Method Development and Validation for Estimation of Bedaquiline and in Tablet Dosage form by HPTLC and RP-HPLC Study

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A diarylquinoline anti-mycobacterial drug, bedaquiline (BDQ), treats mycobacterial infections. It inhibits the proton pump by inhibiting mycobacterial Adenosine Triphosphate (ATP) synthase. ATP enzyme that is energy production requires Mycobacterium tuberculosis. The present study aimed to develop three analytical methods, i.e., High-performance thin-layer chromatography (HPTLC), HPLC, and UV, as BDQ estimation in tablet forms. HPTLC is employed in the first method, which uses ethanol-chloroform-toluene as the mobile phase (6:3.5:0.5). UV spectroscopy at 240nm was used to analyse BDQ at 100-300 micrograms per milliliter. RP-HPLC method based on the separation using a C18 column, Buffer (sodium acetate): Methyl alcohol (70:30) as mobile phase flowing at a rate of 1ml per min. The retention time was determined to be 5.4 minutes. At 240 nm, it could quantify the concentrations of 5-25 μg per ml at concentrations of 5-20 μg per ml. The third method involves the "UV-VISIBLE" spectrophotometric method. A range of BDQ concentrations is determined of 8-40μg/ml, respectively. These methods were checked to ICH guidelines, and as a result, a commercially available pharmaceutical formulation, with the findings of statistical comparison, was conducted between all three methods.

Keywords: Bedaquiline; Estimation; HPTLC; RP-HPLC; Tablet; UV-VISIBLE Spectrophotometry.

BDQ Chemically known as (1R, 2S) 1(6Bromo-2methoxy-3quinolyl) 4-dimethylamino-2(1- naphthyl) 1-phenylbutan-2-ol. The structure of BDQ is shown in fig1. A novel bactericidal anti-mycobacterial drug called bedaquiline. Multidrug-resistant tuberculosis is treated with this diarylquinoline anti-mycobacterial medication by inhibiting mycobacterial ATP synthase, which generates energy for Mycobacterium tuberculosis; it inhibits the proton pump. Bedaquiline causes bacterial death. Its molecular formula is $C_{32}H_{31}BrN_2O_2$, and its molecular weight is 555.504 gm per mol[1,2]. A recent literature review reported that LC-MS and MS had been employed to analyze Bedaquiline and Rifabutin in human plasma[3,4]. A Liquid chromatography-Mass spectroscopy method has been reported to be useful in determining bedaquiline concentrations in human serum[5-7]. Bedaquiline was enantio-separated by polar stationary phases made of polysaccharides[8]. A pharmacokinetic study on bedaquiline[9,10] and

the physical and chemical sustainability of bedaquiline in hydrolytic systems at various pH levels were evaluated by isolating eight variation analog diastereomers by HPLC. They assessed the degradation kinetics of bedaquiline in the solution with UV spectrophotometric methods[11,12]. HPLC can simultaneously determine the levels of bedaquiline and endearing in plasma samples[13]. We have reported on the simultaneous enumeration and quantification by reversed Phase HPLC[14] of bedaquiline (TMC-207), moxifloxacin, and pyrazinamide formulation. As a result of the literature review, we concluded that it is essential to develop analytical methods that are more sensitive, least expensive, take up less time, and use less drug quantity to estimate bedaquiline bulk and its tablet dosage form. As a result, the goal of this work is to provide a precise, accurate, and repeatable method for calculating the concentration of bedaquiline utilizing HPTLC, HPLC, and UV spectrophotometric techniques.

EXPERIMENTAL

Chemicals and reagents

Sirturo tablets labeled to contain Bedaquiline 100 mg. methanol (HPLC grade), glacial acetic acid, sodium acetate, ethanol, chloroform, and toluene were purchased from Loba chem (Mumbai, India). Throughout the project, calibrated glassware was used. All the reagents used in this study were AR Grades.

Instrumentation

In TLC, Silica gel 60 (F254) is coated on an aluminum sheet, and in HPTLC, a Sample applicator (Linomat-V) with a 100L Hamilton syringe. The plates were developed in a 20 cm x 10 cm CAMAG twin trough tank that had been soaked with the extraction solvent for 30 minutes prior to production. An RP-HPLC system with a serial dual plunger pump (micro-volume ten microliters on the primary side, five microliters on the secondary side) and a PDA detector with an injector were developed. As computation, Shimadzu lab solution software was used. A novel UV- spectrophotometry using an Ultra Violet visible double beam spectrophotometer has spectral widths of a nanometer and wavelengths accurate to 0.5 nanometer. 10 mm matched quartz cells in pairs were used. The digital scale Shimadzu AUX220 is utilized for weighing purpose[15].
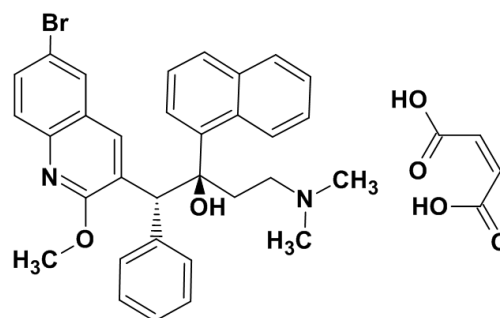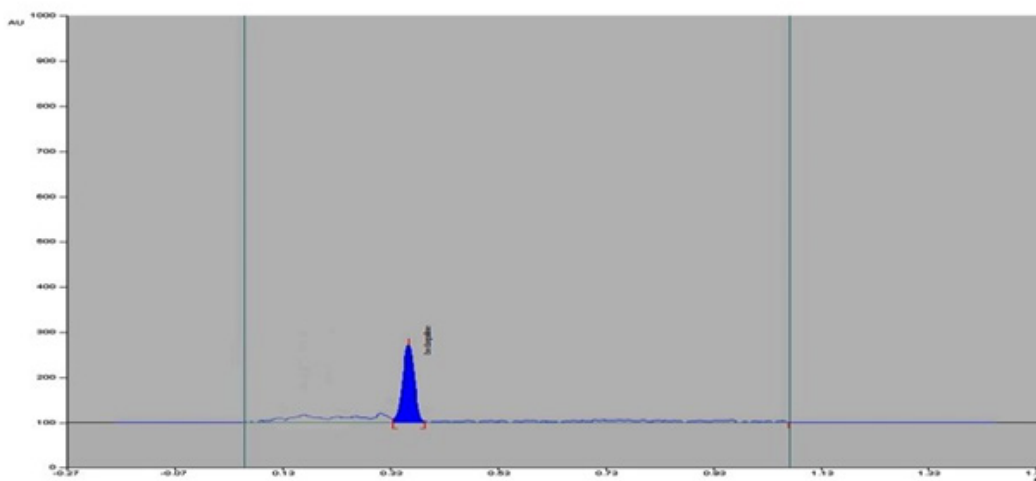

Fig. 1. Structure for Bedaquiline

Fig. 2. Optimized Chromatogram by HPTLC method

Preparation of stock solutions
Standard Solution

        One hundred milligrams of BDQ were accurately weighed and transferred into One hundred milligram volumetric flasks. Methanol adds to dissolve. After ten minutes of sonicating, the volume of the solution was topped up with methanol to achieve 1000 µg per ml of BDQ concentration.

Sample solution

        Weighing 10 tablets accurately and (Sirturo100 mg tablets), approximately the exact weight of the powdered tablet was identified. For dissolving the tablet powder, methanol was added to a flask containing one hundred milligrams of BDQ. Following this, the solution was sonicated for ten minutes. Methanol was used to makeup and then filter paper (Whatman -41) was used for filtering purposes. The finished solution contained 1000 µg per ml.

HPTLC Method

        1, 1.5, 2.0, 2.5, and 3.0 ml of the 1000 µg/ml standard stock solution were pipetted into a 10 ml volumetric flask. The final concentration was 100, 150, 200, 250, and 300 micrograms per ml. Solutions were spotted on a pre-coated aluminum Plate 60 F254 using a CAMAG1000 µl sample applicator syringe. The plate for 20 mins placed in an oven at the temperature of 110°C before spotting the samples for activation. There was a constant flow rate of 0.1L per second, with a space between two bands of 10mm, and a constant application
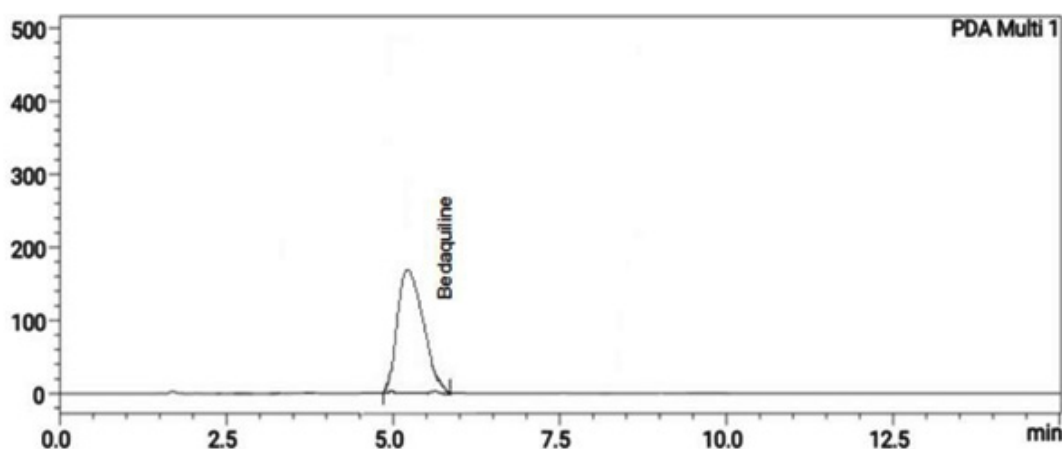

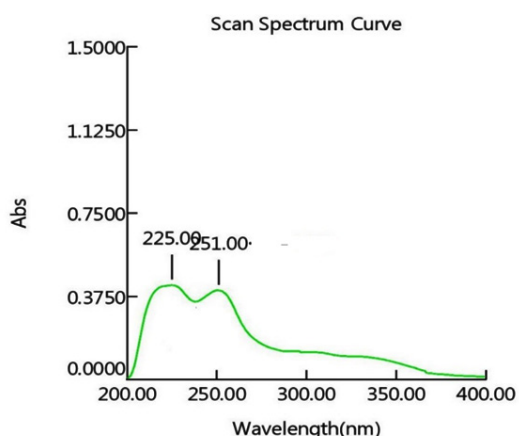**Fig. 3.** Optimized Chromatogram by HPLC method

Fig. 4. UV Spectrum of Bedaquiline

rate of 0.1L per second. Previously, ethanol: chloroform: toluene (6:3.5:0.05 v/v/v) was pre-saturated for 30 min in a twin trough chamber. To a distance of eight cm, it took 15 to 20 minutes to develop the plate. As a result of the development of the plate, it has been dried through passing air from the dryer. In order to scan the spots at 10 mm per second, they were slit at 5 mm by 0.45 mm as they developed at 240 nm[16].

HPLC Method
Chromatographic conditions

        The stationary phase C18 column was used in this method, and Buffer (Sodium acetate at pH-4.5) solvent phase. The samples flow at a speed of 1ml per min. Whattmann filter paper was

used to filter the mobile phase. A detector (PDA) detects the compounds at 240nm. Under vacuum, Acetonitrile was mixed, degassed, and passed to 0.45 micron pore to filter.

Mobile Phase Preparation

About 700 ml of sodium acetate buffer (pH range 4.5) and 300 ml of methanol in an ultrasonic water bath were used to mix and degas the mixture for 5min. A 0.45 pore filter was used to separate the filtrate, which was transferred under vacuum into a volumetric flask containing 1 litre of filtrate.

Preparation of Calibration Curve solution

A 10 ml standard flask was filled with 0.5 ml of standard stock solution (1000 µg/ml). Methanol (100 µg per ml) was then added to the solution, and from above said concentration (100 µg per ml), 0.5, 1.0, 1.5, 2.0, and 2.5 ml pipetted out and transferred to the separate volumetric flask of 10ml capacity. Final concentrations ranged from 5 to 25 µg per ml.

UV – Visible spectrophotometric method
Preparation of Stock solution for calibration

In a standard flask of 250 ml, 5.0 ml of the stock solution (1000 µg per ml) was pipetted and transferred into the standard flask. The solution was made up to volume using methanol (20 µg per ml). Using a pipette, 4, 8, 12, 16, and 20 ml solutions were pipetted from above the concentration (250 µg per ml) into 10 ml standard flasks. The resulting concentrations were 8 to 40 µg per ml.

Validation

The method validation was conducted per International Conference on Harmonization Guidelines[15-16].

Linearity and range

HPTLC calibration curve of BDQ was prepared using methanol solutions containing 100, 150, 200, 250, and 300 µg per ml. To calculate the regression equations for the calibration curve, peak areas were plotted against BDQ concentrations. There were five determinations in each response. To calibrate BDQ with RP–HPLC, various concentration ranges, such as 5 to 25µg/ml, has been made by diluting the stock solution (standard). Under operating chromatographic conditions, an aliquot (10µl) of each solution was injected. UV-visible spectrophotometry was performed in methanol with BDQ solutions containing 8, 16, 24, 32, and 40 µg per ml of BDQ

and measured at 240 nm. By plotting absorbance versus concentration, calibration curves are constructed.

LOQ & LOD

We calculated the detection and quantification limit, by SD and calibration curve. ICH guidelines estimated LOD and LOQ. LOD=3.3×ó/S and LOQ=10×ó/S, where ó is the standard deviation 'S' is the slope.

Content estimation (Assay)

For content estimation, Sirturo100 mg tablets were used. The sample stock solutions were prepared for each HPTLC, HPLC, and UV method from the standard stock. Samples and standards were spotted for HPTLC. A chromatogram was recorded after injecting 20 liters of standard and sample solutions. Standard and sample absorbance were measured for the UV method. The purity percentage was calculated using peak area and absorbance.

Accuracy

BDQ recovery was calculated at three levels (50, 100, and 150%) to determine the method's accuracy. We added known quantities of standard solutions of BDQ 100, 200, and 300g per ml to a pre-analyzed sample solution containing 15µg per ml BDQ before spotting 10µL of each solution on an activated silica plate. A known amount of standard BDQ 7.5, 15, 22.5µg per ml was mixed with pre-analyzed sample solution containing 15µg BDQ for performing reverse phase HPLC. To pre-analyze sample solutions containing 30µg per ml, known amounts of BDQ standard solution were added.

Precision

A procedure's use circumstances will determine how much intermediate precision is required. For the HPTLC method, 200µg per ml was used for the triplicate determination. For the HPLC method, 15µg per ml concentration solution was used. The peak area was measured after the injection of 20µl of solution. The UV method found 24 µg per ml concentration solution absorbance at 240 nm.

Ruggedness

In this definition, reproducibility means that test results can be replicated no matter where they are performed, what analyzers are used, what instruments are used, and how long it takes to run the experiment.

RESULTS

Precision, accuracy, and reproducibility were optimized for HPTLC, HPLC, and UV methods. Toluene: Acetonitrile, methanol: water, and methanol: chloroform ratios were used for HPTLC. Observe spreading and tailing spots. To overcome this problem, we used ethanol-chloroform-toluene (6:3.5:0.5). There was a sharp, symmetrical peak with Rf 0.63 (Fig 2). Different trials for HPLC were performed using different ratios of mobile phases (Water: Acetonitrile (55: 45) & Methanol: H_2O 50: 50), Sodium acetate buffer (3.0): methanol 70%:30%), and it has flowed in range of 0.8, 1.0, 1.2ml per min. There was

no tailing of peak and theoretical plate numbers within the limit (above 2000). In the end, sodium acetate buffer: & methanol (70:30%) was used. Chromatograms showed sharp peaks, and system suitability parameters were within limits. Fig 3 shows the optimized chromatogram. The solvent for the UV method was selected from the solubility study. A concentration solution of 10g per ml was prepared and scanned between 200-400nm. Fig 4 shows the UV spectrum at 240nm with maximum absorbance.

Linearity

The range of analytes in a sample depends on the concentrations of analytes at their highest and lowest concentrations. In the case of HPTLC, a
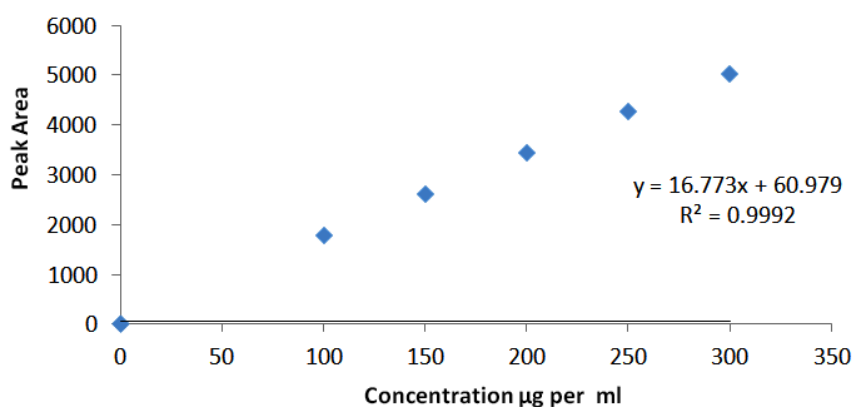

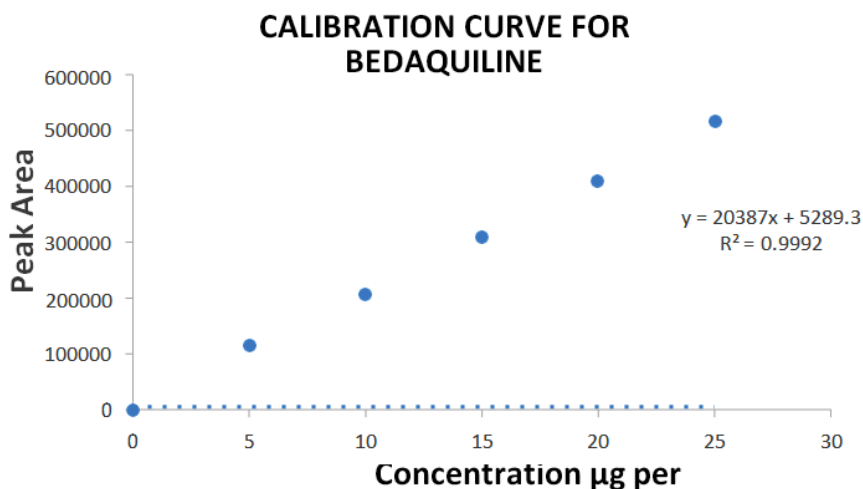**Fig. 5.** Calibration Curve for Bedaquiline by HPTLC method

$y = 16.773x + 60.979$
$R^2 = 0.9992$

Fig. 6. Calibration Curve for Bedaquiline by HPLC method

$y = 20387x + 5289.3$
$R^2 = 0.9992$

CALIBRATION CURVE FOR BEDAQUILINE

$y = 0.014x + 0.0055$
$R^2 = 0.9997$
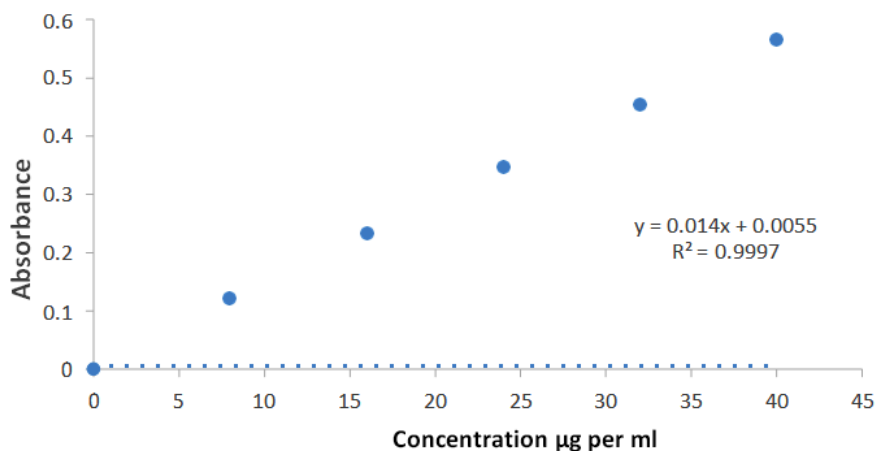
Fig. 7. Calibration curve for Bedaquiline by UV method

Table 1. Reports for Linearity study

Parameters	HPTLC	HPLC	UV
Range(µg/ml)	150-300µg/ml	5-25 µg/ml	8-40 µg/ml
y = mx + c	y = 16.773x + 60.979	y = 20387x + 5289.3	y = 0.014x + 0.0055
r2	0.9992	0.9994	0.9997
Slope (m)	16.773	20387	0.014
Intercept (c)	60.979	5289.3	0.0055
LOD (µg/ml)	0.9081	0.007	0.1791
LOQ(µg/ml)	0.164	0.0212	0.5428

Table 2. Content Estimation of Bedaquiline in marketed formulation by proposed methods

Methods	Standard Peak area	Sample Peak area	Mean (%)	SD	(%) RSD
HPTLC					
1	3447.8	3453.3			
2	3475.3	3481.4	100.37	0.2056	0.2048
3	3463.2	3470.3			
Average	3462.1	3475.3			
HPLC	314092	315223			
1	315324	318401			
2	314962	318230	100.78	0.3707	0.3678
3	314792.6	317284.6			
Average					
	Absorbance of standard solution	Absorbance of sample solution			
UV			100.48	1.4304	1.4235
1	0.346	0.342			
2	0.353	0.357			
3	0.340	0.345			
Average	0.346	0.348			

Table 3. Data for Intermediate Precision by proposed methods

No.	HPTLC	HPLC	UV
1	3447.8	314268	0.346
2	3463.6	314394	0.348
3	3476.5	314942	0.353
4	3404.7	314727	0.333
5	3423.8	314904	0.340
6	3452.9	314749	0.354
Avg	3444.8	314664	0.347
SD%	26.38	274.15	0.0055
RSD	0.7657	0.0871	1.585

linear correlation was observed between peak areas and concentrations in the range (of 100 to 300g/ml) when the HPTLC method was used. The correlation coefficient came out to be r^2=0.9992. A linear concentration value for the HPLC method was 0.9994 µg per ml, with a range from 5-25 µg per ml, and the r^2 value was 0.9994 µg per ml. There was linearity in concentrations between 8 and 40 micrograms/ml of UV solution for the method, and a correlation coefficient value of 0.9997 was identified. Figures 5-7 show the calibration curves for HPTLC, HPLC, and UV methods. Table 1 shows linearity reports.

Table 4. Data for Accuracy study for the proposed methods

Various Methods	Concentration (%)	Added Amount µg/ml	Amount Found µg/ml	Recovery Mean (%)	SD	RSD (%)
HPTLC	50	100	90.1			
	100	200	180.21	100.13	0.0307	0.0305
	150	300	270.45			
HPLC	50	4	3.98			
	100	8	8.12	100.16	1.1547	1.1527
	150	12	11.95			
U0056	50	15	15.09			
	100	30	30.32	100.63	0.411	0.4082
	150	45	45.11			

Table 5. Data for Ruggedness study

Method	Different conditions	Average Percentage	SD	(%) RSD
HPTLC	Analyst-I Analyst-II	101.1 99.28	1.286	1.283
HPLC	Analyst-I Analyst-II	98.42 99.32	0.636	0.643
UV	Analyst-I Analyst-II	99.43 98.04	0.982	0.994

Table 6. Comparison between the reported method and advanced method

HPLC and UV	Reported method (11&13)	Developed method
Linearity	10-100 µg/ml	5-25 µg/ml
LOD LOQ	0.3272µg/ml	0.007
	0.9525µg/ml	0.0212
Linearity	15-75 µg/ml	8-40 µg/ml
LOD LOQ	2.72 µg/ml	0.1791
	8.23 µg/ml	0.5428

LOD and LOD

It was determined that the LOD and LOQ were 0.9081 µg per ml, 0.1640µg per ml, 0.0070µg per ml, 0.0212µg per ml and 0.1791µg per ml, 0.542µg per ml for HPTLC, HPLC and UV methods respectively. The report is shown in table 1.

Content Estimation

It was determined that BDQ is pure in tablet formulation by an assay (content estimation). An estimate of BDQ concentration from a calibration curve was selected and quantified. The

tablet formulation Sirturo (100 mg tablets) was used for identification, and the purity of analytes present in the formulation was analyzed from 100.37 - 100.78% for the proposed methods. The % RSD values were 0.2048, 0.3678, and 1.4235 for the proposed methods. Table 2 shows the result.

Precision

A study of intermediate precision confirmed the method's precision. The analyses of standards were carried out. The % of RSD of the intermediate precision analysis of Bedaquiline was found to be 0.7657, 0.0871, and 1.585 % for HPTLC, RP-HPLC, and UV, respectively. The reports are shown in table -3

Accuracy

The formulation solution previously analyzed was diluted with pure drugs to evaluate the method's accuracy. To analyze the mixture, a method was proposed. A calculation was made to determine the amount of drug recovered. Our study found that the percentage recovery for HPTLC, RP-HPLC, and UV methods was 1000.13% to 100.63%. RSDs were found to be less than 2%. Table 4 shows the results.

Ruggedness

A ruggedness validation was performed on the developed method. An independent lab is rugged if it can maintain high precision over multiple days while using multiple analysts, instruments, and reagents from different sources. The results are shown in Table 5. The % of RSD was 1.283, 0.643, and 0.994% for HPTLC, HPLC, and UV, respectively.

DISCUSSION

The quantitation and detection limits are shallow, indicating that the method is sensitive. Compared with the developed method, the reported Reverse Phase HPLC[11] and Ultra Violet[13] methods had higher LOD and LOQ values. Therefore, the developed method was more sensitive than the reported method (Table -6).

% RSD value below 1.3, considered acceptable, validated the robustness and repeatability of the method. (Acceptance criteria – not more than 2 %). Precision and repeatability were good for the developed method. For the developed RP-HPTLC, HPLC, and UV spectroscopic methods, the RSD values were less than 2%. RSD values were not affected by excipients used in formulations. As a result, it can be concluded that the method is accurate. This resulted in the methods being more accurate as a result of this. With the three methods, the estimation of drugs was accurate, precise, and rapid. HPLC was more sensitive than the other methods when comparing the sensitivity of the three methods since the LOD and LOQ values in HPTLC and UV methods were lower than those of HPLC and UV. Therefore, it is concluded that all three methods can be successfully applied for the routine analysis of the quality control of the BDQ in bulk and as tablets for dosage forms as part of the routine quality control process.

CONCLUSION

A simple, accurate, and reproducible quantitative analysis of BDQ in tablet form was developed and validated by HPTLC, reversed-phase HPLC, and UV visible spectrophotometry. According to the analysis results, the concentration range was linear, and the value of the correlation coefficient was $r^2 < 0.999$ for the three methods. Compared to the developed method, the reported RP-HPLC and UV methods had higher linearity ranges. Thus, by utilizing the developed method, it is possible to estimate BDQ when the least amount of drug needs to be administered.

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