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DEVELOPMENT AND VALIDATION OF ANALYTICAL METHOD FOR QUANTITATION OF BENDROFLUMETHAZIDE CONTENT USING UV-SPECTROSCOPIC TECHNIQUE

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ABSTRACT
A Simple, easy, specific, speedy, precise and accurate UV Spectrophotometric technique have been developed and Validated for quantitation of Bendroflumethazide formulation drug. Bendroflumethazide showed the absorption maxima in at 273.0 nm and was linear for a range of 5 µg/ml–25 µg/ml with correlation coefficient of 0.9993. The validation of the above proposed method was done by carrying out precision and accuracy studies. The analytical method showed good Intra precision (Repeatability) with relative standard deviation 0.896% and Inter precision with relative standard deviation is 0.612% which is less than 2. The percentage recovery at three different levels i.e. 50%, 100% and 150% was found to be 49.3%, 100.0% and 149.2% respectively. The proposed analytical method was validated for the parameter Specificity, Precision, Linearity and range, Ruggedness, Accuracy and recovery. Hence proposed analytical method for quantitation of Bendroflumethazide formulation drug by UV spectrophotometer in pharmaceutical can be applied for the routine quality control analysis.

KEYWORDS: Bendroflumethazide, UV Spectrophotometer, Validation.

INTRODUCTION
Bendroflumethazide is a thiazide which works by inhibiting sodium reabsorption from the distal convoluted tubules in the kidney. The sodium then takes water with it from blood decreasing the amount of fluid flowing through vessels which lowers blood pressure, that's why effective in reducing blood pressure. An anti-hypertensive drug being used for controlling hypertension, hyperkalemia and edema. Diuretics are being used effectively in the management of hypertension and typically prescribed due to their efficacy, low cost and low side effects profile.[1]

The IUPAC name is "3-Benzyl-1, 1-dioxo-6-(trifluoeomethyl)-3, 4-dihydro-2H-1, 2, 4-benzothiadiazine-7-sulfonamide". Bendroflumethazide having molecular formula '$C_{15}H_{14}F_3N_3O_4S_2$' and Molecular weight '421.42 g/mol'. It is official in United States pharmacopoeia,[2] and European/British pharmacopoeia,[3] with Assay method by Chromatographic technique and Potentiometric titration respectively. Literature survey revealed that very few analytical methods are available including Titrimetric,[4] and Chromatographic HPLC.[5-11]

In the present work, a simple, easy, accurate and sensitive method for quantification of Bendroflumethazide content in formulation drug substance pure form was introduced. The simple, easy, and speedy work were not reported for the quantification of Bendroflumethazide formulation drug. All those reported quantitation methods either took a long time for analysis or employ mobile phases with pH adjustment of Buffer solutions for sample preparation, which is monotonous and anomalous,[4-11] especially for routine quantification of quality control samples of assay test study. Hence it was felt necessary to build up a simple, easy, speedy, Low expensive (Cost-effective) and precise UV Spectrophotometric technique for the direct quantitation of Bendroflumethazide formulation drug.

The current research work deals with the development of UV Spectrophotometric technique and its validation as per International Conference on Harmonization (ICH) guideline.[16-18] The developed method was found to be simple, easy, specific, stable, rapid, accurate, precise, reliable, less expensive and time saving by UV Spectrophotometric technique for the quantitation of Bendroflumethazide content in drug substance.

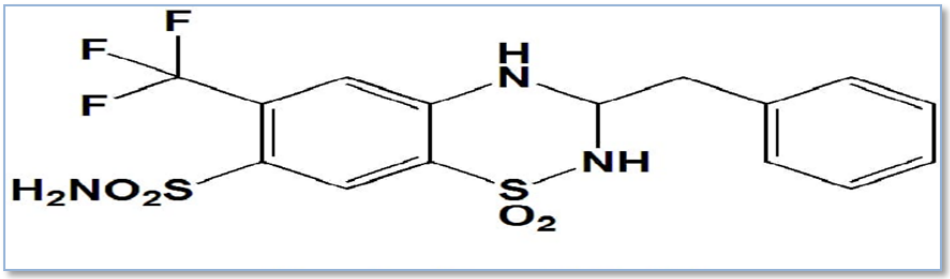

Fig. 1: Chemical structure of Bendroflumethazide.

MATERIALS AND METHODS

Instrumentation and Materials

U.V. visible double beam spectrophotometers of Make Elico, Model SL 210 with Spectra treat software having path length 1cm U.V. matched quartz cells were used. Bendroflumethazide Sample and Standard gifted from Omicron Pharmaceuticals, Surat Gujarat. All chemicals, solvents and reagents i.e. Acetic acid, Ethanol, Water and Acetonitrile used, were analytical grade and purchased from S.D. Fine Chem Ltd/Qualigens, Merck Ltd, India.

Method Development

Preparation of Diluent Solution

Transferred about 100 ml of water to the 1000 ml volumetric flask, then slowly added about 5.0 mL of Acetic acid with constant stirring, then add about 100 ml Ethanol with stirring and, mixed well, then with constant stirring slowly added Acetonitrile up to mark to make volume 1000 ml. used this solution as diluent.

Preparation of Standard Solution

Weighed accurately about 100 mg of Bendroflumethazide and transferred to 250 ml volumetric flask. Dissolved in 10 ml ethanol, then added diluent with intermittent shaking and made up the volume up to 250 ml, further transferred 5 ml of solution to 200 ml volumetric flask and made volume up to mark to get a concentration of 10µg/ml.

Selection of wavelength for analysis of Bendroflumethazide

The standard solution of Bendroflumethazide concentration 10µg/ml was scanned at 200 nm to 400 nm with diluents as the blank to detect maximum wavelength (Figure-2).

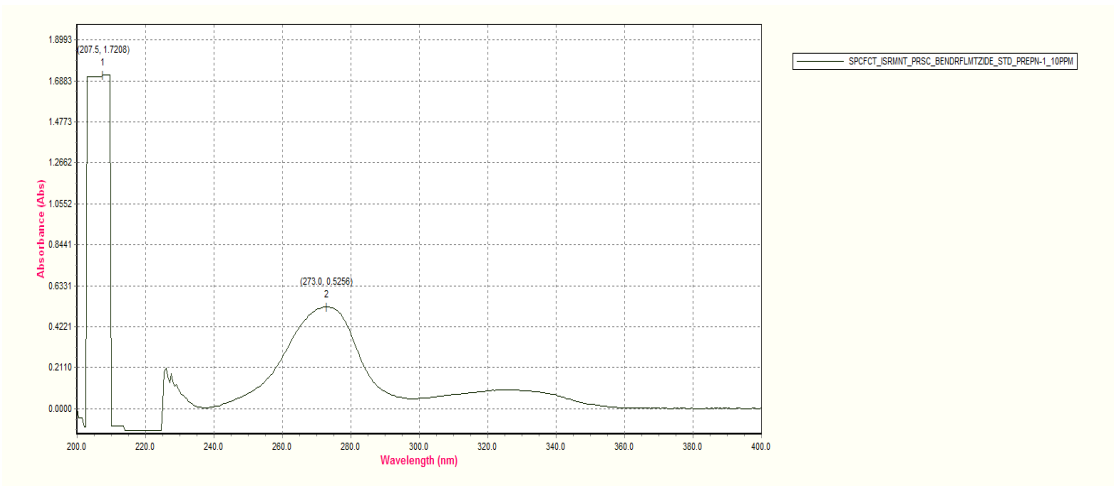

Fig. 2: Estimation of Maxima of Bendroflumethazide.

From the above (Figure-2) spectra of Bendroflumethazide wavelength maxima identified for quantification were 273.0 nm (λmax).

Validation of proposed Analytical Method

The developed quantitation method was validated as per International Conference on Harmonization ("ICH") guidelines referenced for Validation of analytical procedures.[16-18] Analysis of variance was used to ensure the validity and performance effectiveness of the proposed analytical quantitation methods.

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc. Specificity was done by scanning of diluent solution and Standard solution of Bendroflumethazide having concentrations 12 µg/ml in Spectrophotometric range from 200 nm to 400 nm to check specific absorption maxima at predefined wavelength i.e. 273.0 nm and solution stability study performed to evaluate the solution stability at different time interval up to 26 hrs.

Instrument Precision

Instrument precision was assessed to make sure the suitability of the developed proposed analytical method with reverence to capability of instrument constancy to provide the precise wavelength maxim when scanned the Standard solution of Bendroflumethazide having concentrations 10 µg/ml in the UV range from 200 nm to 400 nm. To check specific absorption maxima at predefined wavelength 273.0 nm with reproducible absorption detection. Six separated standard preparations were scanned / analyzed according to the proposed method of analysis. The % RSD due to Bendroflumethazide concentration for the six standards was found 0.593%. The % RSD due to Bendroflumethazide concentration for the instrument precision meets the requirements. Results are tabulated in the Table 1.

Table 1: Instrument Precision.

Sr. No.	Standard Number	Absorbance@273.0 nm	% RSD
1	Standard Preparation -1	0.5245	0.593% Limit < 2%
2	Standard Preparation -2	0.5195	
3	Standard Preparation -3	0.5265	
4	Standard Preparation -4	0.5225	
5	Standard Preparation -5	0.5251	
6	Standard Preparation -6	0.5189	
Average Absorbance		0.5228	

Linearity and Range

The linearity of an assay method is its capability to bring forth test results, which are directly comparative to the concentrations of drug in samples of the offered range. Linearity rationalizes the make use of single-point calibrations. The correlation coefficient of the Regression line for was found that 0.9993.

Five levels of five different concentrations Standard solution of Bendroflumethazide having concentrations 5 µg/ml, 10 µg/ml, 15 µg/ml, 20 µg/ml and 25 µg/ml, in the range relative to the working concentrations, were prepared and read as per proposed analytical method of analysis. A linear regression curve was constructed, the correlation coefficient (R2) and estimation assessment calculated. The correlation coefficient (R2) for Bendroflumethazide obtained is 0.9993. The plot is a straight line and the results are tabulated in the Table 2 and Curve is shown in the Figure 3.

Table 2: Linearity and Range.

Sr. No.	Standard Concentration (µg/ml)	Absorbance @ 273.0 nm	Correlation coefficient
1	5	0.2621	0.9993 Limit ≥0.999
2	10	0.5234	
3	15	0.7794	
4	20	1.0450	
5	25	1.2565	

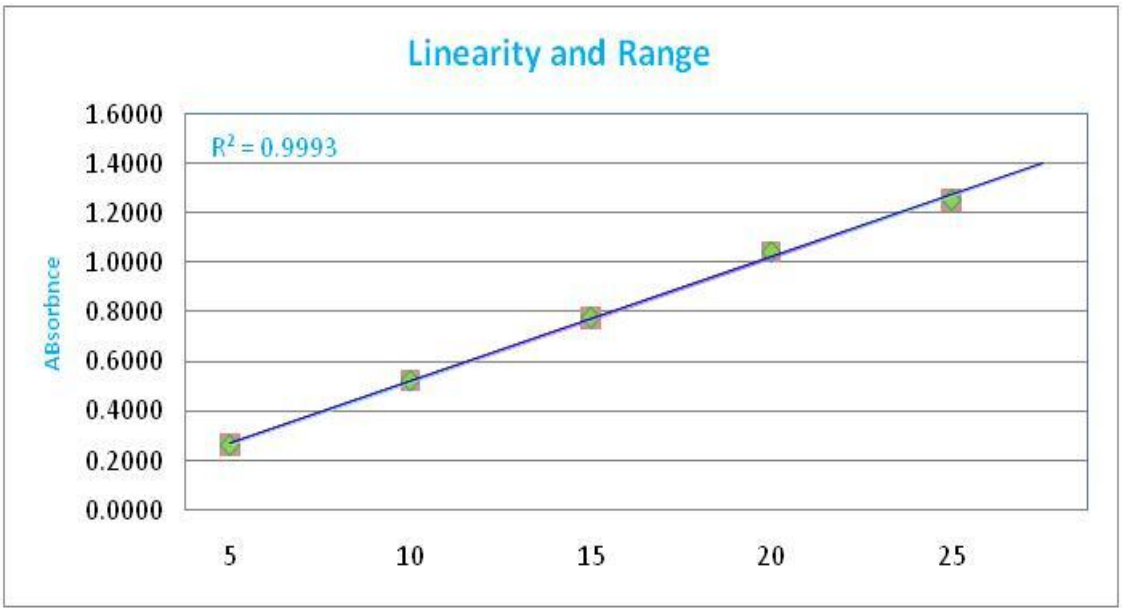

Fig. 3: Linearity and Range of Bendroflumethazide.

Analytical Method Precision

The precision of an analytical procedure expresses the degree of agreement among individual test results when the method is applied to multiple sampling of a homogenous sample.

Procedure for analysis of Sample: Weighed accurately about 100 mg of Bendroflumethazide and transferred to 250 ml volumetric flask. Dissolved in 10ml ethanol, then added diluent and made up the volume to 250 ml, further transferred 5 ml of solution to 200 ml volumetric flask. Made volume up to mark to get a concentration 10 µg/ml.

Intra Precision (Repeatability*)*

This parameter concludes the repeatability of Bendroflumethazide assay results under the same operating conditions over a short period of time. The % RSD due to Bendroflumethazide concentration for the six samples was found to be 0.896%. Six separated sample preparations were analyzed according to the proposed method of analysis. The % RSD due to Bendroflumethazide concentration for the assay meets the requirements. Results are tabulated in the Table 3.

Table 3: Intra Precision (Repeatability) Results.

Sr. No.	Sample Number	Bendroflumethazide % Assay content	% RSD of Six Assay content
1	Sample Preparation -1	99.1	0.896% Limit < 2%
2	Sample Preparation -2	101.0	
3	Sample Preparation -3	99.2	
4	Sample Preparation -4	100.9	
5	Sample Preparation -5	99.6	
6	Sample Preparation -6	99.1	
Average % Assay		99.8	

Inter Precision (Repeatability*)*

This parameter concludes the Intermediate repeatability of Bendroflumethazide assay results under the same operating conditions test performed on a different day, using different makes of reagents and solvents. The %RSD due to Bendroflumethazide concentration for the six samples was found to be 0.612%. Six separated sample preparations were analyzed according to the proposed method of analysis. The % RSD due to Bendroflumethazide concentration for the assay convenes the requirements. Results are tabulated in the Table 4.

Table 4: Inter Precision (Repeatability) Results.

Sr. No.	Sample Number	Bendroflumethazide % Assay Content	% RSD of Six Assay content
1	Sample Preparation -1	100.5	0.612% Limit<2%
2	Sample Preparation -2	99.8	
3	Sample Preparation -3	101.2	
4	Sample Preparation -4	99.7	
5	Sample Preparation -5	99.8	
6	Sample Preparation -6	100.6	
Average % Assay		100.3	

Ruggedness

Ruggedness of the proposed analytical method was assessed by performing the analysis on different days, different makes of reagents and solvents. The respective test assay results of Bendroflumethazide having concentration as 10µg/ml was well-quantitated. The result is expressed as shown in table-3, and 4. The developed analytical method for quantitation of Bendroflumethazide was found to be strong as revealed in table 5.

Table 5: Ruggedness.

Sr. No.	Precision	% RSD of Assay (Six Preparation)	Limit For Ruggedness
1	Intra Precision	0.896	NMT 2%
2	Inter Precision	0.612	
% RSD of Overall 12 Assay content		0.764	

Accuracy

This parameter determines the accuracy of the assay results below the same operating conditions test.

A Bendroflumethazide sample was analyzed for the accuracy with composed known quantity of samples of Bendroflumethazide at 50%, 100%, 150% concentration levels and quantitated as per the method stated in

proposed analytical method respectively. Three estimations were performed over each concentration levels respectively. Results are shown in Tables 6, 7, 8.
The %RSD due to recovery of Bendroflumethazide at 50%, 100%, 150% concentration levels was found to be 49.3%, 100.0% and 149.1% respectively.

Nine sample preparations were analyzed according to the proposed analytical method of analysis. The %RSD due to Bendroflumethazide concentration for the assay meets the requirement and accuracy of recovery is inside 98.0% to 102%. Results are tabulated in the Table 6, 7, 8.

Table 6: Accuracy and Recovery Results @ 50 % Concentration level.

Sr. No.	Accuracy@50% level	Recovery of Bendroflumethazide % Assay content	% Recovery 98.0%-102.0%	% RSD
1	Sample Preparation -1	49.2	98.5	0.486% Limit< 2%
2	Sample Preparation -2	49.1		
3	Sample Preparation -3	49.5		
Average % Assay		49.3		

Table 7: Accuracy and Recovery Results @ 100 % Concentration level.

Sr. No.	Accuracy@100% level	Recovery of Bendroflumethazide % Assay content	% Recovery 98.0%-102.0%	% RSD
1	Sample Preparation -1	98.6	100.0	1.409% Limit < 2%
2	Sample Preparation -2	100.2		
3	Sample Preparation -3	101.4		
Average % Assay		100.0		

Table 8: Accuracy and Recovery Results @ 150 % Concentration level.

Sr. No.	Accuracy@150% level	Recovery of Bendroflumethazide % Assay content	% Recovery 98.0%-102.0%	% RSD
1	Sample Preparation -1	147.6	99.5	1.128% Limit < 2%
2	Sample Preparation -2	150.9		
3	Sample Preparation -3	149.1		
Average % Assay		149.2		

Solution Stability

Solution stability of the Bendroflumethazide solution was performed up to 26 hrs with set of different time interval and found the bench top solution is stable showing cumulative % RSD of different time interval is 1.011 which is less than the 2. Hence the Bendroflumethazide bench top solution is found to be stable up to 24 hrs at room temperature and suggested 24 hrs solution stability.

RESULTS AND DISCUSSION

The proposed quantitation analytical method for Bendroflumethazide discussed in the present work provides a simple, easy, speedy, stable, accurate, precise, reliable, low expensive (Cost- effective), time *saving* and convenient method for the quantitation analysis of Bendroflumethazide using U.V. Spectrophotometry. λ max estimated for quantitation was 273.0 nm. In the developed analytical method, the linearity was observed 0.9993 in the concentration range of 5 µg/ml -25 µg/ml.

Method precision for the Bendroflumethazide at concentrations level 10µg/ml was found in the range of 99.1%-101.2%. Accuracy of the proposed analytical method was established by recovery studies and the results were expressed as percent recovery and were found in the Range of 98.1%-101.4%.Values of standard deviation and coefficient of variance was satisfactorily indicating the accuracy of the analytical methods. Intra-day and Inter-day precision studies were carried out by analyzing the sample of Bendroflumethazide different time interval on the same day and on different days respectively. Standard deviation and coefficient of variance for Intra-day and Inter-day precision studies was found to be less than 2 indicating precision of the proposed method.

based on the consequences of proposed assessment for analytical method development and analytical validation study test results, it was originate that, the proposed analytical method for quantitation of Bendroflumethazide using UV Spectrophotometry is Precise, Reproducible, Accurate, Simple, Easy, Speedy, Stable, Time saving and low expensive (Cost-effective). This analytical method can be employed for routine quality control quantitation of Bendroflumethazide formulation drug in pharmaceutical analysis.

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