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ANALYTICAL METHOD DEVELOPMENT, OPTIMIZATION AND VALIDATION OF COMBINATION OF AZITHROMYCIN AND BENZOYL PEROXIDE BY RP-HPLC USING DESIGN OF EXPERIMENT AS PER ICH GUIDELINE

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Abstract

A simple, fast, accurate and precise RP-HPLC method were developed and validated for the estimation of Azithromycin & Benzoyl peroxide per ICH guidelines. Potassium dihydrogen phosphate and Acetonitrile (50:50) are commonly used as solvents. The method was developed in Eclipse C_{18} column (Waters XTerra®, 4.6X250 mm, particle 5µ) with Potassium dihydrogen phosphate and Acetonitrile are commonly used solvents in RP -HPLC having low UV cut- off of 200-400 nm respectively. In RP-HPLC method was found to be linear in the range of Azithromycin/Benzoyl peroxide is 1-5µg/ml with a correlation coefficient value of 0.99. The accuracy studies of RP-HPLC method was performed at three different levels, i.e., 80%, 100%. The limit of detection (LOD) and Limit of Quantification (LOQ) for Azithromycin 0.009µg/ml & 0.028µg/ml and for Benzoyl peroxide 0.015µg/ml & 0.046µg/ml is to be were found. The Percentage RSD should not be more than 2 which indicate the accuracy and precision of the method. Hence the method was robust.Thus this shows that the method is capable to give a good detector response, the recovery calculated was within the range of 98% to 101% of the specification limits. Hence the method was a rapid tool for routine analysis of Azithromycin/Benzoyl peroxide in the bulk and in the pharmaceutical dosage form.

Keywords: Method validation, RP-HPLC, Azithromycin & Benzoyl peroxide.

Introduction

Azithromycin is an antibiotic useful for the treatment of a number of bacterial infections. This includes middle ear infections, strep throat, pneumonia, traveler's diarrhea, and certain other intestinal infections. It may also be used for a number of sexually transmitted infections including Chlamydia and gonorrhea infections. Along with other medications, it may also be used for malaria. It can be taken by mouth or intravenously with doses once per day. Azithromycin prevents bacteria from growing by interfering with their protein synthesis. It binds to the 50S subunit of the bacterial ribosome, thus inhibiting translation of mRNA but Nucleic acid synthesis is not affected.

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Azithromycin was first discovered in 1980. It is on the World Health Organization's List of Essential Medicines, the most effective and safe medicines needed in a health system.

Benzoyl peroxide is a medication and industrial chemical and a medication which used to treat mild to moderate acne. Other uses include hair bleaching, teeth whitening, and textile bleaching. Benzoyl peroxide is used as an acne treatment. Benzoyl peroxide is lipophilic drug. When applied to the skin it is capable of penetrating into the pilosebaceous follicle. After penetration in the skin, benzoyl peroxide releases free radical oxygen and benzoic acid. The free radicals oxidize bacterial proteins thus, higher concentrations of benzoyl peroxide applied to the skin result in larger amounts of drug in the skin. The benzoic acid is cleared rapidly by the kidneys and excreted unchanged in the urine. [22] It improves both inflammatory and non-inflammatory lesions of acne. Benzoyl peroxide was first made in 1905 and came into medical use in the 1930s. It is on the World Health Organization's List of Essential Medicines. Keeping this point into consideration, an attempt was made to develop a simple, accurate and validated stability indicating RP-

HPLC method for the estimation of Azithromycin and Benzoyl peroxide in pure and tablet form. The proposed Method was validated as per ICH guidelines.

Experimental

Chemicals and Reagents: Analytically pure sample of Azithromycin and Benzoyl peroxide with purity greater than 98% manufactured by Heliox Pharma and SGPTC Pvt. Ltd.was used. Acetonitrile (HPLC grade) was obtained from Fischer, Potassium dihydrogen phosphate ((HPLC grade, Thomas Baker, Mumbai), Methanol (HPLC & Spectroscopy grade was used.

Instruments and apparatus

RP-HPLC was performed using RP-HPLC instrument equipped with an UV-Visible detector and a photodiode array detector (LC-2010, Shimadzu, Japan), an auto-sampler; column used was Welchrome C18 (4.6 × 250 mm i.d., 5 µm particle size) and an LC-solution software.

Chromatographic conditions

Preliminary experiments were conducted to identify the critical attribute factors (CQAF) and to set their minimum and maximum levels using Taguchi screening method for design of experiments. Initially following parameters were investigated: selection of a column (C_8 and C_{18}), mobile phase (ratio of 30mM Potassium dihydrogen phosphate buffer: Acetonitrile), column temperature, detector wavelength, pH of mobile phase, mode of flow (isocratic/gradient) as well as ideal flow rate and the injection volume and evaluated for the risk management method process parameters viz. Number of theoretical Plates (TP), Assay, and tailing factor (TF).

Standard Stock Solution Preparation (100 µg/ml)

An accurately weighed quantity of about 10 mg of Azithromycin and 10 mg of Benzoyl peroxide 1ml appended, about 1 ml of diluents was added and sonicate to dissolve. From this took Suitable amount of sample further dilute to form 100 µg/ml.

Preparation of mobile phase of Azithromycin and Benzoyl peroxide

A filtered and degassed mixture of 300Mm Potassium Dihydrogen Phosphate Buffer and Acetonitrile (50:50) was prepared and used as mobile phase. Selection of flow rate of Azithromycin/Benzoyl peroxide. As increase in the flow rate results in decrease in the retention time. Hence sufficient flow rate of 0.8 ml/min. was chosen to avoid overlap between the peaks and the loss of its acceptable resolution values.

Preparation of sample solution of Azithromycin and Benzoyl peroxide

20 Tablets of contents were weighed and the average weight was determined. They were crushed in to fine powder with glass mortar. The tablet powder equivalent to 100 mg of Azithromycin and Benzoyl peroxide was transferred to a 100 ml volumetric flask and dissolved in mobile phase and the content was made up to mark with mobile phase. Then the sample solution kept in Sonicator for 15 min and the solution was filtered through the Whatman filter paper no. 41. (Whatman International Ltd., England). The filtrate contains 10µg/ml of of Azithromycin and Benzoyl peroxide to give the respective concentrations as par with standard solution.

System Suitability studies of Azithromycin/Benzoyl peroxide

According to the USP, system suitability tests are an integral part of chromatographic methods. These tests are used to verify that the resolution and reproducibility of the system are adequate for the analysis to be performed. System suitability tests are based on the concept that the equipment, electronics, analytical operations, and samples constitute an integral system that can be evaluated as a whole. The purpose of the system suitability test is to ensure that the complete testing system (including instrument, reagents, columns, analysts) is suitable for the intended application.

Assay Procedure for Azithromycin/Benzoyl peroxide

From the standard stock solution, each level solution was injected into the chromatographic system and the peak area was measured. A graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) was plotted and the correlation coefficient was calculated. The linearity of the method was demonstrated over the concentration range of Azithromycin/Benzoyl peroxide 1 µg/ml to 5 µg/ml. The solutions were injected in to HPLC system as per test procedure. HPLC Chromatogram was recorded of each concentration and the calibration curve was plotted (area vs. concentration). The regression equation and correlation coefficient were obtained.

Preparation of sample stock solution of Azithromycin/Benzoyl peroxide

About 10mg of Azithromycin/Benzoyl peroxide samples was weighed in to volumetric flask, it was dissolved with Potassium Dihydrogen Phosphate Buffer and Acetonitrile (50:50) and the volume was made up to the mark with same diluents (10µg/ml of Azithromycin/Benzoyl peroxide).

Validation of Spectrophotometric (RP-HPLC) method

The proposed RP-HPLC method was validated as per ICH guidelines.

Linearity and Range studies of Azithromycin/Benzoyl peroxide

The linearity of analytical method is its ability to elicit test results that are directly proportional to the concentration of analyte in sample within a given range. The range of analytical method is between the upper and lower levels of analyte that have been demonstrated to be determined within a suitable level of precision, accuracy and linearity. Selected linearity range for Azithromycin was 1-5 µg/ml and for Benzoyl peroxide it was 1-5 µg/ml.

Accuracy studies of Azithromycin/Benzoyl peroxide

Accuracy of the method was determined in terms of % recovery of standard. Recovery studies were carried out by addition of standard drug solution at the level of 80%, 100% and 120% to the pre-analyzed sample. Results of the recovery study were found to be within the acceptance criteria 100±10 %, indicating a good degree of sensitivity of the method towards detection of analyte (s) in sample. In this method the known concentration of standard drug was added to the assay sample.

Precision studies of Azithromycin/Benzoyl peroxide

The intra-day and inter-day variation for determination of Azithromycin and Benzoyl peroxide hydrochloride were carried out Six times in the same day and six consecutive days using concentration 5 µg/ml of Azithromycin and Benzoyl peroxide % RSD was calculated. The method was found to be precise due to low values of the %RSD.

LOD and LOQ studies of Azithromycin/Benzoyl peroxide

The LOD and LOQ of developed method were studied as per ICH guidelines. Several approaches for determining the LOD & LOQ are possible, depending on the procedure i.e, a non-instrumental or instrumental. Among them here employed method was,

LOD=3.3σ/Sand

LOQ= 10σ/S

Where, σ = the standard deviation of response

S = the slope of calibration curve.

Robustness studies of Azithromycin/Benzoyl peroxide

The robustness was studied by analyzing the same samples of Azithromycin and Benzoyl peroxide concentration 5 µg/ml by deliberate variation in the method parameters. The change in the responses of Azithromycin and Benzoyl peroxide were noted in terms of %RSD. Robustness of the method was studied by change in wavelength or change in flow rate, change in Ph of mobile phase. .

Results and Discussion

Selection of mobile phase and flow rate of Azithromycin/Benzoyl peroxide

Initially various mobile phase compositions were tried, to separate title ingredients. Mobile phase composition and flow rate selection was based on peak parameters (height, tailing, theoretical plates, capacity or symmetry factor) and run time. The optimized mobile phase ratio (v/v) consisted of Acetonitrile and buffer (50:50), pH 6.5 with 1.1 ml/min of flow rate. Flow rate was found to be quite robust. The optimum wavelength for detection was 235nm for Benzoyl peroxide and 215nm for Azithromycin at which better detector response was obtained. The retention time was found to be 22.0min for Azithromycin and 2.5min for Benzoyl peroxide and the total runtime for this method along with the elution of and indicates that the developed method is quite fast and economical.

Calibration curve for Azithromycin/Benzoyl peroxide

Appropriate aliquots from standard Azithromycin/Benzoyl peroxide stock solutions were transferred into different volumetric flasks of 10ml capacity and the volume is adjusted to the mark to obtain concentrations of 1 µg/ml to 5 µg/ml of Azithromycin/Benzoyl peroxide.

Method Validation

Linearity

The correlation coefficient (R) obtained was calculated and it was found to be greater than 0.99 for Benzoyl peroxide and Azithromycin given in below table, which is well within the acceptance criteria. The results are shown in Table 1, 2, 3. The concentration was found to be proportional to the area and the response of the detector was determined to be linear over the range of 1-5 µg/ml for both Benzoyl peroxide and Azithromycin shown in Figure 1 & 2.

Table 1: Regression coefficient value for both drugs

S.No.	Name of drug	Linearity range	Regression coefficient value
1	Benzoyl peroxide	1-5µg/ml	0.998
2	Azithromycin	1-5µg/ml	0.997

Table 2: Linearity of Benzoyl peroxide

S.No.	Concentration (µg/ml)	Retention Time	Area
1	1	2.5	86427±1.24
2	2	2.5	154040±1.34
3	3	2.5	225070±0.93
4	4	2.54	307274±0.54
5	5	2.5	382581±1.19

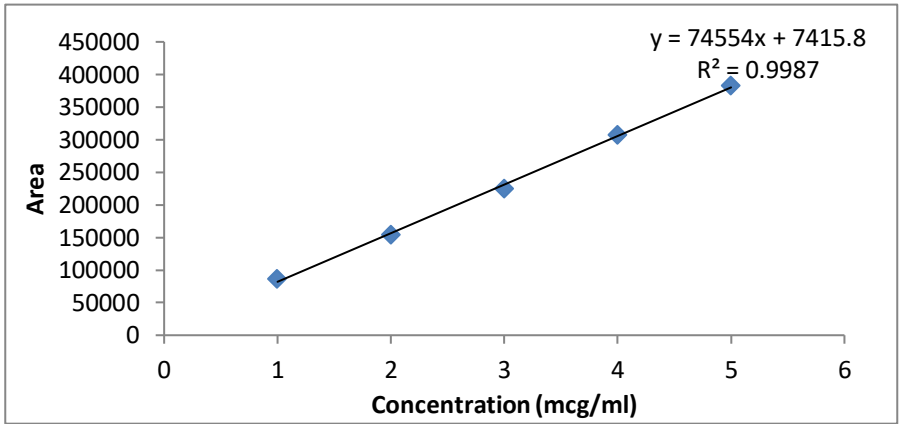

Figure 1: Linearity of Benzoyl peroxide

Table 3: Linearity of Azithromycin

S.No.	Concentration (µg/ml)	Retention Time (min.)	Area
1	1	22.033	125359±0.68
2	2	22.136	206308±1.12
3	3	22.221	308004±1.03
4	4	22.156	413253±0.93
5	5	22.156	512581±1.30

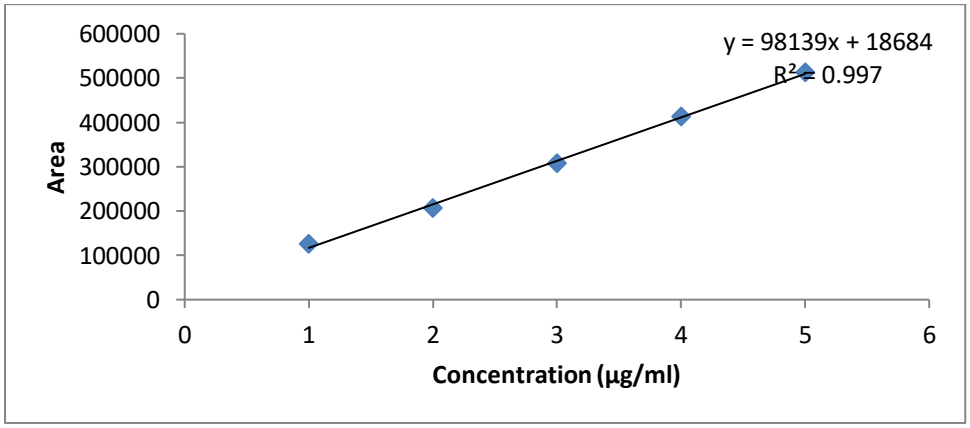

Figure 2: Linearity of Azithromycin

Accuracy

The results indicate that the recoveries are well within the acceptance range of 80 – 120%, therefore, method is accurate and it can be used for the simultaneous estimation of Benzoyl peroxide and Azithromycin. Accuracy of both drugs was given in below table 4, 5, 6, 7, 8, 9.

Table 4: Accuracy of 80% sample of Benzoyl peroxide

Concentration ((µg/ml)	Retention Time	Area
8µg/ml	2.551	233217
8µg/ml	2.551	233217
8µg/ml	2.549	233317
	Mean	233250.3333
	S.D.	57.73502687
	%RSD	0.024752388

Table 5: Accuracy of 80% sample of Azithromycin

Concentration (µg/ml)	Retention Time	Area
8µg/ml	22.121	284135
8µg/ml	22.021	286741
8µg/ml	22.034	289434
	Mean	286770
	S.D.	2649.619029
	%RSD	0.923952655

Table 6: Accuracy of 100 % sample of Benzoyl peroxide

Concentration	Retention Time	Area
10µg/ml	2.549	303657
10µg/ml	2.537	307274
10µg/ml	2.542	307944
	Mean	306291.6667
	S.D.	2306.149677
	%RSD	0.752926027

Table 7: Accuracy of 100 % sample of Azithromycin

Concentration	Retention Time	Area
10µg/ml	22.156	410191
10µg/ml	22.101	413253
10µg/ml	22.056	411233
	Mean	411559
	S.D.	1556.813412
	%RSD	0.378272231

Table 8: Accuracy of 120 % sample of Benzoyl peroxide

Concentration	Retention Time	Area
12µg/ml	2.461	396553
12µg/ml	2.462	394973
12µg/ml	2.460	398143
	Mean	396556.3333
	S.D.	1585.002629
	%RSD	0.399691669

Table 9: Accuracy of 120 % sample of Benzoyl peroxide

Concentration	Retention Time	Area
12µg/ml	22.230	447899
12µg/ml	22.241	447327
12µg/ml	22.233	440002
	Mean	445076
	S.D.	4403.510304
	%RSD	0.989383904

Method Precision

It is a measure of degree of repeatability of an analytical method under normal operation and it is normally expressed as % of relative standard deviation (% RSD). The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits. Similarly % RSD for Interday Precision and Intraday Precision was given below that was found to be within the specified limits. The percentage RSD values for Area and Retention Time in precision study were calculated. Chromatogram of Repeatability, Interday precision and Interday precision was given below.

Table 10: Repeatability of Benzoyl peroxide and Azithromycin

S.No.	Concentration (µg/ml)	Benzoyl Peroxide		Azithromycin	
		Retention time (min.)	Area	Retention time (min.)	Area
1	5	2.461	398542	22.230	472613
2	5	2.468	398561	22.198	472408
3	5	2.452	398554	22.261	472554
4	5	2.391	398497	22.268	472621
5	5	2.398	398562	22.201	472406
6	5	2.460	398599	22.231	472513
Mean			398552.5	Mean	472519.166
Standard Deviation			33.21897048	Standard Deviation	95.4890917
%RSD			0.008334905	%RSD	0.02020851

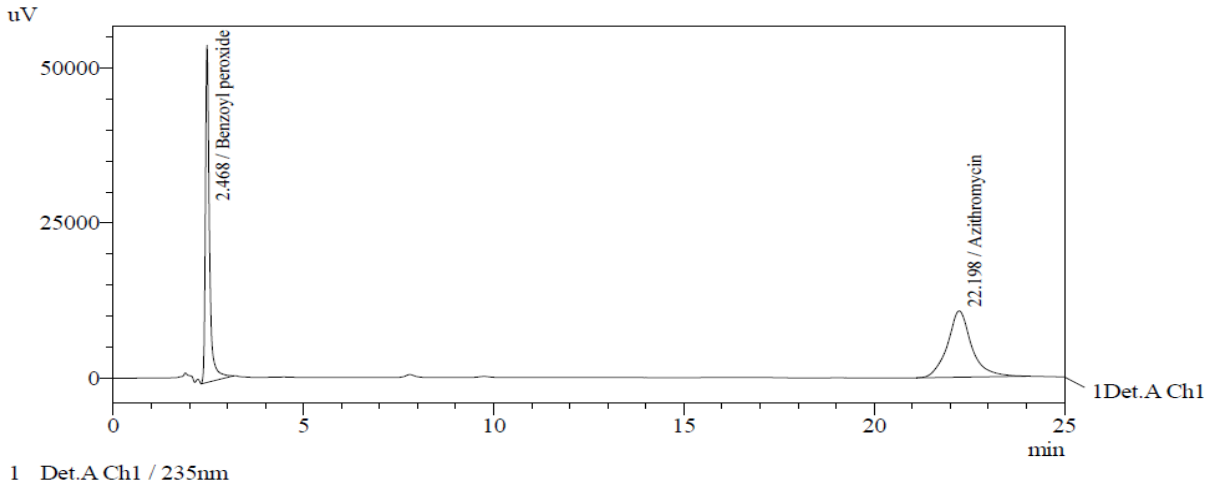

Figure 3: Chromatogram of repeatability of Benzoyl peroxide and Azithromycin

Table 11: Interday Precision of Benzoyl peroxide and Azithromycin

S.No.	Concentration (µg/ml)	Benzoyl Peroxide		Azithromycin	
		Retention time (min.)	Area	Retention time (min.)	Area
1	5	2.461	393118	22.230	445826
2	5	2.468	388054	22.198	441695
3	5	2.452	388259	22.261	441715
4	5	2.391	385556	22.268	424972
5	5	2.398	391289	22.201	430122
6	5	2.460	391281	22.231	442054
Mean			389592.8333	Mean	437730.6667
Standard Deviation			2778.374735	Standard Deviation	8203.558586
%RSD			0.713148317	%RSD	1.874111003

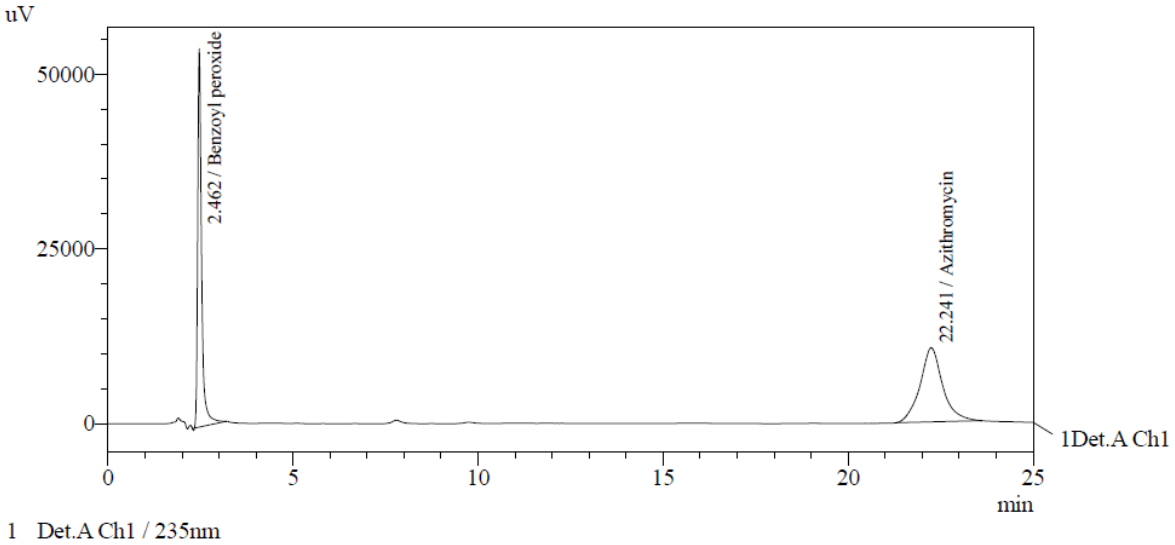

Figure 4: Interday Precision of Benzoyl peroxide and Azithromycin

Table 12: Intraday Precision of Benzoyl peroxide and Azithromycin

S.No.	Concentration (µg/ml)	Benzoyl Peroxide		Azithromycin	
		Retention time (min.)	Area	Retention time (min.)	Area
1	5	2.461	390594	22.23	450254
2	5	2.461	398561	22.230	472408
3	5	2.460	390277	22.232	446708
4	5	2.462	387714	22.241	451804
5	5	2.460	398143	22.232	458372
6	5	2.462	390216	22.241	456409
Mean			392584.1667	Mean	455992.5
Standard Deviation			4587.672238	Standard Deviation	9077.224108
%RSD			1.168583103	%RSD	1.990652063

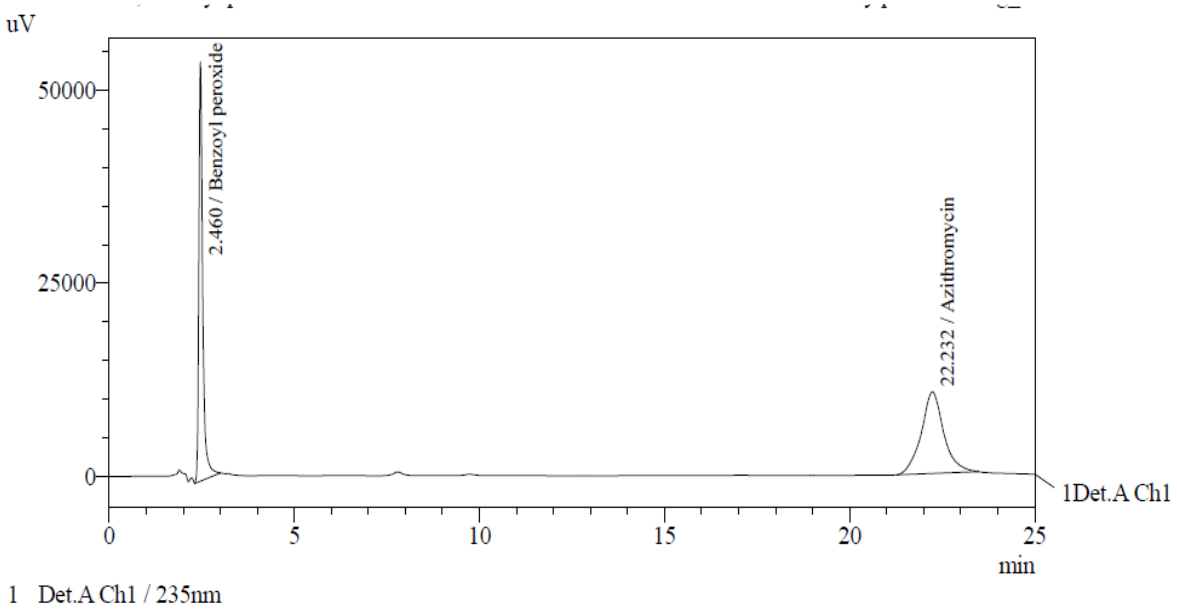

Figure 5: Intraday Precision of Benzoyl peroxide and Azithromycin

LOD and LOQ

The Limit of detection and limit of quantification of the method were calculated basing on standard deviation of the response and the slope (s) of the calibration curve at approximate levels of the limit of detection and limit of quantification. The data were represented in Table 13. The results obtained were within the limit.

Table 13: LOD and LOQ of Benzoyl peroxide and Azithromycin

S.No.	Parameter	Benzoyl peroxide	Azithromycin
1	LOD	0.015µg/ml	0.009µg/ml
2	LOQ	0.046µg/ml	0.028µg/ml

Robustness

The Robustness of the method was found out by testing the effect of small deliberate changes in the chromatographic conditions in the chromatographic conditions and the corresponding peak areas. The factors selected for this purpose were flow rate, Change in wavelength and pH variation in the mobile phase. The method was found to be robust enough that the peak area was not apparently affected by small variation in the chromatographic conditions. The system suitability parameters were within the limits and shown in Table 14-19 and chromatograms were represented in Figure 6-13.

The Percentage RSD should not be more than 2. The %RSD obtained for change of flow rate, Change in wavelength and pH variation in the mobile phase was found to be below 2, which was within the acceptance criteria. Hence the method was robust.

(A) Change in flow Rate

Table 14: Robustness of Benzoyl peroxide and Azithromycin at 0.9ml/min

S.No.	Concentration (µg/ml)	Benzoyl Peroxide		Azithromycin	
		Retention time (min.)	Area	Retention time (min.)	Area
1	5	2.511	393329	22.403	441848
2	5	2.460	398143	22.232	447800
3	5	2.560	398143	22.232	447800
Mean			396538.3333	**Mean**	445816
Standard Deviation			2779.364196	**Standard Deviation**	3436.388802
%RSD			0.700906813	**%RSD**	0.770808765

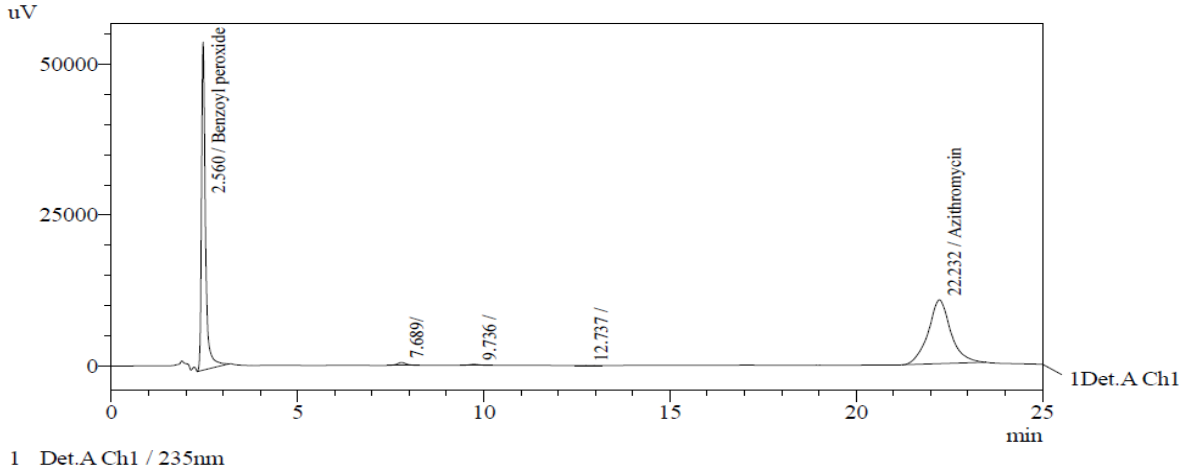

Figure 6: Table: Robustness of Benzoyl peroxide and Azithromycin at 0.9 ml/min

Table 15: Robustness of Benzoyl peroxide and Azithromycin at 1ml/min

S.No.	Concentration (µg/ml)	Benzoyl Peroxide		Azithromycin	
		Retention time (min.)	Area	Retention time (min.)	Area
1	5	2.461	398542	22.230	472613
2	5	2.468	398561	22.198	472408
3	5	2.452	398554	22.261	478554
Mean			398552.3333	Mean	474525
Standard Deviation			9.609023537	Standard Deviation	3490.721559
%RSD			0.002410982	%RSD	0.735624374

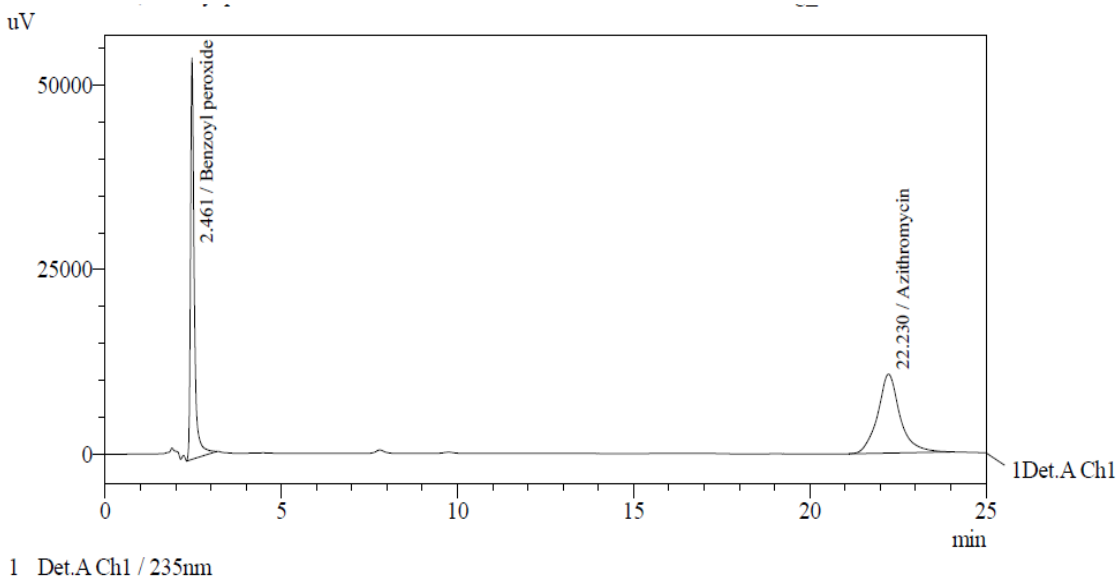

Figure 7: Robustness of Benzoyl peroxide and Azithromycin at 1ml/min.

Table 16: Robustness of Benzoyl peroxide and Azithromycin at 1.1ml/min.

S.No.	Concentration (µg/ml)	Benzoyl Peroxide		Azithromycin	
		Retention time (min.)	Area	Retention time (min.)	Area
1	5	2.461	398542	22.230	472613
2	5	2.468	398561	22.198	472408
3	5	2.452	398554	22.261	478554
Mean			398552.3333	Mean	474525
Standard Deviation			9.609023537	Standard Deviation	3490.721559
%RSD			0.002410982	%RSD	0.735624374

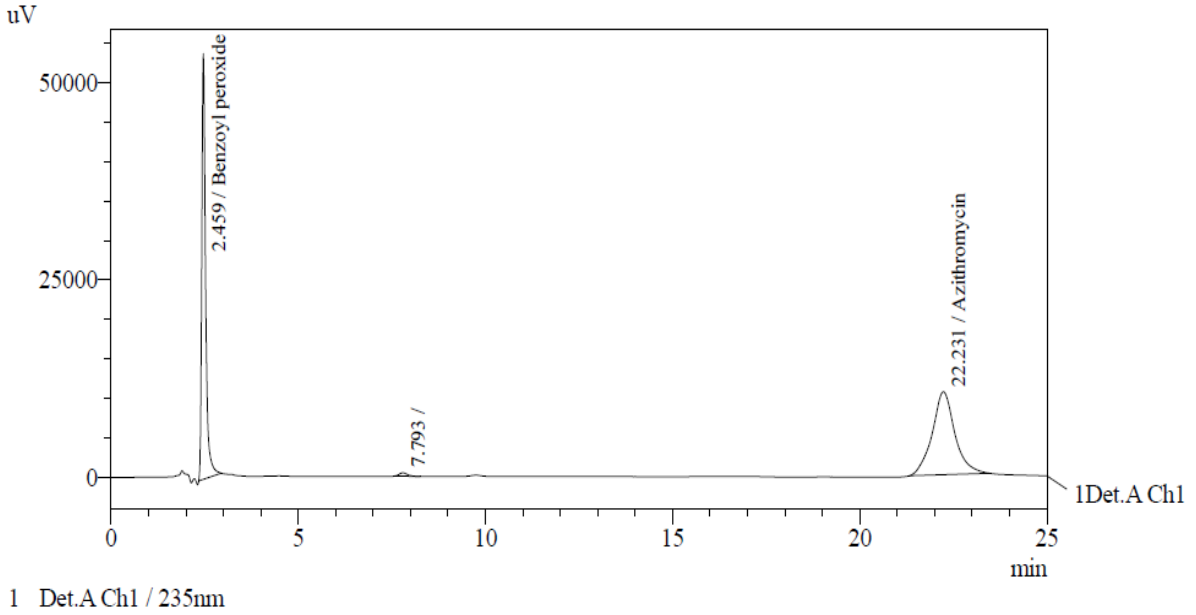

Figure 8: Robustness of Benzoyl peroxide and Azithromycin at 1.1ml/min

Change in wavelength

Table 17: Robustness of Benzoyl peroxide and Azithromycin at 230nm

S.No.	Concentration (µg/ml)	Benzoyl Peroxide		Azithromycin	
		Retention time (min.)	Area	Retention time (min.)	Area
1	5	2.461	395237	22.230	428676
2	5	2.461	391998	22.230	432408
3	5	2.460	395239	22.232	432217
Mean			394158	Mean	431100.3333
Standard Deviation			1870.615139	Standard Deviation	2101.705101
%RSD			0.4745851	%RSD	0.487521103

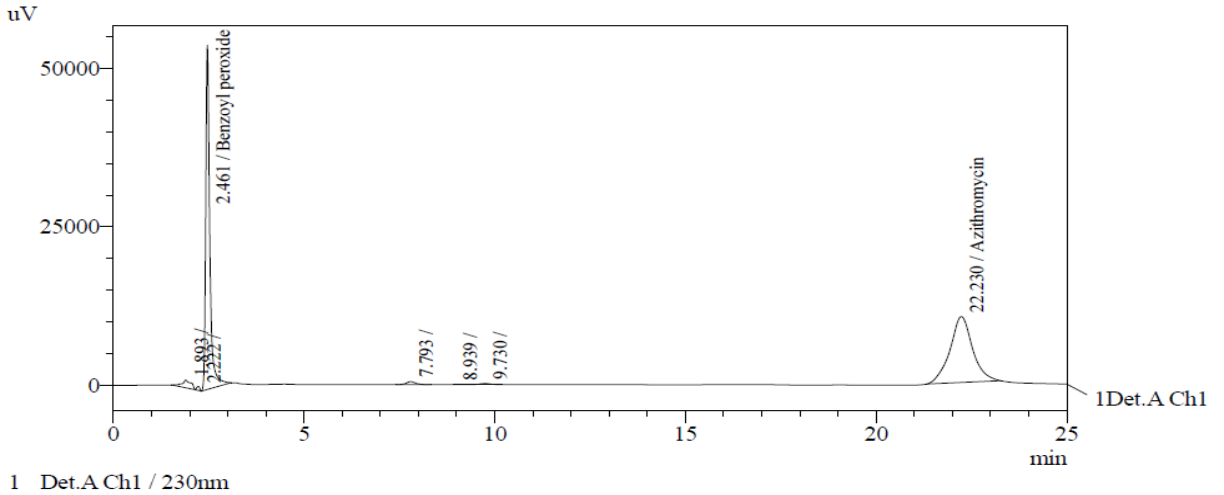

Figure 9: Robustness of Benzoyl peroxide and Azithromycin at 230 nm

Table 18: Robustness of Benzoyl peroxide and Azithromycin at 240 nm

S.No.	Concentration (µg/ml)	Benzoyl Peroxide		Azithromycin	
		Retention time (min.)	Area	Retention time (min.)	Area
1	5	2.461	398561	22.230	472408
2	5	2.460	396557	22.232	488372
3	5	2.460	389518	22.232	484185
Mean			394878.6667	Mean	481655
Standard Deviation			4749.375152	Standard Deviation	8277.25794
%RSD			1.202742906	%RSD	1.718503481

Table 19: Robustness of Benzoyl peroxide and Azithromycin at 235 nm

S.No.	Concentration (µg/ml)	Benzoyl Peroxide		Azithromycin	
		Retention time (min.)	Area	Retention time (min.)	Area
1	5	2.391	398497	22.268	472621
2	5	2.398	398562	22.201	472406
3	5	2.460	398599	22.231	472513
Mean			398552.6667	Mean	472513.3333
Standard Deviation			51.63655046	Standard Deviation	107.5003875
%RSD			0.012956017	%RSD	0.022750763

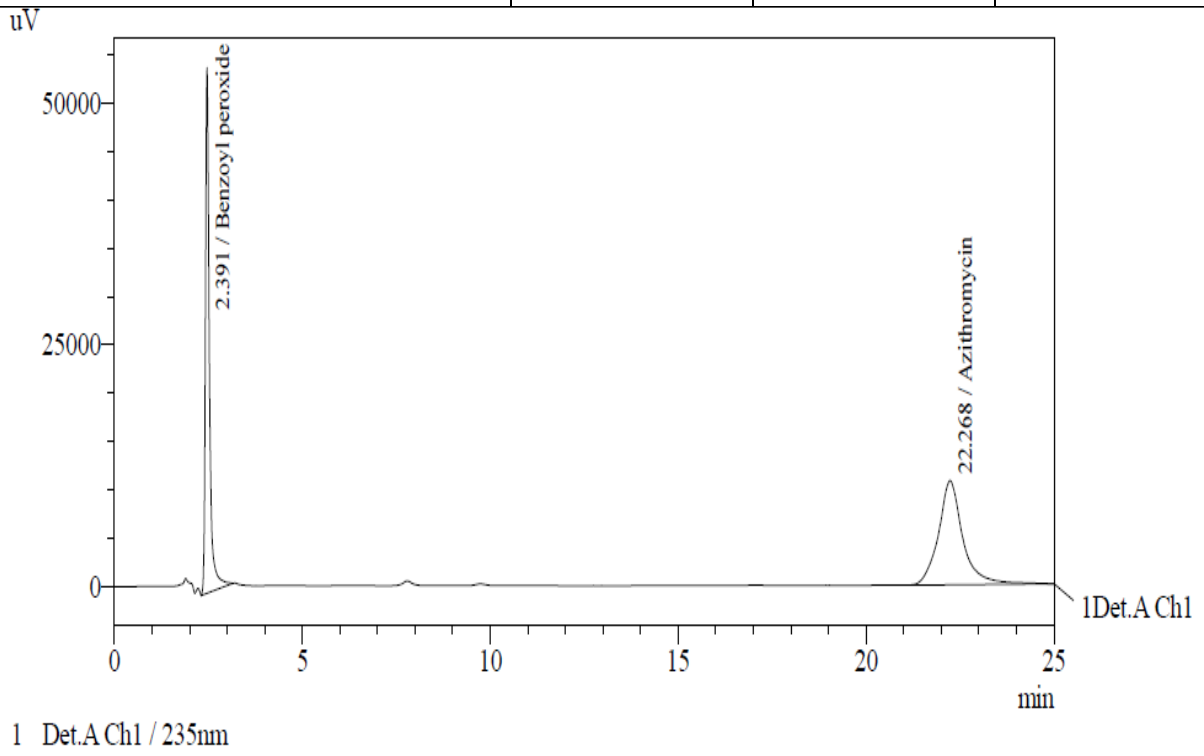

Figure 10: Robustness of Benzoyl peroxide and Azithromycin at 235 nm

Change in PH of mobile phase

Table 20: Robustness of Benzoyl peroxide and Azithromycin at 6.4 pH

S.No.	Concentration (µg/ml)	Benzoyl Peroxide		Azithromycin	
		Retention time (min.)	Area	Retention time (min.)	Area
1	5	2.516	393329	22.413	441848
2	5	2.460	398143	22.232	447800
3	5	2.560	398143	22.232	447800
Mean			396538.3333	Mean	445816
Standard Deviation			2779.364196	Standard Deviation	3436.388802
%RSD			0.700906813	%RSD	0.770808765

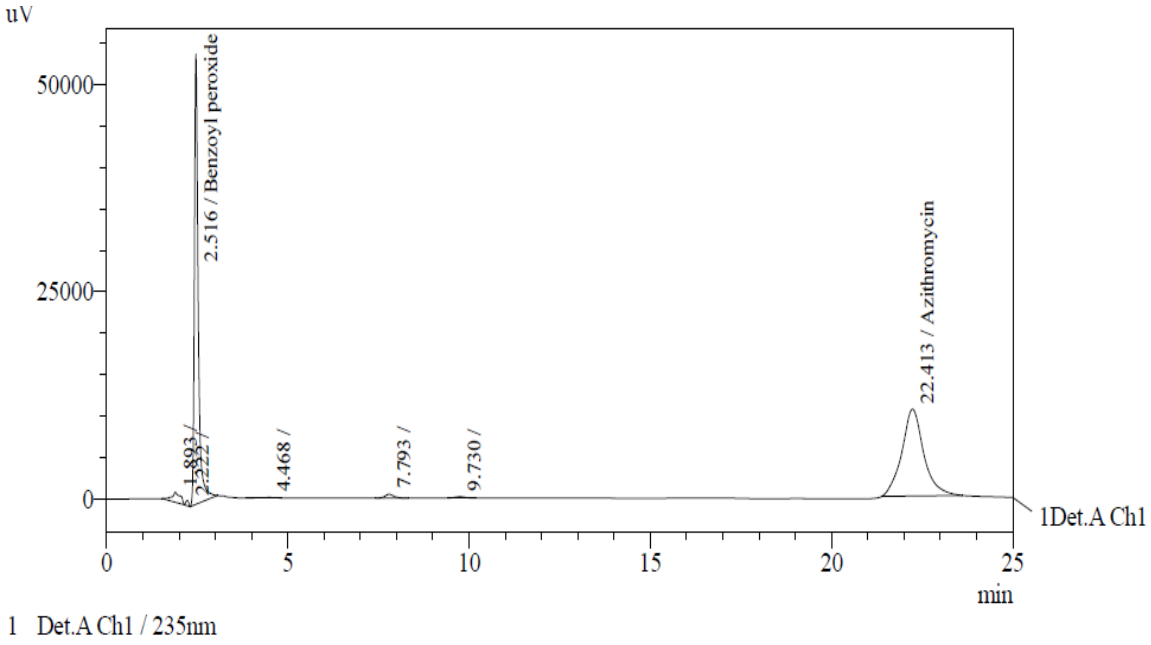

Figure 11: Robustness of Benzoyl peroxide and Azithromycin at 6.4 pH

Table 21: Robustness of Benzoyl peroxide and Azithromycin at 6.5 pH

S.No.	Concentration (µg/ml)	Benzoyl Peroxide		Azithromycin	
		Retention time (min.)	Area	Retention time (min.)	Area
1	5	2.391	398497	22.268	472621
2	5	2.398	398562	22.201	472406
3	5	2.460	398599	22.239	472513
Mean			398552.6667	Mean	472513.3333
Standard Deviation			51.63655046	Standard Deviation	107.5003875
%RSD			0.012956017	%RSD	0.022750763

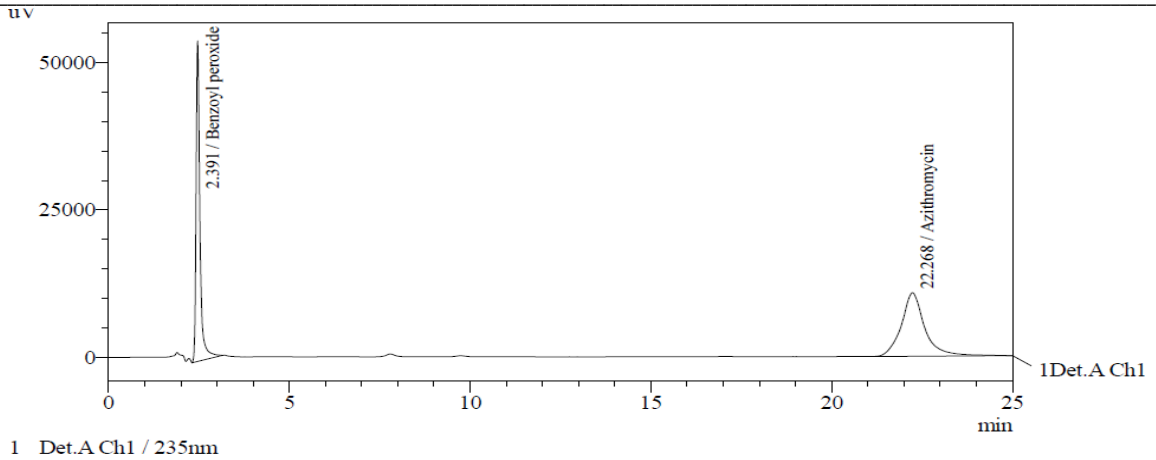

Figure 12: Robustness of Benzoyl peroxide and Azithromycin at 6.5 pH

Table 22: Robustness of Benzoyl peroxide and Azithromycin at 6.6 pH

S.No.	Concentration (µg/ml)	Benzoyl Peroxide		Azithromycin	
		Retention time (min.)	Area	Retention time (min.)	Area
1	5	2.459	398738	22.231	443032
2	5	2.461	398561	22.230	430015
3	5	2.463	398561	22.235	442408
Mean			398620	Mean	438485
Standard Deviation			102.1909976	Standard Deviation	7341.867542
%RSD			0.025636194	%RSD	1.674371425

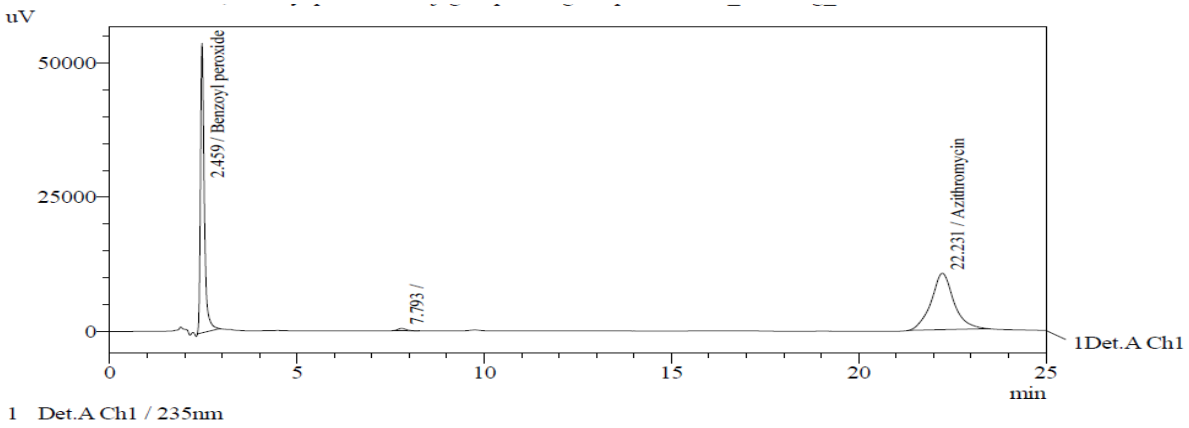

Figure 13: Robustness of Benzoyl peroxide and Azithromycin at 6.6 pH

Ruggedness

The Ruggedness of the method was found out by testing the effect of small deliberate changes in the analyst. The method was found to be robust enough that the peak area was not apparently affected by small variation in the chromatographic conditions. The system suitability parameters were within the limits and shown in Table No. 23, 24 and chromatograms were represented in Figure 14, 15

The Percentage RSD should not be more than 2. The %RSD obtained for change of analyst was found to be below 2, which was within the acceptance criteria. Hence the method was rugged.

Table 23: Ruggedness of Benzoyl peroxide and Azithromycin by analyst one

S.No.	Concentration (µg/ml)	Benzoyl Peroxide		Azithromycin	
		Retention time (min.)	Area	Retention time (min.)	Area
1	5	2.569	398545	23.230	482613
2	5	2.568	398561	23.198	482438
3	5	2.602	398554	23.248	488565
Mean			398553.3333	Mean	484538.6667
Standard Deviation			8.020806277	Standard Deviation	3488.004635
%RSD			0.00201248	%RSD	0.719860947

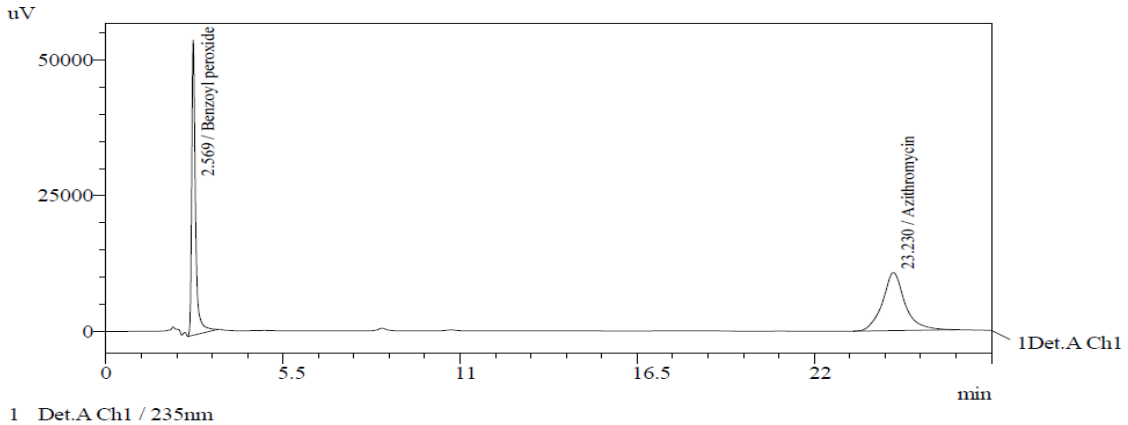

Figure 14: Ruggedness of Benzoyl peroxide and Azithromycin by analyst one

Table 24: Ruggedness of Benzoyl peroxide and Azithromycin by analyst two

S.No.	Concentration (µg/ml)	Benzoyl Peroxide		Azithromycin	
		Retention time (min.)	Area	Retention time (min.)	Area
1	5	2.578	398546	23.331	472513
2	5	2.578	388561	22.268	473408
3	5	2.496	398564	23.268	487554
Mean			395223.6667	Mean	477825
Standard Deviation			5770.045609	Standard Deviation	8437.436637
%RSD			1.459944355	%RSD	1.765800583

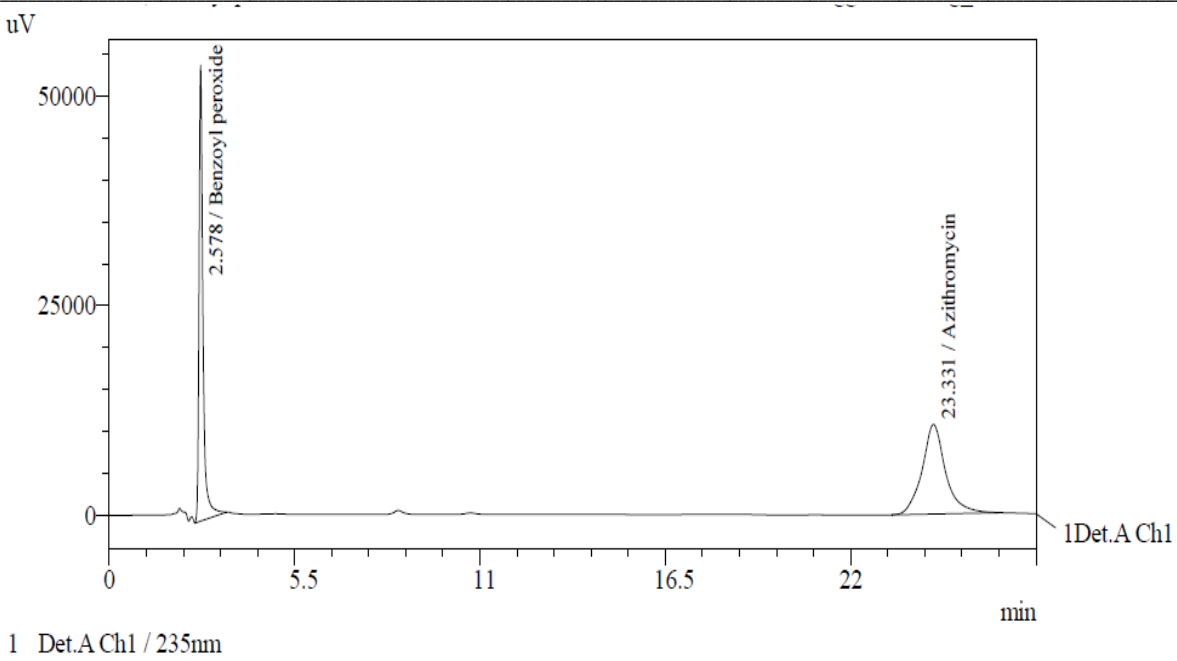

Figure 15: Ruggedness of Benzoyl peroxide and Azithromycin by analyst two

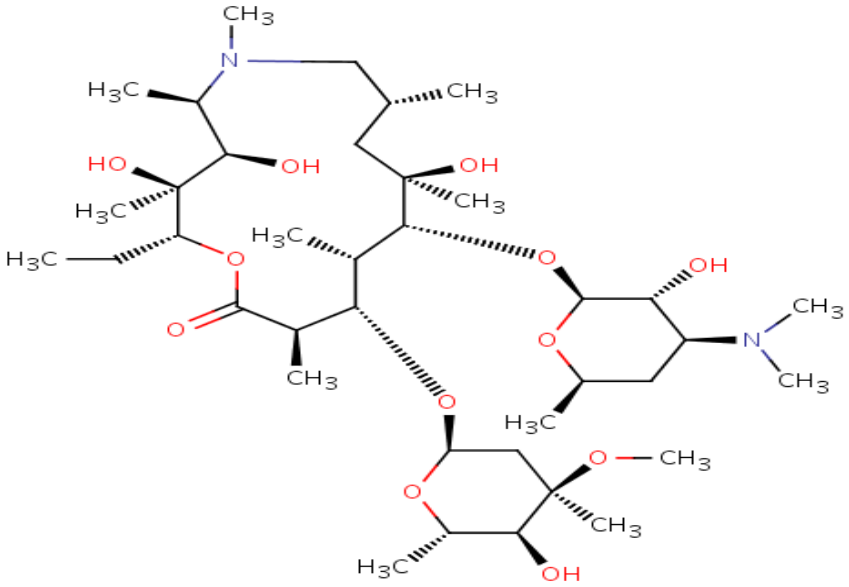

Figure 16: Molecular Structure of Azithromycin

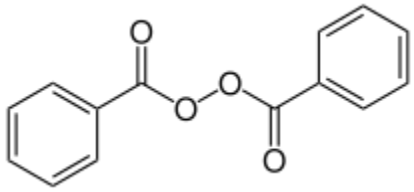

Figure 17: Molecular Structure of Benzoyl peroxide

Melting point: Melting point of Azithromycin was found to be 114-116°C and for benzoyl peroxide 104-106°C
FT-IR: FT-IR spectra of both drugs and mixture of both drugs were given below.

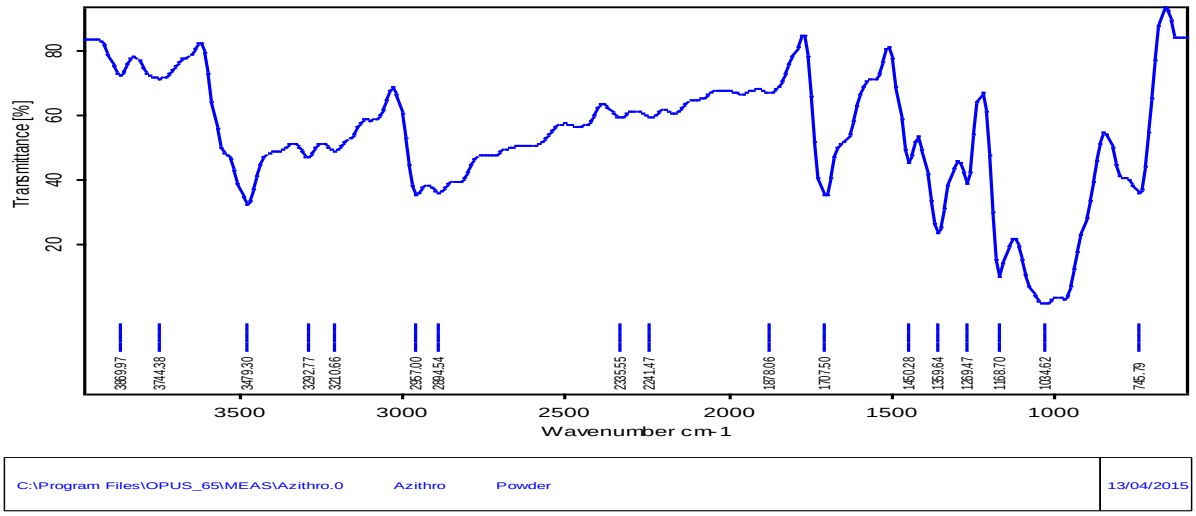

Figure 18: IR spectra of Azithromycin

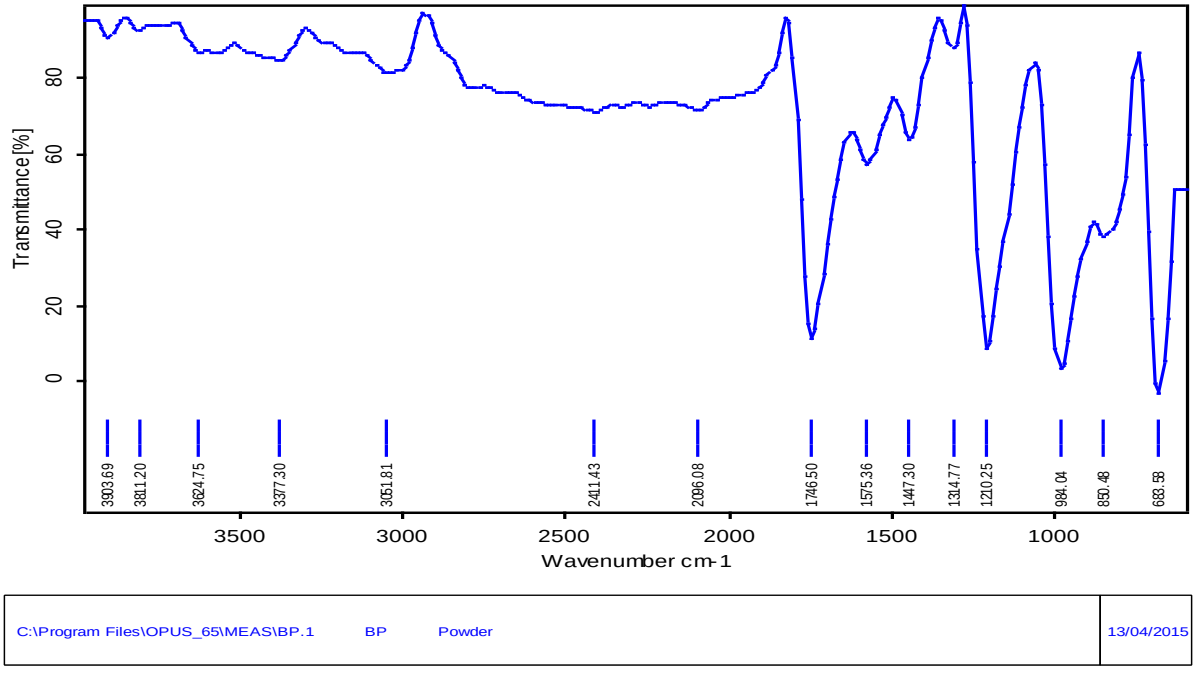

Figure 19: IR spectra of Benzoyl Peroxide

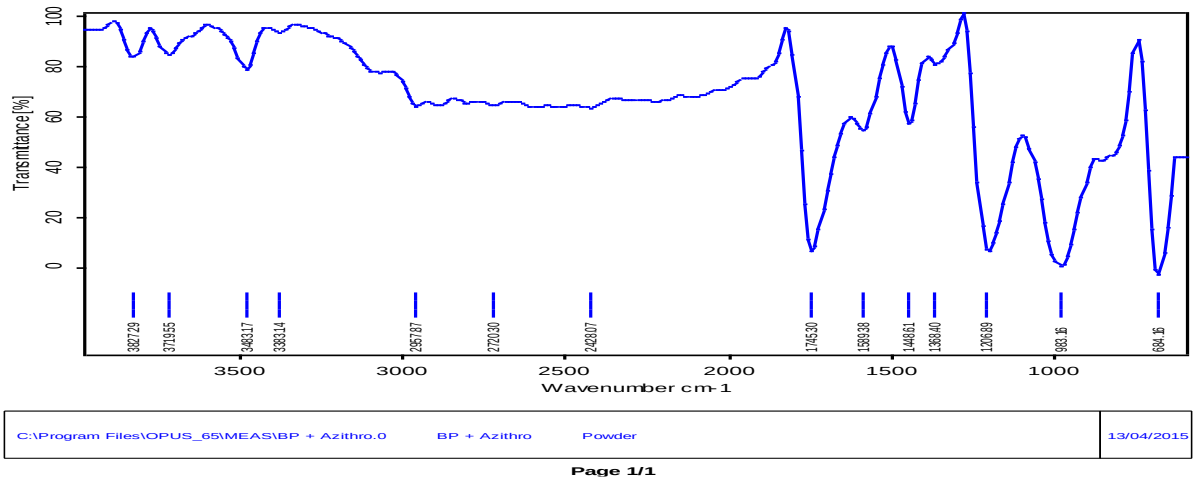

Figure 20: IR spectra of mixture of Azithromycin and Benzoyl Peroxide

From the figure no. 18, 19, 20 it was found that there was no interaction either physical or chemical in between both drugs.

Conclusion

High performance liquid chromatography at present one of the most sophisticated tool of the analysis. The estimation of Imatinib Azithromycin and Benzoyl Peroxide was done by RP-HPLC Method. The mobile phase was optimized with consists of Potassium Dihydrogen Phosphate Buffer and Acetonitrile (50:50). A C18 column (4.6X250 mm, particle 5µ, Make: Waters XTerra®) was used as stationary phase. The solutions were chromatographed at a constant flow rate of 1.1 ml/min. The linearity range of Azithromycin and Benzoyl Peroxide were found to be from 1-5 µg/ml. In this method, correlation coefficient was found to be 0.998 for Benzoyl peroxide and 0.997 for Azithromycin. The maximum absorbance is found to be at 235nm for Benzoyl peroxide and 215nm for Azithromycin. The value of % RSD was found to be within the specified limits which indicating accuracy and precision of the method. LOD and LOQ were found to be within limit. The results obtained on the validation parameters met ICH and USP requirements. The method was found to be accurate, precise, repeatable and reproducible with different instruments and analysts. So, simple, sensitive, accurate, precise RP- HPLC methods were developed and validated for the simultaneous estimation of Benzoyl peroxide and Azithromycin.

The method also finds use in clinical, biological and pharmacokinetic studies for the drug Azithromycin and Benzoyl peroxide. The method was validated as per ICH guidelines, and validation acceptance criteria were met in all cases. Hence, this method was specific, stability-indicating and can be successfully used for the estimation of drug in bulk and pharmaceutical dosage forms.

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