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Stability-Indicating RP-HPLC Method Development for Simultaneous Determination and Estimation of Bendroflumethiazide and Nadolol in Raw and Tablet Formulation

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ABSTRACT

A sensitive, feasible RP-HPLC method has developed and validated for the analysis of Bendroflumethiazide and Nadolol in raw and tablet formulation. Successful separation of drugs products is developed on a C (18) column reversed-phase using and using mobile phase composition of Acetonitrile: Methanol: Water (30:20:50 v/v/v). The flow rate was adjusted to 1 mL/minute and the absorption maxima were observed at 250 nm utilizing Shimadzu SPD-20A Prominence UV-Vis detector. Good linearity was obtained in the range of 2-10 µg/ ml, 5-25 µg/ml, for Bendroflumethiazide, Nadolol respectively. The HPLC, tablet formulation assay shows percentage purity ranging from 100.12 to 99.94% for Bendroflumethiazide, 100.97 to 99.73%for Nadolol. The mean percentage purity is 100.01% and 100.07% for Bendroflumethiazide and Nadolol respectively. The chromatographic retention time of Bendroflumethiazide and Nadolol was found to be 2.2 and 5.7 minutes respectively. The tailing factor was 0.779 and 0.980 for Bendroflumethiazide and Nadolol respectively. The developed method validated according to the ICH guidelines. The method was found to be applicable for determination and validation of Bendroflumethiazide and Nadolol in combined tablet form.

Keywords: *Bendroflumethiazide (BDT), Nadolol (ND), HPLC and UV.*

1. INTRODUCTION

Multiple therapies are becoming extremely useful in pharmaceutical dosage forms [1]. As the result, numerous and various combinations of drugs are being introduced into the market. Bendroflumethiazide belongs to a group of medicines called thiazide diuretics [1,2]. A diuretic is a medicine, which increases the amount of urine that you pass out from your kidneys. Diuretics are a common treatment for high blood pressure (hypertension)[2]. The mechanism of action of Bendroflumethiazide results in an interference with the renal tubular mechanism of electrolyte reabsorption [2,3]. The mechanism of the antihypertensive effect of thiazides is unknown. Diuretics like Bendroflumethiazide work by interfering with the transport of salt and water across certain cells in your kidneys [3]. Bendroflumethiazide is designated chemically as 3-benzyl-3, 4-dihydro-6-(trifluoromethyl)-2*H*-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide [3,4]. Nadolol is a nonselective beta-adrenergic receptor blocking agent. Nadolol used in the treatment of high blood pressure and chest pain [5]. Additionally, it is often prescribed in the treatment of atrial fibrillation, migraine headaches, and complications of cirrhosis [5-9]. Nadolol is designated chemically as 1-(*tert*-butylamino)-3-{(5,6,7,8-tetrahydro-*cis*-6,7-dihydroxy-1-naphthyl)oxy} -2-propanol [4].

Literature review shows several methods has been developed and reported for BDT and ND estimation in biological fluids and there are some methods reported by [10-13], spectroscopy [14-17], HPTLC HPLC, UPLC and capillary electrophoresis [18-21]. Two methods were reported for estimation of this combination first is UV spectroscopy [22-24] and the other is HPTLC method [25,26]. Method development of HPLC estimation for this combination is new method will fulfil all requirements of validation according to ICH guidelines.

2. MATERIALS AND METHODS

The working standard of Bendroflumethiazide and Nadolol was purchased from Sigma, UK. The Marketed sample of Corzide strength Bendroflumethiazide 5mg, Nadolol 40mg manufactured and marketed by King Pharamcceuticals, Bristol. Acetonitrile and Methanol HPLC grade was purchased from Merck, Darmstadt, Germany.

3. INSTRUMENTATION

HPLC instrumentation and chromatographic condition:

HPLC system of Shimadzu LC-20 AT, with an auto sampler (SIL-20AC HT, Shimadzu, Japan) and SPD-10 detector (SPD- M20A, Japan) was used. For data recording the LC-solution software used. A Zorbax Eclipse Plus, Agilent Technology column (150mm x 4.6mm, 5µm) was used Pore size of the column 95Å. For degassing mobile phase, power sonic 505 ultrasonic baths (Hwashin technology, Seoul, Korea) was used. By using oven (CTO-20AC) column was maintained at a temperature of 37ºC and 1 ml/min was the flow rate. Analysis was carried over with 20µl injection volume using SPD-10 detection at 250nm. 15 minutes was set as run time.

Preparation of Mobile phase

Mix a mixture of Acetonitrile 300mL (30%), 200ml Methanol HPLC (20%) and water 500mL (50%), the resulting solutin was filtered with 0.45µ membrane filters and degassed in an ultrasonic bath for 10 minutes. The final ratio of mobile phase is Acetonitrile: Methanol: Water (30:20:50 v/v/v).

Preparation of Bendroflumethiazide (BDT), Nadolol (ND) Stock solution

Accurately 1 mg of BDT (RS) and ND (RS) was taken separately in 100 ml volumetric flasks and mixed with 25 ml of mobile phase solution and sonicated for 10 minutes and 75ml of mobile phase was added to the mark and cooled to room temperature. To get the concentration of 2-10 µg/ml of BDT and 5-25 µg/ml of ND varying quantities of standard stock solution was diluted with mobile phase. The two BDT and ND powder freely soluble in methanol and does not have any interference in the absorption peaks.

Preparation of sample solution

10 tablets of marketed sample of Corzide weighed accurately and powder equivalent of 5 mg of BDT and 40mg of ND transferred into 25ml volumetric flasks and dissolved with 25 ml mobile phase and the resulting solution was filtered through Whatman filter paper. Further dilutions were made based on the required concentrations.

Method validation

The present method was preceded to obtain new, sensitive and easy method for simultaneous estimation by HPLC from capsule formulation. According to the ICH guidelines recommendations the experimental was validated and USP-30 for parameters such as, system suitability, accuracy, precision, linearity and specificity.

System suitability

System suitability parameters like resolution, retention time, tailing factor and column theoretical plates was performed by injecting six replicates of standards and two replicates of sample preparation at a 100% level to cross verify the accuracy and precision of the chromatographic system.

Linearit

The chromatographic method linearity was established by plotting a graph to concentration vs peak area of BDT and ND standard and determining the correlation coefficients (R2) of the two compounds. For the linearity studies of 2-10 µg/ml of BDT, 5-25 µg/ml of ND respectively were injected into the HPLC system. For 45 minutes column was equilibrated with the mobile phase before injection of the solutions.

Accuracy

The recovery experiments show the accuracy of the method. The recovery was performed by adding BDT and ND working standards to placebo (excipients mixture) in the range of test concentration (60%, 80% and 100 %) and expressed as percent (%) recovered. Three samples were prepared for each recovery level. The recovery statistical results are within the acceptance range (S.D. < 2.0) value for BDT and ND.

Precision

In the proposed method **t**he intraday and interday precision was determined by analyzing the sample responses 4 repeats on the same day and 4 different days of a week for 4 different concentrations of standard solutions of BDT and ND. 4-10 µg/ml of BDT and 10-25 µg/ml of ND respectively and results are represented in terms of % RSD.

Specificity

The analytical method specificity is to measure the compound accurately in presence of interferences like excipients, degradants and matrix components. The HPLC of standard mixture and formulation shows specificity of method. The HPLC method is able to access the analyte in presence of excipients.

Statistical Parameters

The results of assay obtained are subjected to the following statistical analysis, standard deviation, relative standard deviation, coefficient of variation and standard error.

4. RESULTS AND DISCUSSION

The HPLC chromatogram of BDT and ND are presented in figure 1, 2. Wavelength 250nm was selected by scanning all standard drugs over a wide range of wavelength 200-400 nm. Linearity was evaluated by plotting peak area as a functional of analyte concentration for BDT and ND. The graphical representation was given in figure 3 and 4 data is presented in table 1.

The system suitability parameters like resolution, tailing factor, retention time and theoretical plates for the developed RP-HPLC method data are presented in table 2. The limit of detection and limit of quantification for BDT and ND are presented in table 3.

The specific range was determined from linearity studies, for both drugs and found to be 2-10 µg/ml of BDT and 5-25 µg/ml of ND. The data was analyzed by linear regression least square fit method. The slop, intercept, correlation coefficient and regression equation were also determined and the data presented in table 4.

The BDT and ND chromatographic retention time found to be 2.2 and 5.7 minutes respectively. This is well within the specific limits of 15 minutes. The high – resolution of BDT and ND indicates complete separation of the drugs. The tailing factor was found to be was 0.779 and 0.980 for BDT and ND respectively. The peaks are symmetrical and

theoretical plates for BDT and ND were 5674 and 9867 respectively, which shows the column efficient performance. The quantitative estimation of BDT and ND tablet formulation was carried out by RP-HPLC method using Acetonitrile: Methanol: Water (30:20:50 v/v/v) using C18 column as the stationary phase. Chromatogram of BDT and ND tablet formulation shown in the figure 5. Quantitative estimation (Assay) data of BDT and ND presented in table 5. Recovery studies of BDT and ND tablet formulation shown in table 6.

The tablet formulation shows percentage purity ranging from 100.12 to 99.94% for BDT and 100.97 to 99.73% for ND. The mean percentage purity is 100.01% and 100.07% for BDT and ND respectively. The percentage deviation was found to be -1.0 to +1.0% and -0.6 to +0.7, for BDT and ND respectively. The RSD values are below 2% indicating the method precision and the accuracy of the method shown by the low standard error values. This shows a good index of accuracy and reproducibility of the developed method. All the parameters including flow rate, detection wavelength sensitivity was maintained constant.

5. CONCLUSION

The proposed and developed RP-HPLC method is precise, accurate, and sensitive. The method is rapid, reproducible, and economical and does not have any interference due to the excipients in the pharmaceutical preparations.

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TABLES

Table 1: HPLC linearity data for Bendroflumethiazide and Nadolol

SNo	Concentration (µg/ml) of BDT	Peak area	Concentration (µg/ml) of ND	Peak area
1	2	4357	5	2574
2	4	8564	10	5123
3	6	12432	15	7644
4	8	16697	20	10124
5	10	21149	25	12657

Table 2: Results of system suitability parameters

SNo	Parameters	BDT	ND
1.	Theoretical plates	5674	9867
2.	Tailing factor	0.779	0.980
3.	Resolution factor	11.7	11.7
4.	Retention time	2.2	5.7
5.	Linear dynamic range	2-10 µg/ml	5-25 µg/ml

Table 3: Results of Limit of detection (LOD) & limit of quantification LOQ

Parameters	BDT	ND
LOD (µg/ml)	0.900	0.500
LOQ (µg/ml)	1.280	1.500

Table 4: Results of statistical parameters Statistical parameters

SNo	Parameters	BDT	ND
1.	Standard deviation (SD)	4.77	5.92
2.	Relative standard deviation (RSD)	0.00716	0.00652
3.	% RSD	0.516	0.421
4.	Standard error (SE)	0.02154	0.02375
5.	Correlation Coefficient (r)	0.9797	0.9252
6.	Slope (a)	23.791	17.423
7.	Intercept (b)	17.176	11.234
8.	Regression equation Y = (a X + b)	Y = 23.791X + 17.176	Y = 17.423X + 11.234

Table 5: Quantitative estimation (Assay) data of Bendroflumethiazide and Nadolol

S No	Drug	Label claim (mg/Tab)	Amount found (mg/Tab)	Mean amount found (mg/ Tab)	Percentage purity (% w/w)	Mean percentage purity (% w/w)	% Deviation
1.	**BDT**	5	5.07 4.98 4.94 4.98 5.12	5.01	100.07 99.98 99.94 99.97 100.12	100.01	+ 0.7 +0.2 +0.6 -0.2 +0.12
2.	**ND**	40	40.42 40.57 39.73 39.90 39.76	40.07	100.42 100.57 99.73 99.90 99.76	100.07	+0.58 +0.57 +0.23 +0.10 +0.24

Table 6: Recovery studies of Bendroflumethiazide and Nadolol tablet formulation

S No	Drug	Amount of Drug in preanalyzed Sample	Amount of Standard drug (RS) added (µg/ml)	Amount of drug recovered (µg/ml)	% Recovery	Mean recovery in Percentage
1.	**BDT**	6	4.00 6.00 8.00	10.73 12.54 14.32	100.73 100.54 100.32	100.53
2.	**ND**	15	15.00 20.00 25.00	30.32 34.98 40.74	100.92 99.98 100.74	100.54

FIGURES

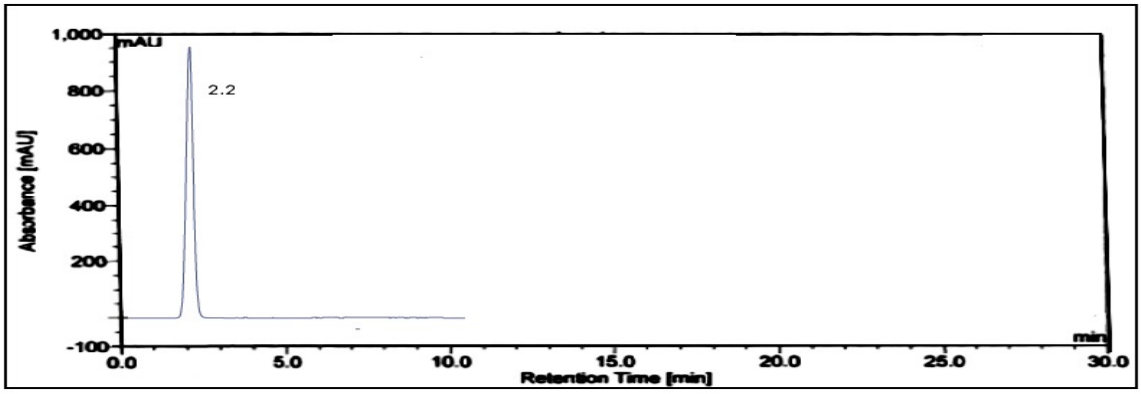

Figure 1: A Typical Chromatogram of Bendroflumethiazide Standard

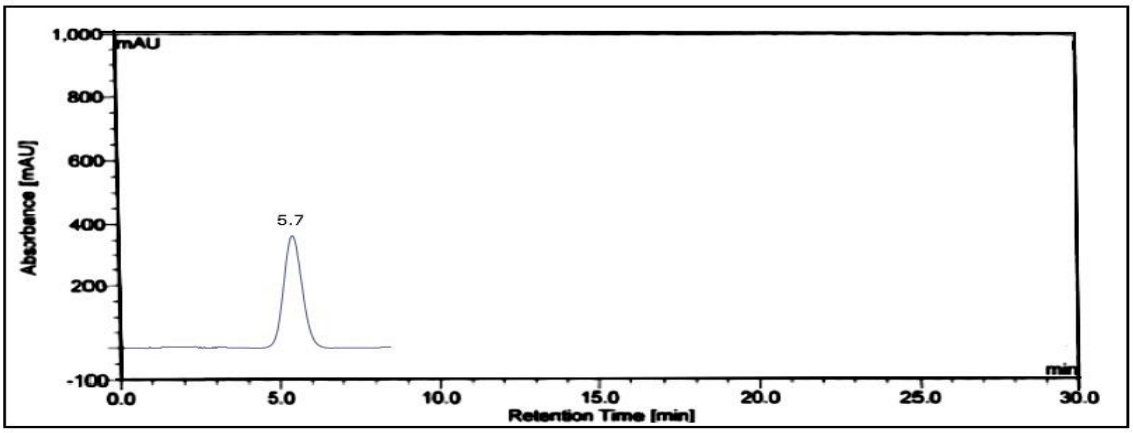

Figure 2: A Typical Chromatogram of Nadolol Standard

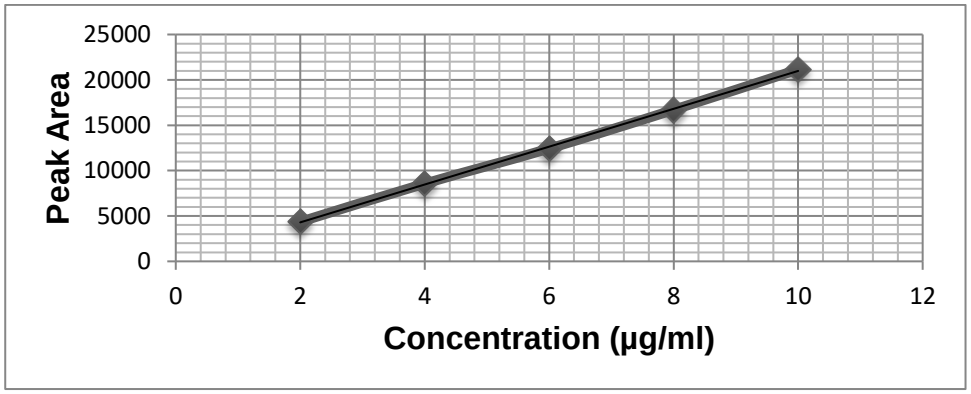

Figure 3: Calibration graph of Bendroflumethiazide 2-10µg/ml precision

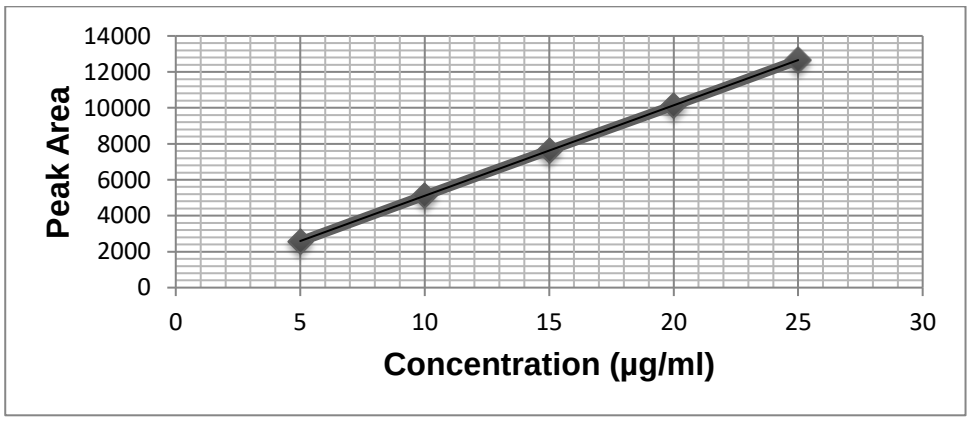

Figure 4: Calibration graph of Nadolol 5-25 µg/ml precision

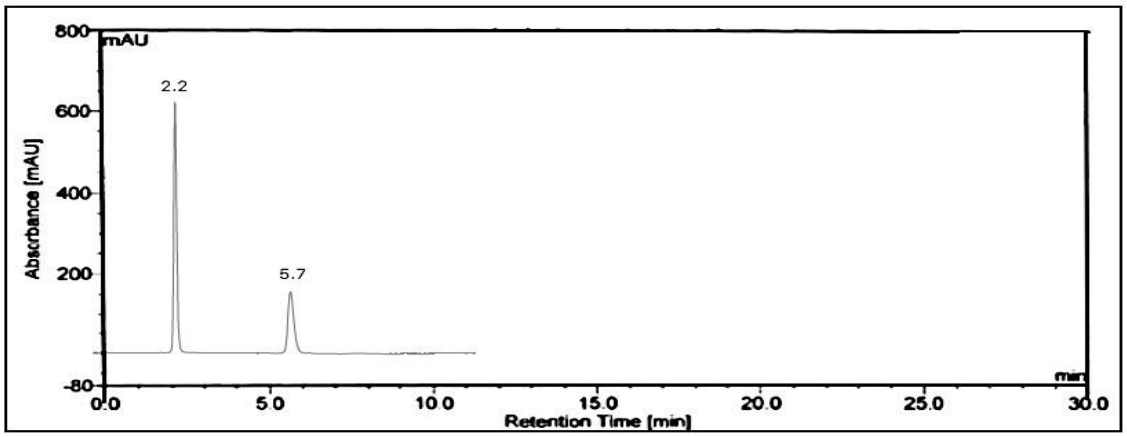

Figure 5: Chromatogram of Bendroflumethiazide and Nadolol in tablet formulation

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