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Simultaneous Estimation Of Azithromycin, Fluconazole And Ornidazole In Combined Dosage Form Using Hptlc Method

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Abstract
A rapid, stable high-performance thin-layer chromatography (HPTLC) method was created and validated for the simultaneous quantification of azithromycin (AZM), fluconazole (FLZ), and ornedizole (OZ) in Combi-kit amounts from tablets. The stationary phase was made up of HPTLC silica gel 60 F254 plates, while the mobile phase was a combination of toluene, methanol, and 1,4-dioxane in a volume ratio of 2:2:6. A CAMAG TLC Scanner 3 was used to perform densitometric scanning at a wavelength of 210 nm. The validation of the created technique adhered to the standards established by the International Council for Harmonization. The RF values of AZM, FLZ, and OZ were 0.202, 0.382, and 0.522, respectively. In this case, the statistical tests previously described for determining the suitability of the simple linear regression model are applied to our models AZM (y = 4.506x + 2189.4), FLZ (y = 4.506x + 2189.4), and OZ (y = 4.8969x + 240.53). LOD= 3s/S and LOQ = 10s/S (2) Where s is the standard deviation of y-intercept and S is the slope of the calibration curve. The LOD and LOQ were found to be 0.0467and 0.141 g/zone, respectively. These three drugs can be routinely analyzed in their pharmaceutical dosage form using these methods. Results for analysis of both methods were tested and validated for various parameters according to ICH guidelines.

Keywords: *Azithromycin, Fluconazole, Ornedizole, High Performance Thin Layer Chromatography, Pharmaceutical dosage form, Stability, Validation.*

1. INTRODUCTION

New measuring technologies can be employed in industries only if a good scientific explanation for the application has been produced, demonstrated, and justified, and the proposed technique has been accepted by internal business processes (Neville Broad, 2002). Every year, a number of medicines are put into the market, either as new medications or as modifications to existing compounds. Because of the probable uncertainties in the long-term use of these treatments, the development of new toxicities and patient resistance, or the introduction of a superior drug, there is a time lag between the dates of drug launch to the date of inclusion in pharmacopoeias. As a result, fresh analytical procedures for such medications must be developed and validated may not be available in pharmacopoeias (Rashmin Bharatbhai Patel, 2008).

It is well acknowledged that a created technique must be validated since validation procedures demonstrate the analytical laboratory's certification and ability (Isabel VJ Taverniers, 2004). Analytical measures are involved with every element of society, and there are several reasons for performing these measurements. Clearly, it is critical to discover the proper outcome and be able to demonstrate that it is accurate. As a result, method validation is necessary. For example, the increasing usage of new botanical substances in nutritional supplements and meals has resulted in a frenzy of research targeted at the development and validation of analytical methods for the correct quantification of active compounds (Ashok Kumar, 2006). Because drugs involve the taking of human life, analysis is essential for any good or service (Hema, Swati Reddy, 2017). Analytical chemistry is the study of the separation, measurement, and identification of chemical additives in synthetic and herbal materials made up of one or more

chemicals or elements. Analytical chemistry is divided into two main categories: qualitative evaluation and quantitative evaluation. The former identifies the presence of chemical additives in the sample and the latter calculates the amount of positive detail or compound present in the substance, or sample (Ravisankar Panchumarthy,2015). Each year, there are more medications introduced to the market. These medications may also be brand-new things or slight structural changes made to the ones we already have. Medicines should be available in a way that guarantees their quality as well as bioavailability, acceptable plasma concentration, desired timeframe, the commencement of action, appropriate dose, safety, efficacy, and stability throughout product storage (A Patel, 2016). Preclinical testing, clinical testing, regulatory registration, drug discovery, research lab trials, and other steps are all involved in the long process of developing a pharmaceutical.

Numerous administrative organizations, such as the United States Food and Drug Administration (USFDA), also mandate that the drug product be evaluated for its identification, potency, characteristics, quality, stability, and purity before it can be released for use in order to further improve the sufficiency and protection of the medication after acceptance. In order to avoid such problems, pharmaceutical validation and process controls are crucial (Elsie Jatto, 2020). A medication's debut to the market and the date it is taken into consideration for inclusion in pharmacopoeias sometimes occur at different times. This is due to potential flaws in the continued and extensive use of those pharmaceuticals, claims of ongoing toxicity (leading to their removal from the market), the emergence of patient resistance, and the advancement of more advanced medical treatments in an effort to compete. In some cases, there may be requirements and analytical methods for certain medications that are outside the scope of pharmacopoeias. It becomes required in order to create novel analytical techniques for such drugs (R Pathuri, 2013). The development and validation of analytical approaches play crucial roles in the research, development, and production of pharmaceuticals. Obtaining accurate, realistic, and consistent information is the basic goal of an analytical measure. Validated analytical techniques are crucial to reaching this objective. Results from technique validation may be used to determine the quality, consistency, and dependability of analytical findings, which are essential components of any sane analytical procedure. Most laws and quality standards that affect laboratories require validation of analytical techniques (R patil, 2014).

1.1 Analytical method development

In the absence of established approaches, new methodologies are being developed for the evaluation of innovative products. Innovative procedures are created to decrease the value aside from time for greater precision and strength in order to analyse the existence of either pharmacopoeial or nonpharmacopoeial product. Through test runs, these approaches have been optimised and proven to be reliable. Alternative methods are developed and put into use to replace the current approach in the context of comparing laboratory data with all available benefits and drawbacks.

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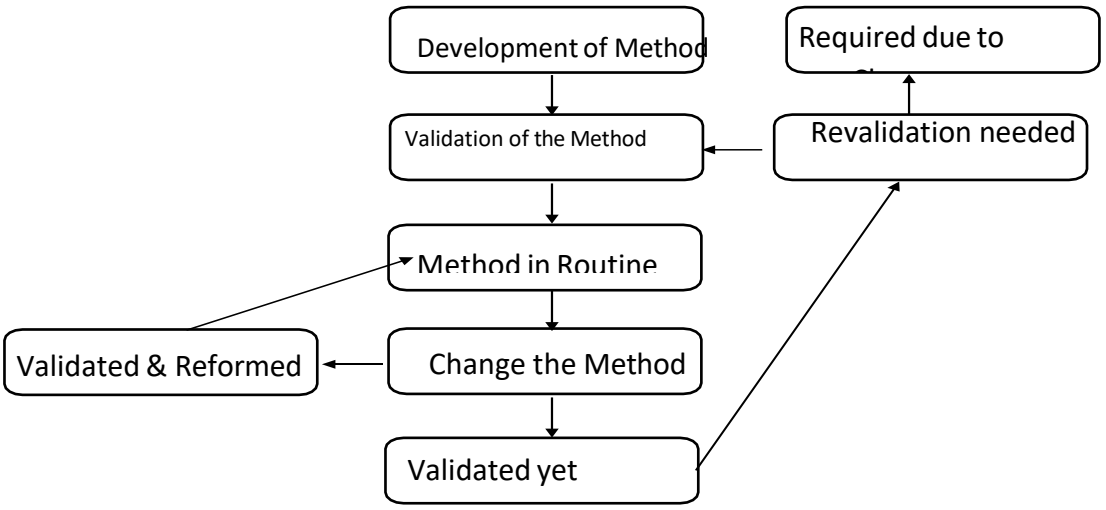


Figure 1. Life Cycle of the analytical method

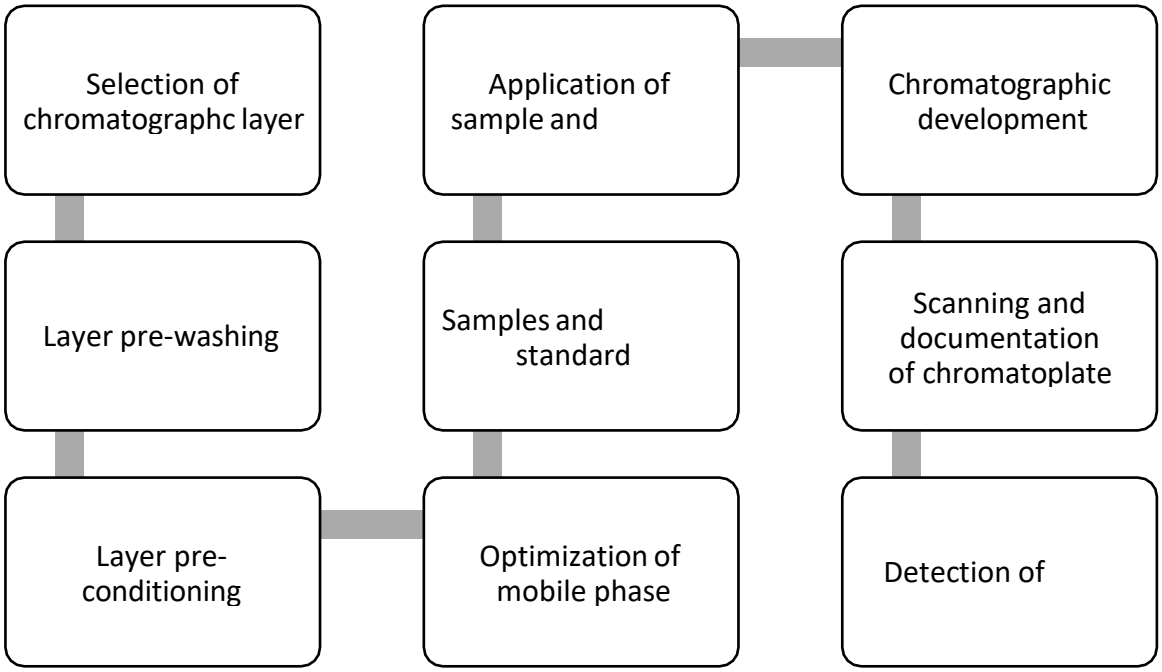


Figure 2. Schematic procedure for HPTLC method Development

2. MATERIALS AND METHODS

2.1 Preparation of mobile phase

A mixture of 1,4-dioxane, methanol, toluene 2:2:6 (V/V/V) was made, and it was then filtered through a 0.45 µ millipore nylon filter. Using an ultrasonic cleaner, the solution was degassed for 15 minutes. The ensuing mixture served as the mobile phase.

2.2 Chromatographic conditions

In HPTLC, chromatographic separation of drug was performed with silica gel 60 F254 (10.0 × 10.0 cm with 250 mm layer thickness) from E. Merck, Germany. Samples were applied as 8 mm bands by means of Camag 100 μL, sample syringe (Hamilton, Switzerland) with Linomat 5 applicator (Camag, Switzerland). Densitometric scanning was performed in the absorbance/reflectance mode at 210 nm using Camag TLC scanner 3 with deuterium source, slit dimension settings of length 2 mm, width 0.1 mm, monochromator band width 30 mm, and scan rate of 4 mm s^{-1}. Win CATS software (V 1.4.2, Camag, Switzerland) was used for scanner control and data processing.

2.3 Preparation of a standard mixture of azithromycin, fluconazole and ornidazole

To prepare the stock solutions, 100 mg of azithromycin was accurately weighed and dissolved in 10 mL of methanol to obtain a concentration of 10,000 ppm (10 mg/mL). Similarly, 15 mg of fluconazole was dissolved in 10 mL of methanol to prepare a 1,500 ppm (1.5 mg/mL) solution. Additionally, 75 mg of ornidazole was dissolved in 10 mL of methanol to yield a stock solution of 7,500 ppm (7.5 mg/mL). For the preparation of the working standard solution, 1 mL each of the azithromycin, fluconazole, and ornidazole stock solutions were pipetted into a 10 mL volumetric flask. The volume was made up to the mark with methanol to obtain a mixed working standard solution containing azithromycin (1,000 ppm), fluconazole (150 ppm), and ornidazole (750 ppm). All solutions were freshly prepared, stored in amber-colored glass containers to prevent photodegradation, and used within their stability period.

2.4 Preparation of sample mixture of azithromycin, fluconazole and ornidazole

For the preparation of the sample solution, one tablet each containing azithromycin, fluconazole, and ornidazole was accurately weighed and finely powdered. The powdered content of each tablet was transferred into separate 100 mL volumetric flasks, and
approximately 70 mL of methanol was added to each. The flasks were sonicated for 10 minutes to ensure complete dissolution of the active pharmaceutical ingredients and then made up to volume with methanol. The resulting solutions were filtered through Whatman filter paper to remove any insoluble

excipients. From each of these stock solutions, 1 mL was pipetted and transferred into a common 10 mL volumetric flask. The final volume was adjusted to the mark with methanol to prepare a combined working solution containing 1,000 ppm of azithromycin, 150 ppm of fluconazole, and 750 ppm of ornidazole.

2.5 Selection of detection wavelength

Each drug concentration was measured against a solvent blank in a standard stock solution of azithromycin, fluconazole, and ornidazole at a concentration of 10 µg/ml was scanned with a 10 mm path length in the UV region (200-400 nm) against a solvent blank the three- dimensional overlay spectrum.

3. METHOD VALIDATION

When a method has been developed it is important to validate it to confirm that it is suitable for its intended purpose. The validation tells how good the methods are, specifically whether it is good enough for the intended application. The method validation is today an essential concern in the activity of analytical chemistry laboratories. It is already well implemented in pharmaceutical industry. However, in other fields like food, petrol chemistry or in the biotechnological field, regulations have not reached such a level of requirement. The International Conference on Harmonisation (ICH) has provided definitions of validation issues included in "analytical procedures" for the fields of bioanalytical methodology, pharmaceutical and biotechnological procedures. Likewise the US Pharmacopeia (USP) has published guidelines for method validation for analytical methods for pharmaceutical products. However the guidelines form ICH and USP are not as detailed as those from the FDA, and in the analytical biotechnology area there, exists no detailed validation guidelines. The most common validation parameters will be briefly described below

4. RESULT AND DISCUSSION

The impact of chromatographic variables, such as mobile phase composition and ratio, was investigated in order to fine-tune the chromatographic parameters. The chromatographic parameters, such as resolution and retention factor (RF), were determined, and the resultant chromatograms were documented. For estimation, the parameters with the highest resolution and retention period were chosen.

4.1 Selectivity

To assess the method's selectivity, chromatography was carried out, and a densitogram of azithromycin AZM, FLZ, and OZ was measured. The retardation factor (Rf) value of the AZM, FLZ, and OZ site was compared to that of the norm to confirm its location. Studies on selectivity showed that, under the circumstances outlined, none of the excipients exhibited the same retardation factor as the AZM, FLZ, and OZ standards. In contrast, the Rf values and the area under the curve values measured for AZM, FLZ, and OZ in matrix were comparable to those derived from the standard, demonstrating the validity of the method for identifying AZM, FLZ, and OZ. the line of calibration. Calibration data also yielded $r2 \approx 1$, as seen, however the connection is not linear. Therefore, when evaluating a linear relationship, the correlation coefficient should be provided, but the linearity should be assessed using suitable statistical tests, as advised by the FDA guidance for the validation of analytical methods. In this instance, the statistical tests previously described for determining the suitability of the simple linear regression model are applied to our models AZM ($y = 4.506x + 2189.4$), FLZ ($y = 4.506x + 2189.4$), and OZ ($y = 4.8969x + 240.53$).

4.2 Accuracy

Accuracy The amounts of additional standards recovered into dosage form at 80%, 100%, and 120% were 106.3%, 101%, and 100.83%, respectively. The correctness of the devised HPTLC method was confirmed by the average recovery of 102.7% at three levels. The observed average recovery satisfies the acceptance criterion for percentage recovery (98– 102%).

4.3 Recovery study

S. No.	Amount of drug added (ng/band)			Amount of drug added			% Recovery		
	AZM	FLZ	OZ	AZM	FLZ	OZ	AZM	FLZ	OZ
1.				250.02	37.87	187.5			
2.	250	37.5	187.5	264.95	52.44	202.3	99.46	99.84	99.97
3.	500	75	375	269.76	58.96	212.8	98.8	99.92	99.97
4.	750	112.5	562.5	283.89	73.37	224.6	99.56	99.79	100.01
Mean							99.273333	99.85	99.98333
S.D.							0.4129568	0.06557439	0.023094
% RSD							0.4159	0.0663	0.023

Table 1. Recovery study of AZM, FLZ ,OZ

4.4 Lower limits of detection and quantification

The parameters limits of detection (LOD) and quantification (LOQ) were determined on the basis on the calibration curve as described in the ICH Q2R1 guidelines [23] as follow: LOD = 3s/S and LOQ = 10s/S (2) Where s is the standard deviation of y-intercept and S is the slope of the calibration curve. The LOD and LOQ were found to be 0.0467and 0.141 g/zone, respectively

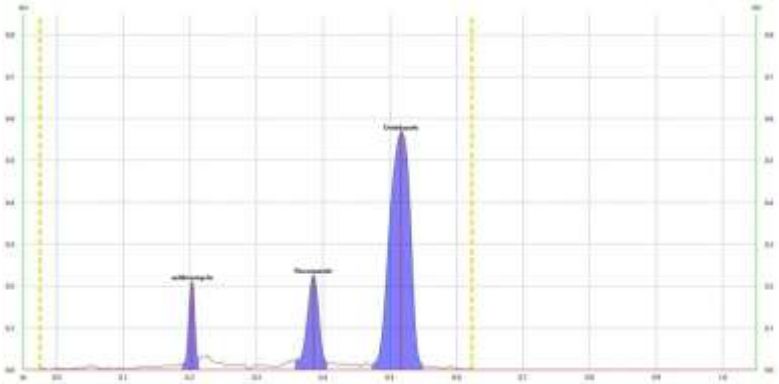


Figure 3. Chromatogram of mixed standard solution containing AZM, FLZ and OZ

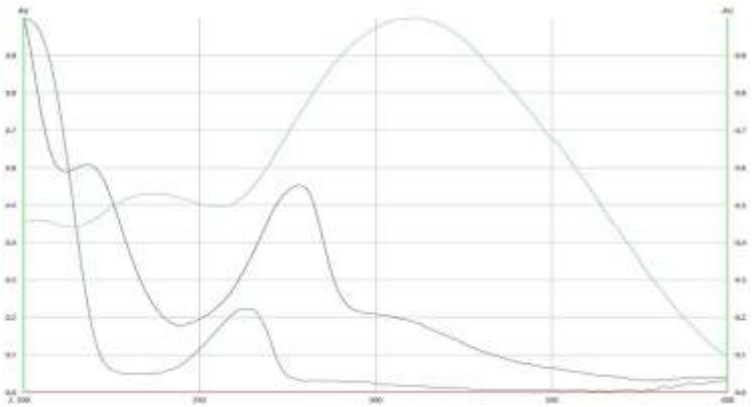
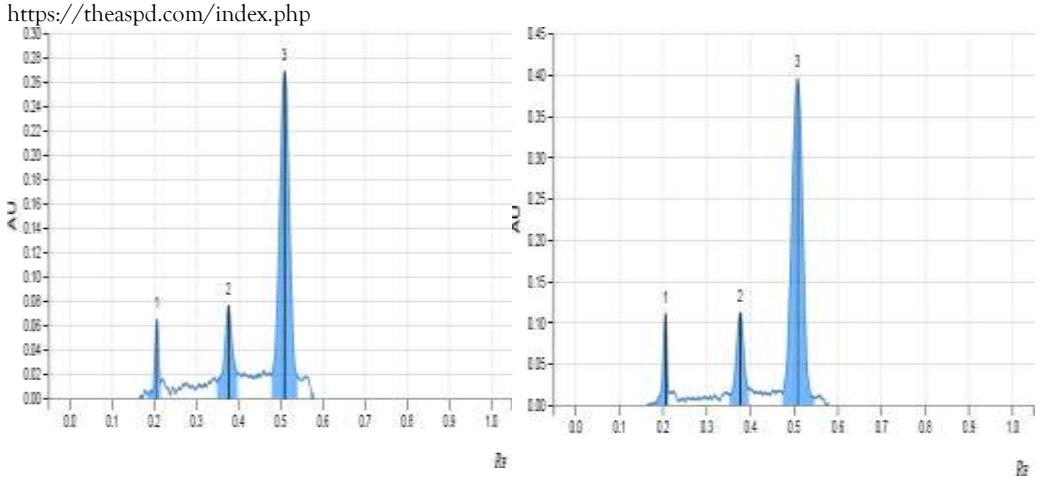
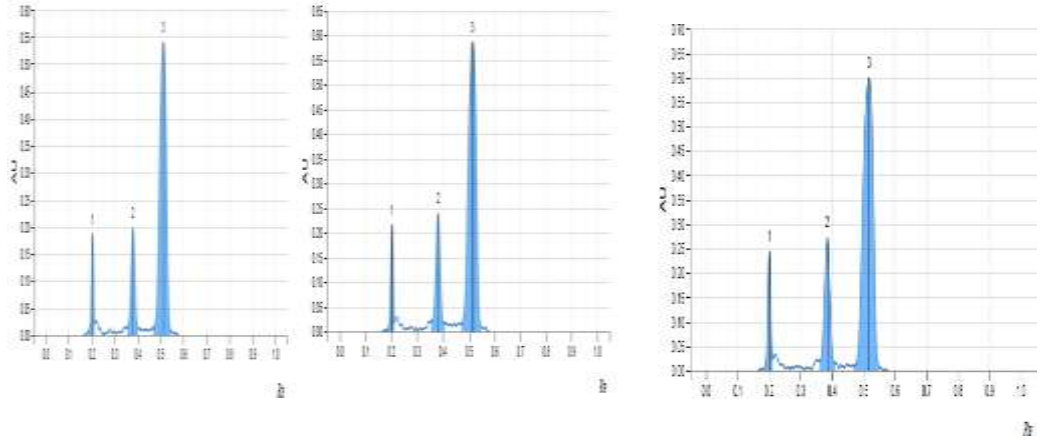


Figure 4. Overlain spectrum of mixed standard solution containing AZM, FLZ and OZ



2.0 µL **4.0 µL**



8.0 µL **10.0 µL** **12.0 µL**

Figure 5. Chromatogram of mixed standard solution containing 2-12 µL AZM, FLZ and OZ using mobile phase as 1,4-Dioxane, Methanol, Toluene 2:2:6 (V/V/V)

Table 2: Linearity study of AZM

S. No.	Concentration (ng/band)				
	1000	2000	3000	4000	5000
Replica 1	6431.2	11200.5	16100.6	20485.9	24318.4
Replica 2	6411.1	11153.3	16111.2	20236.1	24125.1
Replica 3	6222.5	11412.1	16025.4	20365.3	24563.8
Replica 4	6376.8	11023.4	16123.1	20156.3	25236.8
Replica 5	6291.3	11135.6	15910.3	21063.6	24235.3
Replica 6	6345.2	11125.5	16165.2	19995.4	24632.4
Mean	6346.35	11175.07	16072.63	20383.77	24518.63
S.D	78.345306	129.8635	91.61669	373.449	401.4937
% R.S.D	1.2344939	1.162083	0.570017	1.83209	1.637504

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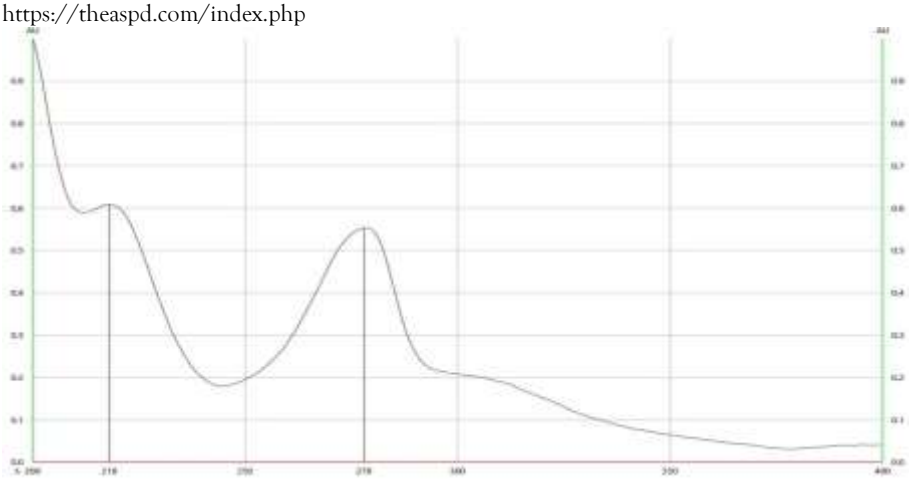

Figure 6. Overlain spectrum of AZM

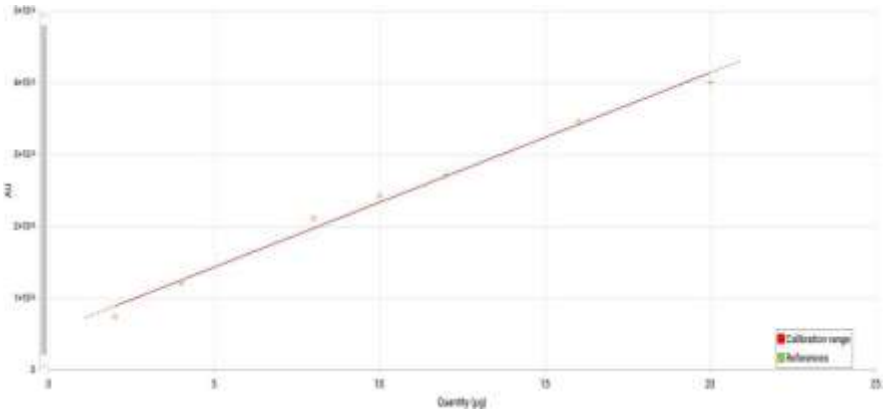

Figure 7. Calibration Curve of AZM

Table 3: Linearity study of FLZ

S. No.	Concentration (ng/band)				
	500	1000	1500	2000	2500
Replica 1	7552.5	13070.3	18223.6	22863.8	27142.8
Replica 2	7512.5	13252.6	18356.6	22750.8	27050.8
Replica 3	7485.1	13096.6	17865.8	21860.6	26230.6
Replica 4	7491.7	13751.7	17965.2	22956.6	27450.7
Replica 5	7568.6	13251.8	18365.8	22940.8	27325.7
Replica 6	7486.2	13259.9	18756.7	22751.1	27287.4
Mean	7516.1	13280.48	18255.62	22687.28	27081.33
S.D	36.178834	245.7882	319.608	414.6016	439.7881
% R.S.D	0.4813511	1.850747	1.750738	1.827463	1.623953

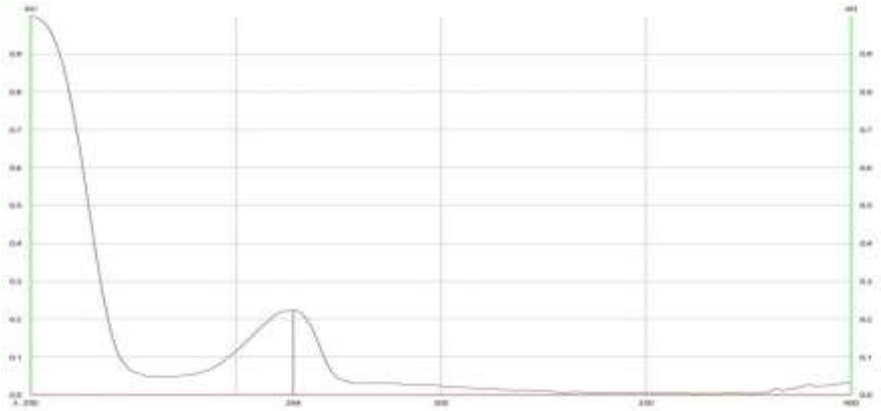

Figure 8. Overlain spectrum of FLZ

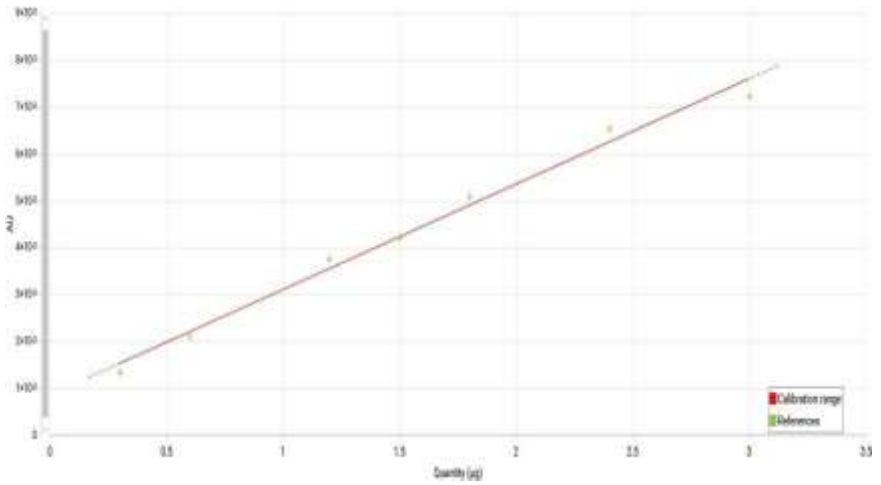

Figure 9. Calibration Curve for FLZ in Mobile phase

Table 4: Linearity study of OZ

S. No.	Concentration (ng/band)				
	500	1000	1500	2000	2500
Replica 1	2679.1	5393.9	7528.8	10225.8	12457.7
Replica 2	2695.6	5390.6	7569.5	10147.4	12056.7
Replica 3	2598.6	5295.4	7495.7	10220.1	12465.7
Replica 4	2612.3	5397.2	7510.8	10520.1	12380.1
Replica 5	2645.5	5311.3	7486.1	10143.8	12350.1
Replica 6	2609.7	5283.8	7536.9	10009.9	12488.8
Mean	2640.1333	5345.367	7521.3	10211.18	12366.52
S.D	40.123941	53.91826	30.42203	170.2037	160.9629
% R.S.D	1.5197695	1.008691	0.404478	1.666836	1.301603

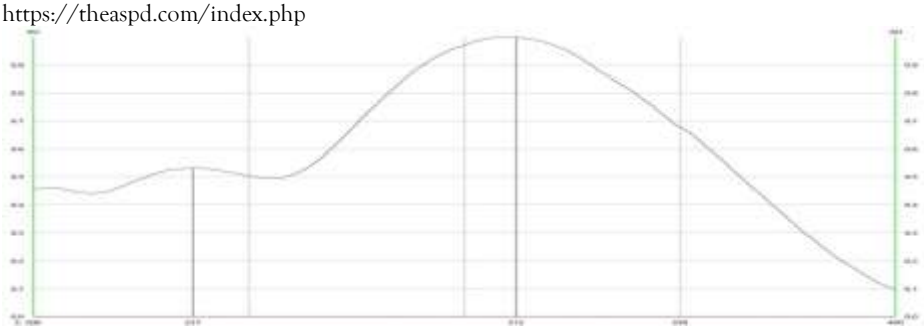

Figure 9. Overlain spectrum of OZ

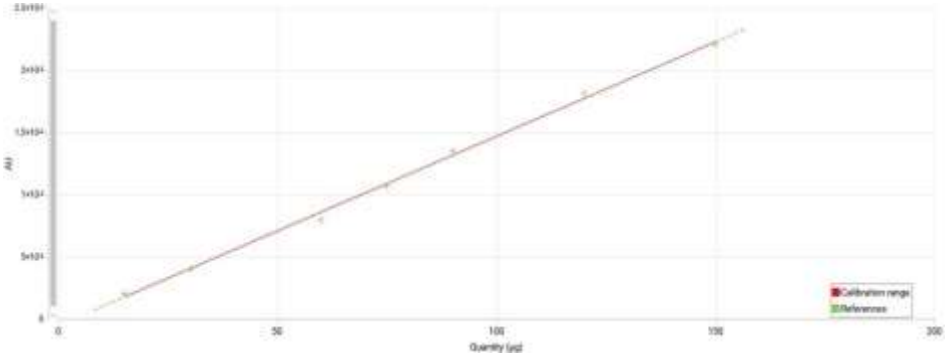

Figure 10. Calibration Curve for OZ in Mobile phase

5. CONCLUSION

The pharmaceutical industry needs a simple, quick, and cost-effective approach to simultaneously analyze multicomponent formulations without the need to separate or extract the analyte from the excipients or from themselves. In the current investigation, a novel, straightforward, quick, economical, precise, and selective HPTLC technique has been devised for the simultaneous quantitative determination of Paracetamol, Hydrochlorothiazide, and Enalapril maleate in bulk and tablet dosage form, taking all of this into account.

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