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MULTIVARIATE CALIBRATION TECHNIQUE AIDED UV SPECTROPHOTOMETRIC METHOD FOR THE ESTIMATION OF BENEDIPINE HYDROCHLORIDE IN PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

The current work focuses on the creation and validation of a quick, sensitive, and accurate mulitivariate calibration approach utilizing UV spectrophotometric techniques for the assessment of Benedipine. The λmax of benedipine is at 237 nm. The multivariate calibration technique applies the linear regression equation by evaluating the link between concentration and absorbance at five distinct wavelength. The findings were processed statistically. The devised approach was verified as per the ICH criteria. The approach is accurate, precise, and linearity within the range of 7-13 µg mL^{-1}. This statistical technique delivers optimal findings by reducing the variation arising from the instrumental or experimental circumstances.

Keywords: Benedipine Hydrochloride, Multivariate Calibration, Hypertension, UV Spectrophotmetric

INTRODUCTION

Nearly 1.13 billion people worldwide have hypertension. Hypertension, also known as high blood pressure. Hypertension is a potentially fatal medical disorder that can endanger the brain, heart, kidneys, and other organs **[1]**. Reversible myocardial ischemia causes angina pectoris, which is a type of visceral discomfort. Ischemic attacks are typically asymptomatic. Pain arises late in the

ischemia event when it manifests. When enough blood is not pumped to the heart, angina may occur. To treat hyper tension and angina pectoris benidipine hydrochloride is used.

Benidipine hydrochloride (BEN) is a dihydropyridine calcium channel blocker that is both highly effective and long acting. BEN is chemically 5-O-((3R)-1-benzylpiperidin-3-yl) 3-O-methyl(4R)-2-6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate. The molecular formula is $C_{28}H_{32}CIN_3O_6$ while 542.0 is the molecular weight **[2]**. BEN inhibits L, N, and T type calcium channels, making it a calcium channel inhibitor. BEN has a relatively long-lasting pharmacological impact, which may be explained by the DHP binding site's great affinity for cell membranes. BEN is also vascular selective for peripheral blood vessels.

It was invented in Japan by Kyowa Hakko, and it is nowadays accessible in various Asian countries such as India and Japan. It was patented in 1981 and approved for medical use in 1991 **[3]**. It consists of orally active antihypertensive medicines as well as T,N, and L-type that displace a large range of activites invitro and invivo. It is generally used in the treatment of hypertension, renal parenchymal , coronary heart disease, cardiac arrhythmias, and angina pectoris as a racemate **[4]**. BEN is mentioned in the Japan Pharmacopoeia 2 official monograph, which provides potentiometric titration and analytical protocols for its measurement. BEN pill contains 95.0 percent to 105.0 percent of the indicated quantity of BEN **[5]**. The commercially available formulations include 4mg, 8mg tablets for oral use. The general properties of BEN have similar to those of nifedipine. The structure was depicted in **Figure 1** and instruments used were represented in **Table 1**.

The literature review for BEN is like UV visible **[4-9]**, HPLC **[10]**, RP-HPLC **[11, 12]**, LC-MS **[13]**, LC-tandem-MS **[14]**, Capillary GC-MS**[15]**, UPLC **[16]**, Spectroflurimetric **[17]**, Spectrophotometric method was developed. When the absorbance of an analyte (X) at five distinct wavelengths is measured, (λ = 227, 232, 237, 242 and 247 nm), For each wavelength, the following equation may be constructed.

$A_{\lambda 227} = a X C_x + k_1$ … .(1)

$A_{\lambda 232} = b X C_x + k_2$ ..... .(2)

$A_{\lambda 237} = c X C_x + k_3$ …(3)

$A_{\lambda 242} = d X C_x + k_4$ … (4)

$A_{\lambda 247} = e X C_x + k_5$ … (5)

where A_λ the absorbance of the analyte; sloping of the analyte's linear regression functions are a, b, c, d, e.; the intercepts of the linear regression functions are k_1, k_2, k_3, k_4, k_5 at the five specified wavelengths; and analyte's concentration is C_x. The five

equation systems (1–5) listed above may be summarized as follows

$A_T = a \times C_x + b \times C_x + c \times C_x + d \times C_x + e \times C_x + K_T$… (6)

which can be further simplify to AT = C x (a+b+c+d+e) + K_T…… (7)

$$C_x = \frac{A_T - K_T}{(a+b+c+d+e)}$$

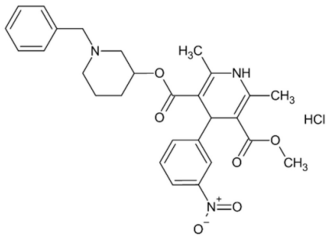


Figure 1: Chemical Structure of BEN

MATERIALS AND METHODS

Chemicals

Methanol, Niksan Pharmaceutical Ahmedabad, India, gifted BEN.

Instruments used

Table 1: Instruments used

Name	Make model
UV Spectrophotometer	LABINDIA UV 3092 double beam UV –VIS spectrophotometer (200-400 range)
Sonicator	Soni clean sonicator (model 160T, Thebarton-Australia)
Weighing balance	Analytical balance (AS 245, Mettler Toledo, India)

Standard preparation

Consider 10 mg of BEN API, place it in a 10ml volumetric flask, and dissolve it in methanol. Sonicate for 10 minutes, make up the volume with methanol, and filter the solution with Whatmann grade 42 circular filter paper. Fill a 100ml volumetric flask with specified stock solution of 1ml. Fill a 100ml volumetric flask with 1ml of the specified stock solution. Make up the volume with methanol, which gives 10 µg/ml.

Working Solution

From the above stock solution 7-13 µg mL^{-1} solution were prepared by using methanol as solvent.

Extraction of BEN from tablet and sample preparation

Consider 10 tablets of 4mg BEN tablets. Weigh tablets individually and note the readings. Crush the tablets in the motor with a pestle. Take 10mg of powdered tablet and dissolve in methanol in a 10ml volumetric flask. Sonicate for 10minutes, make up the volume with methanol, and filter the solution with Whatman grade 42 circular filter papers. From the stock solution pipette out 1ml and transfer it into a volumetric flask of 100ml. Make up the volume with methanol, which gives 10 µg mL^{-1} solution. From the 10 µg mL^{-1} solution prepare 7, 8, 9, 10, 11, 12, 13µg mL^{-1} solutions.

Determination of absorption maxima (λ max)

The maximum was reported to be 237 nm for a 10 µg mL^{-1} solution produced from standard stock solution. To maximize the correlation coefficient and eliminate instrumental fluctuations, the absorbance of

the solution was recorded from the λmax (237 nm) range, i.e., 227, 232, 237, 242, 247 nm.

Method validation

The technique was tested for linearity, sensitivity, precision, and accuracy applying ICH Q2B criteria.

Linearity

BEN stock solution and sample solution were diluted with methanol to achieve concentrations of 7-13 μg mL^{-1} (0.7, 0.8, 0.9, 1.0, 1.1, 1.2 and 1.3 μg mL^{-1}). The solution's absorbance was measured throughout wavelength range. i.e., 227, 232, 237, 242, and 247 nm, in order to improve correlation and reduce instrumental variability. In addition, with the MVC approach, the absorbance of the linearity solution across the specified wavelength was recorded and analysed.

Precision

The repeatability of the precision was evaluated using intraday and interday precision. To test different accuracy levels, a typical standard solution of BEN at a concentration of 10 μg mL^{-1} was utilised. Six solutions were tested at seven distinct wavelengths for the repeatability research. The absorbance of produced solution was determined three times in the intraday form on the same day at defined time intervals. Furthermore, the absorbance on three consecutive days is also used to create inter-day variance.

Limit of Detection (LOD)

The LOD of BEN was calculated using equations based on the standard deviation and calibration slopes of reactions for each wavelength.

$$\mathbf{LOD} = \frac{3.3\,\sigma}{S}$$

Limit of Quantification (LOQ)

The LOQ of BEN was calculated using equations based on standard deviation and calibration slope of response for a particular wavelength.

$$\mathbf{LOQ} = \frac{10\,\sigma}{S}$$

Accuracy

At 80, 100 and 120 percent the accuracy of BEN was assessed for the pre-analyzed test solution and also estimated the percent recovery values.

RESULTS AND DISCUSSION

The UV Spectrum of 10 μg mL^{-1} solution of BEN in methanol is presented in **Figure 2** which depicts the λmax value of BEN at 237 nm.

Stability of solutions:

For sample solutions of 10 μg mL^{-1} BEN the durability of the sample was carried out for two weeks, for 6hr at room temperature. Without spectrophotometric alterations in the settings the analysis of the solutions confirmed the drug permanence, this had no noticeable impact on the drug's maximum wavelengths or absorbance values. The RSD percent values were not more than 1%. Stock solutions have been

declared safe in freezers for at least two weeks. Using the described method, the concentrations of freshly produced and old solutions were monitored for two weeks. The discrepancy was noted to be less than 2%

Linearity

In agreement with ICH Q2 (R1) criteria, within the concentration range of 80-120 percent of 10 µg mL^{-1} (8-12 µg mL^{-1}) the linearity findings of the suggested technique were discovered. A calibration graph was created based on the absorbance of dilute standard solutions at seven distinct wavelengths. The resultant regression equations were derived and found straight for BEN within the concentration range obtained, and an average of MVC regression equations yielded y = 0.0636x+0.0222, with an R^2 of 0.9998, suggesting that the recommended approach obeys Beers rule and yielded results. The overlay spectra was represented in **Figure 3**, calibration graph in **Figure 4** and result was depicted in **Table 2**.

Limit of Detection and limit of Quantification

LOD and LOQ concentrations were determined by computing the linearity slope and confirmed through independent sample analyses, which proved that detection is realistically achievable at such ranges. The results were calculated for BEN the LOD value was 0.2023 and LOQ was 0.6132.

Precision

The percent RSD of interday and intraday accuracy was revealed to be less than 1percent, indicating that the method for estimating the drug is precise, and the results are shown in **Table 3** and spectra was represented in **Figure 5, 6, and 7** When compared to the previously mentioned approaches, the new methodology has the same accuracy.

Accuracy:

The recommended UV approach was used on a pure sample of BEN spanning the concentration range of 8–12 µg mL^{-1}. There was no notable discrepancy in precision and accuracy between the successes of the suggested comparison techniques. The spectra was represented in **Figure 8** and **Table 4**.

Assay of marketed formulations:

The proposed spectrophotometric technique examined the presence of BEN in tablet formulations. The UV absorption spectrums of the commercial tablet were attained in three replicates. Higher analytical recovery values represent that the pharmaceutical formulation extraction and filtering technique does not result in significant loss. Furthermore, the findings reported for each active substance are consistent with those claimed by manufacturers. The comparable methods

employing statistical analysis such as the variance ratio F-test value was 1.3122 and t-test value was 0.5211 this values were compared with that of table values of F-test and T- test which was 19 and 2.776. The **Table 5** illustrates the findings, and spectra in **Figure 9** which demonstrate a better outcome when compared to the previously reported approaches. Furthermore, the suggested approach might be extended to other pharmaceutical formulations incorporating BEN.

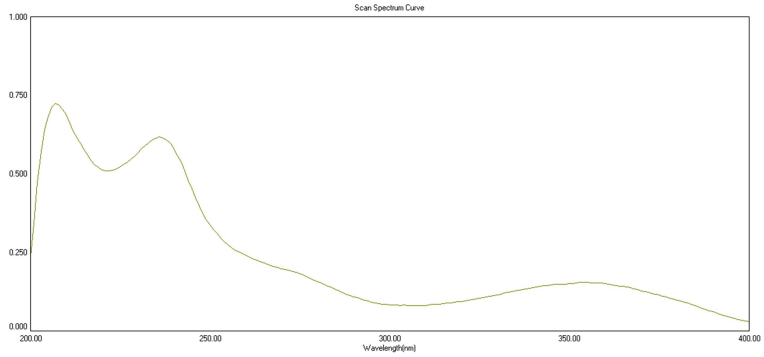


Figure 2: UV spectrum of standard BEN (10 µg mL^{-1}) by employing methanol as blank

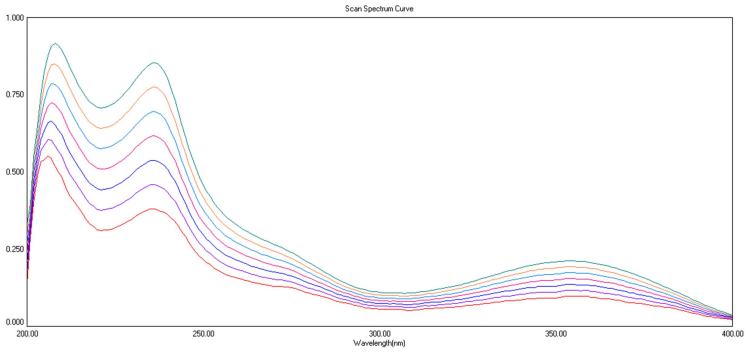
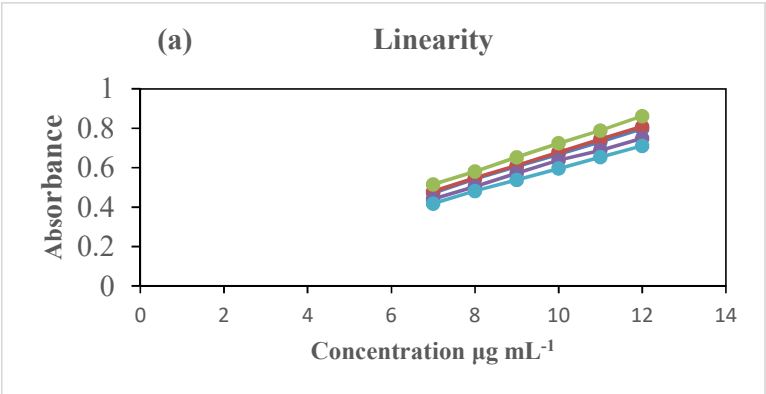


Figure 3: Linearity spectrum of BEN (7-13 µg mL^{-1}) by using methanol as a blank



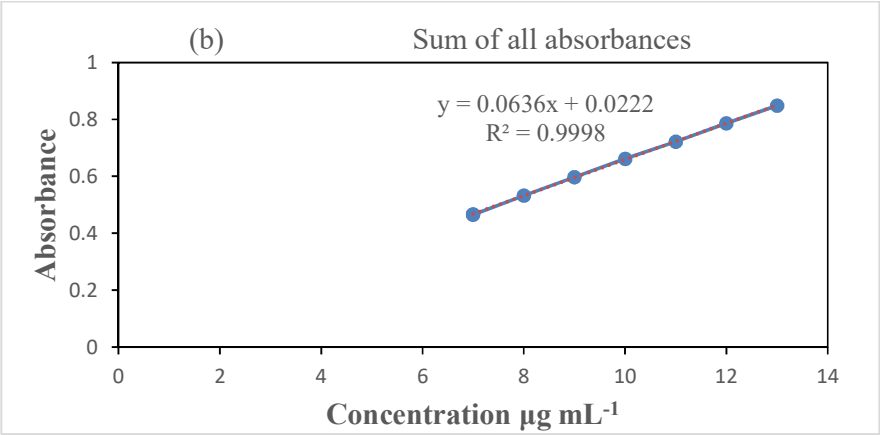


Figure 4: (a) Linearity plot at different wavelength and (b) Sum of all Absorbance

Table 2: Linearity data for developed method of BEN

Best - fit values	227 nm	232 nm	237 nm	242 nm	247 nm
Slope	0.0644	0.0651	0.0697	0.0610	0.0577
Y-Intercept when X=0	0.0240	0.0253	0.0255	0.0182	0.0176
r^2	0.9999	0.9997	0.9998	0.9997	0.9997

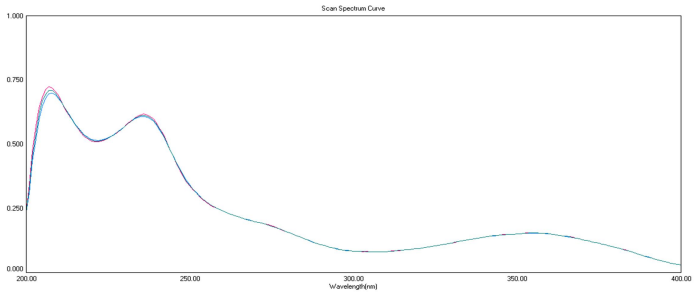


Figure 5: System precision overlay spectra of BEN (10 µg mL^{-1})

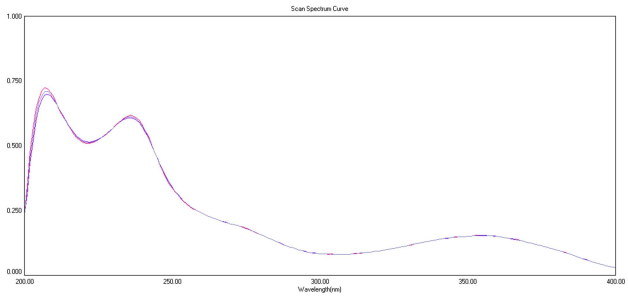


Figure 6: Interday precision overlay spectra of BEN (10 µg mL^{-1})

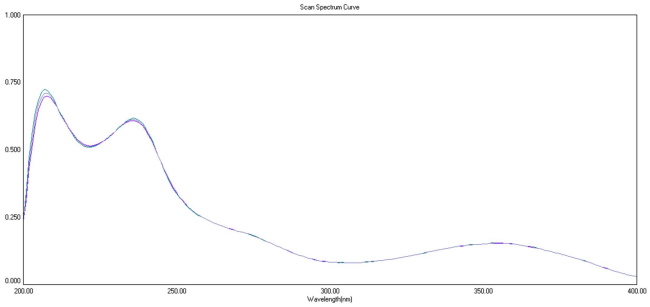


Figure 7: Intraday precision overlay spectra of BEN (10 µg mL^{-1})

Table 3: System precision, Inter - day and Intra - day Precision data for the proposed technique of BEN

	System precision	Interday and Intraday precision		
	Absorbance of standard for 10 µg mL^{-1}	% Recovery of sample equivalent to 10 µg mL^{-1}		
		Day 1	Day 2	Day 3
1	0.724	99.34	99.55	99.47
2	0.726	99.15	98.72	98.74
3	0.730	98.43	99.42	98.42
4	0.723	98.96	98.49	99.18
5	0.719	99.63	98.67	98.77
6	0.721	98.95	99.21	98.37
Mean	0.724	99.08	99.89	98.83
SD	0.004	0.41	0.44	0.43
%RSD	0.53	0.41	0.44	0.43

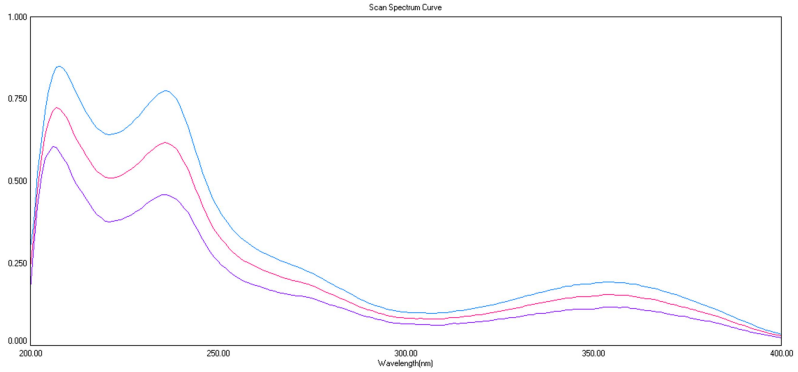


Figure 8: Overlay spectra of accuracy of BEN 80, 100 and 120 percent spiking

Table 4: Accuracy data for proposed method of BEN

Concentration levels (%)	Quantity present	Amount added (µg mL^{-1})	Amount recovered (µg mL^{-1})	Mean % recovery	SD
80	5	3	7.82	97.83	0.5051
100	5	5	9.90	99.03	0.6658
120	5	7	11.82	98.56	0.8007

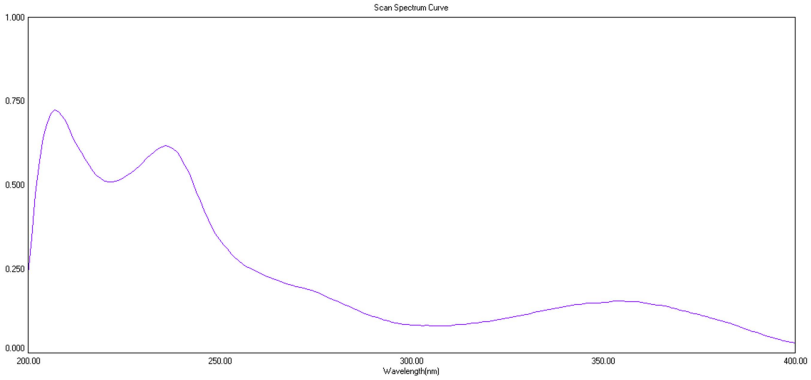


Figure 9: UV spectrum of Sample BEN (10µg mL^{-1}) by using methanol as blank

Table 5: Assay of BEN with two marketed formulations

Marketed formulation	Label claim (mg)	Mean ± SD (n = 3)	% RSD
Batch 1	4	98.83 ± 0.38	0.386
Batch 2	4	99.00 ± 0.50	0.505

CONCLUSION

The proposed MVC approach was a simple, novel, accurate, precise technique for estimating BEN in Pharmaceuticals. It is strongly advised to create a new approach for routine BEN analysis in Quality control department. All validation parameters were evaluated and confirmed to be within limits when compared to ICH guidelines.

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CONFLICT OF INTEREST

The authors report no conflicts of interest on the study**.**

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