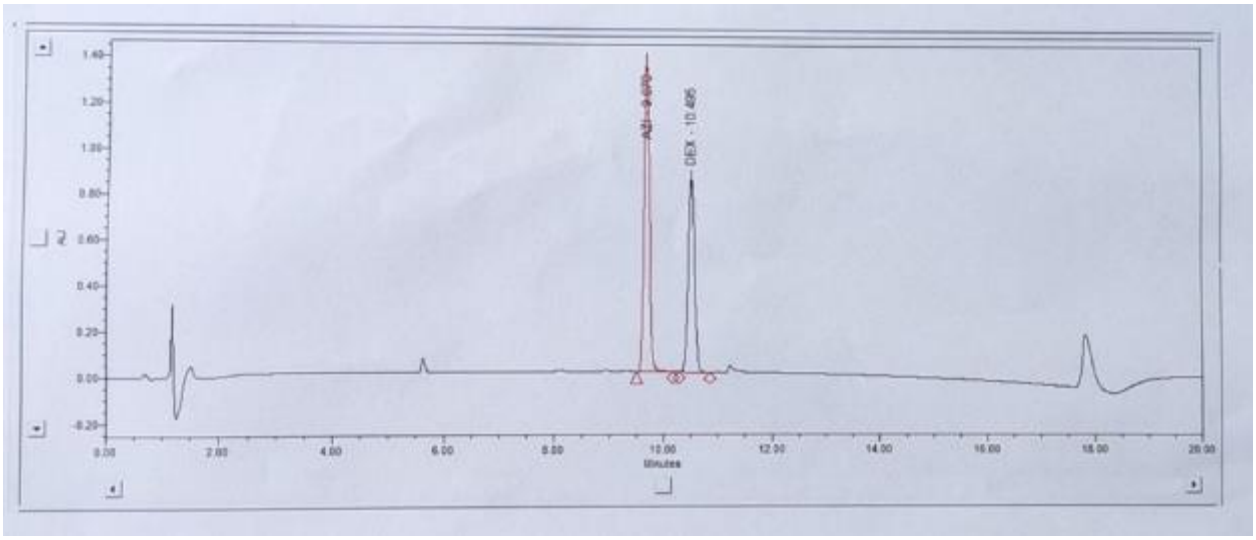

Figure 3 Chromatogram of AZI and DEX Standard Solution

3.2. Results of validated analytical method

3.2.1. Specificity

The analytical approach demonstrated specificity, since there was no interference from the diluent during the retention of AZI and DEX. Figure 4 illustrates the overlay graph of the blank beside the conventional answer.

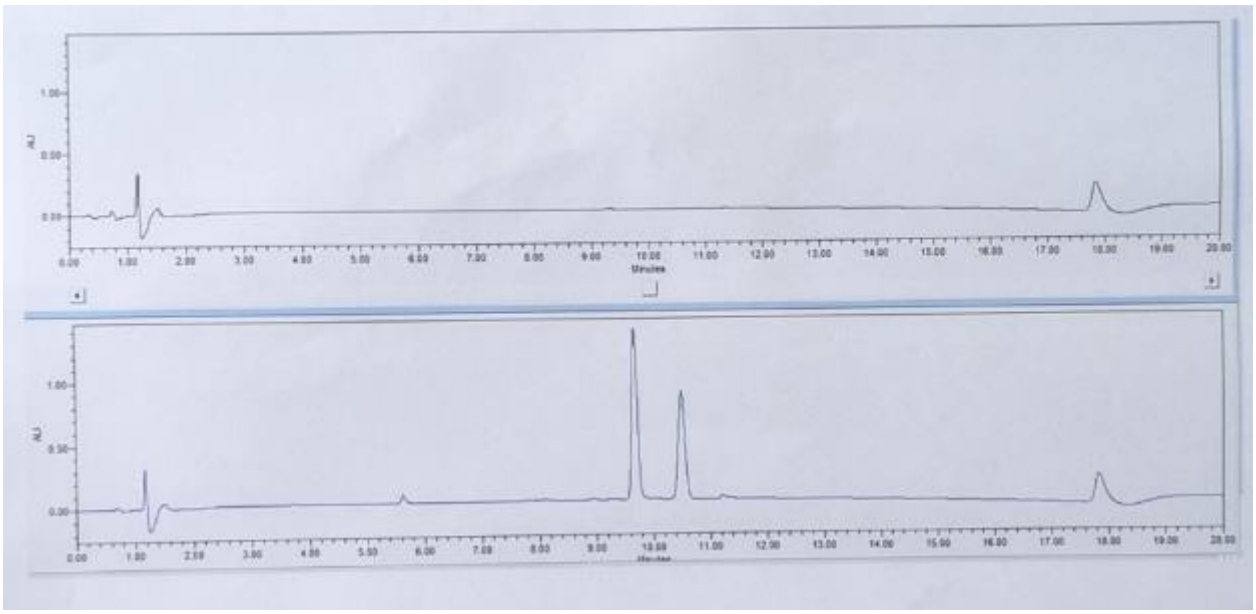

Figure 4 Overlaid Chromatogram of AZI and DEX for Specificity**.**

3.2.2. Linearity

The peak area versus concentration was used to establish linearity, with a concentration range of 20-120 µg/ml for AZI and 2-12 µg/ml for DEX. The correlation coefficient was determined to be 0.999, consistent with the guidelines shown in the linearity plots presented in Figures 5 and 6, with corresponding results displayed in Table 1.

Table 1 Result of AZI and DEX Linearity

Parameter	Result for AZI	Result for DEX
Linearity Range	20-120µg/ml	2-12µg/ml
Linearity	Y = 7240.5x - 66618	Y = 12367x + 55654
Correlation coefficient	0.9996	0.9995
LOQ µg/mL	1.80(µg/mL)	0.25 (µg/mL)
LOD µg/mL	0.60(µg/mL)	0.08 (µg/mL)

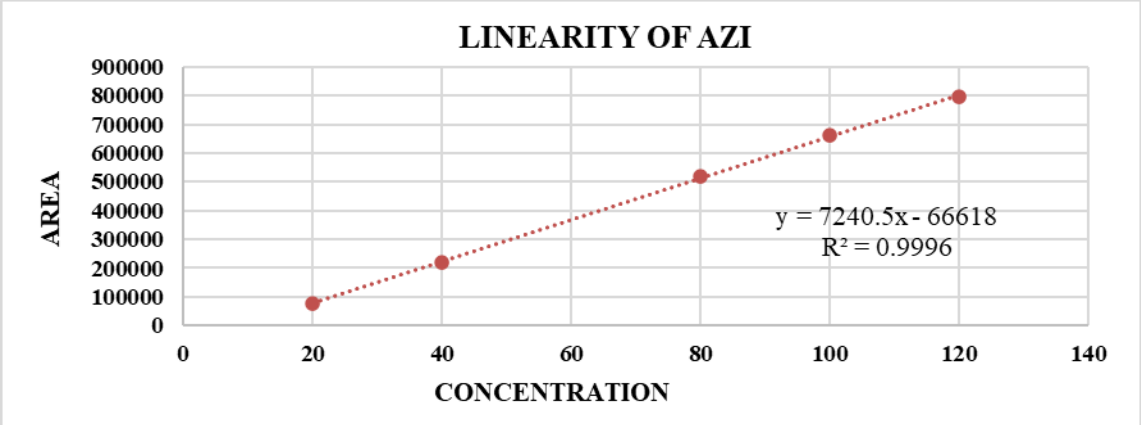

Figure 5 Linearity plot of AZI

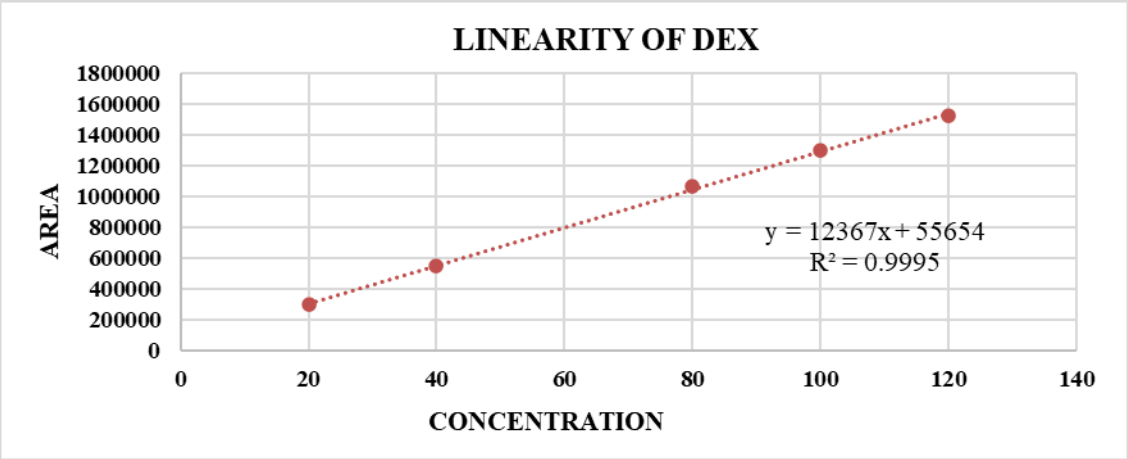

Figure 6 Linearity plot of DEX

3.2.3. Accuracy

Recovery was conducted at medication concentrations of 50%, 100%, and 150% for AZI and DEX. The recovery values were determined to be within the anticipated range of 95% to 105%. The findings are shown in Table 2.

3.2.4. Precision

The % RSD of six replicates of the standard solution were determined to be 0.76% for AZI and 1.08% for DEX. The % RSD for inter-day precision for AZI and DEX was determined to be 0.89% and 1.13%, respectively, while the % RSD for intra-day precision was found to be 1.21% and 1.51%, respectively. The findings demonstrate that the procedure is accurate.

Table 2 Result of AZI and DEX Recovery

Name of Compound	Recovery Level	% Recovery			%Recovery Avg.	%RSD
		Set- 1	Set- 2	Set- 3		
AZI	50	99.5	98.5	99.2	99.1	0.51
	100	100.5	99.1	101.8	100.5	1.34
	150	100.1	101.1	99.9	100.4	0.64
DEX	50	99.1	98.9	100.5	99.5	0.88
	100	101.7	100.5	100.1	100.8	0.83
	150	101.1	100.5	98.9	100.2	1.13

3.2.5. Robustness

The approach was deemed reliable since minor changes in the mobile phase's composition, flow rate, and detector wavelength does not significantly affect the findings. The findings are shown in Table-3.

Table 3 Result of AZI and DEX Robustness

Drug Name	Condition	As Such (Normal)	Flow Plus 1.3 mL/min	Flow Minus 1.1 mL/min	Plus Wavelength (217nm)	Minus Wavelength (213nm)	Plus Organic ACN: Buffer (77:23 %v/v)	Minus Organic ACN: Buffer (73:27 %v/v)
AZI	%RSD	0.89	0.97	1.13	1.11	0.85	1.45	1.10
	USP Plates	5896	6012	5987	5569	5753	4523	6352
	USP Tailing	0.9	1.1	1.0	1.2	1.1	0.9	0.8
DEX	%RSD	1.40	1.21	1.18	0.99	1.03	1.12	1.35
	USP Plates	8752	8145	7896	7632	7965	8963	6521
	USP Tailing	1.0	1.1	1.0	1.0	0.9	0.9	1.1

3.3. Estimation of AZI and DEX in Marketed Formulation

Table 4 Market formulation analysis (n=5)

Dosage	AZI			DEX		
Eye Drop	Label Value (10	% Drug	Mean ± SD	Label Value (1	% Drug	Mean ± SD
Spl-1	9.951	99.51	99.58 ± 0.52	0.986	98.6	100.16 ± 1.14
Spl-2	9.896	98.96		1.01	101	
Spl-3	10.011	100.11		0.996	99.6	
Spl-4	9.921	99.21		1.001	100.1	
Spl-5	10.01	100.1		1.015	101.5	

The suggested RP-HPLC technique for the measurement of AZI and DEX was successfully used for the eye drop formulation. The % assay of AZI and DEX was found to be satisfactory and consistent with the label claim. The analytical results were shown in Table 4.

4. Conclusion

An accurate, selective, and specific RP-HPLC technique was successfully developed and validated for the detection of AZI and DEX in the commercial formulation. The procedure's dependability is shown by the results of precision and accuracy. There exists No substantial interference was detected at the retention times of AZI and DEX, and a linear response was noted at a calibration range of 20-120 µg/ml for AZI and 2-12 µg/ml for DEX, with a correlation value of around 0.999. The recovery for both components was within the acceptable range of 95%-105%. The aforementioned approach is dependable and may be used to estimate AZI and DEX in laboratory environments for future applications.

Compliance with ethical standards

Disclosure of conflict of interest

The authors affirm that they have no known financial or interpersonal conflicts that would have appeared to have an impact on the research presented in this study.

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