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RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF BENZTHIAZIDE AND TRIAMTERENE IN THEIR COMBINED DOSAGE FORM

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ABSTRACT

The RP-HPLC method describes developed simple, rapid, specific, selective, accurate and precise method for the simultaneous estimation of Benzthiazide and Triamterene in tablet form. Benzthiazide falls under the category of Thiazide Diuretic and Antihypertensive Agents whereas Triamterene falls under the category of potassium-sparing diuretic and is used in the management of edema associated with congestive heart failure, hepatic cirrhosis with ascites, nephrotic syndrome, and idiopathic edema. RP-HPLC method for Benzthiazide and Triamterene were developed using mobile phase buffer (pH3.5): methanol (70:30v/v), flow rate 1mL/min, injection volume 20µL, temperature 25 ºC detection wavelength 222nm. Linearity range was found to be 5-15µg/ml for Benzthiazide and 10-30µg/ml for Triamterene. LOD and LOQ values were found to be 1.513 and 4.587 µg/ml for Benzthiazide and 0.695 and 2.108 µg/ml for Triamterene respectively. The assay result found to be 99.75 % and 99.97 % for Benzthiazide and triamterene respectively. The developed method was validated according to ICH guidelines.

KEYWORDS: Benzthiazide, Triamterene, RP-HPLC Method, simultaneous estimation.

INTRODUCTION

Benzthiazide is chemically known as 6-chloro-1,1-dioxo-3-(phenylmethylsulfanylmethyl)-4*H*-benzo[*e*][1,2,4] thiadiazine-7-sulfonamide and is used in the treatment of high blood pressure and edema.[1] Chemical structure of benzthiazide is shown in Fig. 1.Triamterene is chemically known as 6-phenylpteridine-2,4,7-triamine and is used for the management of

edema associated with congestive heart failure, hepatic cirrhosis with ascites, nephrotic syndrome, and idiopathic edema.[2] Chemical structure of triamterene is shown in Fig. 2. Literature survey reveals that spectrophotometric method[3,4,5,6,7,8], HPTLC[9] methods was reported for estimation of Benzthiazide alone and with other combination in Bulk and Dosage form. Literature survey reveals that by spectrophotometric[11,12,13], HPLC[14,15,16] methods was performed for estimation of Triamterene alone and with other combination in Bulk, and Dosage form. As per our best effort of literature review, it reveals that no method has been reported for simultaneous estimation of Benzthiazide and Triamterene by UV-VIS spectrophotometry and RP-HPLC in combined dosage form. Hence, the present study was aimed to develop and validate accurate and precise method for simultaneous estimation of Benzthiazide and Triamterene.

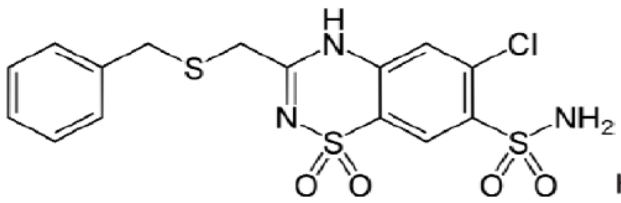

Fig :1. Benzthiazide

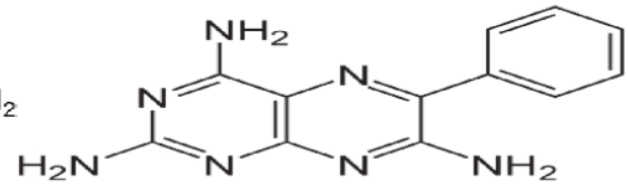

Fig:2 triamterene

MATERIALS AND METHODS

The present work was carried out HPLC (LC -20AT SHIMADZU Cosmosil packed MS II column 5 C18). The detector used is typically a photomultiplier tube, a photodiode, a photodiode array or a charge coupled device. Ditide Tablet (Benzthiazide 25mg, Triamterene 50mg) was obtained from GlaxoSmithKline.

REAGENTS AND CHEMICALS

Benzthiazide and Triamterene were obtained as API gift sample from GlaxoSmithKline. Solvents and reagents were used as HPLC grade.

EXPERIMENTAL CONDITIONS

Preparation of Standard Stock Solution of Benzthiazide

Accurately weighed 10 mg of Benzthiazide was taken in 10ml volumetric flask and diluted with methanol up to the mark. Benzthiazide working standard stock solution (100 µg/ml): Prepared by transferring 1 ml from stock solution, and diluted up to the mark with methanol.

Preparation of Standard Stock Solution of Triamterene

Accurately weighed 10mg of Triamterene was taken in 10ml volumetric flask and diluted with methanol up to the mark. Triamterene working standard stock solution (100µg/ml): prepared by transferring 1ml from stock solution and diluted up to the mark with methanol.

Preparation of Sample Solution

Twenty tablets were weighed and powdered. The tablet powder equivalent to 10 mg of Benzthiazide was transferred to 100ml conical flask, dissolved and sonicated for 20 min and diluted up to mark with methanol. The solution was filtered through 0.45 µ filter paper and first few ml of filtrate were discarded. From this solution take 1 ml and diluted up to 10 ml (10µg/ml).

PROCEDURE

Chromatographic Condition

Chromatographic separation was achieved by using mobile phase consisting of Methanol: Phosphate Buffer (30:70), pH 3.5 adjusted with o-Phospharic acid (70:30v/v) at a constant flow rate of 1.0 ml/min. A RP-C18 column was used as the separation phase. Detection was carried out using a photomultiplier tube, photo diode array detector at 222 nm.

Linearity

To establish the linearity a series of dilutions ranging from 5-15 µg/ml for Benzthiazide and 10-30 µg /ml for Triamterene were prepared separately and the peak areas were plotted against the concentration.

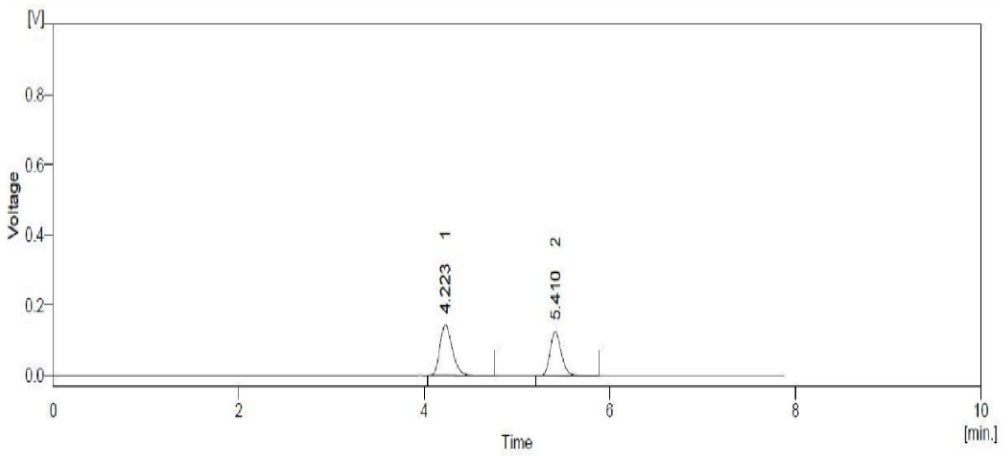

Fig:3 Chromatogram of Benzthiazide and Triamterene (Methanol: Buffer 30:70 pH 3.5)

Validation of RP-HPLC Method

1.Accuracy

For both drugs recovery studies were carried out by applying the method to drug sample to which known amount of Benzthiazide and Triamterene corresponding to 80%, 100% and 120% were prepared and to measure the area and put into the linear calibration curve equation on the basis of these to calculate concentration of mple and %Recovery.

2.Precision

The precision of method was verified by repeatability, Inter-day and intra-day precision. Precision was expressed in terms of %Relative standard deviation (%RSD).

Intraday Precision

Three different concentration of Benzthiazide (5, 10 and 15µg/ml) and Triamterene (10, 20 and 30µg/ml) were analysed on the same day three times at different time interval and % R.S.D was calculated.

Interday Precision

Three different concentration of Benzthiazide (5, 10 and 15µg/ml) and Triamterene (10, 20 and 30µg/ml) were analysed on three different days and % R.S.D was calculated.

3. LOD and LOQ

Calibration curve was repeated for 3 times and the standard deviation (SD) of the intercepts was calculated. LOD=3.3 * SD/slope of calibration curve and LOQ=10 * SD/slope of calibration curve where, SD = Standard deviation of intercepts.

4.Robustness

To evaluate robustness of the developed method, few parameters were deliberately varied. These parameters included variation in flow rate, organic phase ratio, detection wavelength and pH of mobile phase. The average value of % RSD was calculated.

System Suitability Test

System suitability is the checking of a system to ensure system performance before or during the analysis of unknowns. Parameters such as plate count, tailing factors, resolution and reproducibility in retention time for six repetitions of 100% concentration of Benzthiazide and Triamterene were determined.

RESULTS AND DISCUSSIONS

Pure drug of Benzthiazide and triamterene along were injected into HPLC system, and run in different solvent systems such as Methanol: Water (50:50 pH3.5) 1ml/min in which broad peak was observed for Benzthiazide and Water(pH3.5): Methanol: Triethylamine(60:40:01) in which peak shape for Triamterene was asymmetrical.

Finally the system containing Methanol: Phosphate Buffer (30:70) pH3.0 adjusted with o-Phospharic acid, 1ml/min was found to be satisfactory and both drugs gave sharp & symmetrical peaks.

Table: 1 Parameters of System Suitability.

Sr. No.	Parameters	Benzthiazide	Triamterene
1	Theoretical Plates	5164	7059
2	Tailing factors	1.473	1.4
3	% R.S.D Of Peak Area	0.49	0.65

Table: 2. Accuracy data Benzthiazide and Triamterene.

Initial Concentration (mg)		Level Of Recovery	Quantity Of Std Added (mg)	Total Amount (mg)
BNZ	TRI		BNZ TRI	BNZ TRI
5	10	80%	4 8	9 18
5	10	100%	5 10	10 20
5	10	120%	6 12	11 22

Precision

Precision for method were measured in terms of % RSD for Benzthiazide and Triamterene and was found to be 0.4908 and 0.649 respectively.

Intraday precision

The % RSD of Benzthiazide and Triamterene for intraday precision was found to be 1.34 and less than 1.13 respectively.

Interday Precision

The % R.S.D of Benzthiazide and Triamterene for interday precision was found to be 0.69 and less than 1.04 respectively.

Robustness

Table 3: Area at Different Flow Rate, Mobile Phase Ratio, pH for Benzthiazide.

Benzthiazide 10	Area					
	1	2	3	Mean	SD	%RSD
Flow Rate 1.2	1264.748	1287.610	1295.513	1282.624	15.977	1.25
Flow Rate 0.8	1343.654	1366.852	1372.079	1360.859	15.134	1.11
Mobile Phase	1259.805	1285.029	1294.216	1279.683	17.8174	1.39
	1331.36	1349.563	1357.518	1346.147	13.4094	0.99
pH 3.7	1239.47	1261.204	1266.54	1255.738	14.3389	1.14
pH 3.3	1333.069	1353.633	1358.881	1348.528	13.6423	1.01

Table 4: Area at Different Flow Rate, Mobile Phase Ratio, pH for Triamterene.

Triamterene 10	AREA					
	1	2	3	MEAN	SD	%RSD
Flow Rate 1.2	1465.73	1447.77	1472.11	1461.873	12.6230	0.86
Flow Rate 0.8	1559.51	1547.57	1571.50	1559.527	11.9650	0.76
Mobile Phase	1462.792	1456.862	1482.323	1467.326	13.3222	0.90
	1542.815	1520.815	1554.827	1539.509	17.2102	1.11
pH 3.7	1438.38	1423.41	1450.61	1437.469	13.6238	0.94
pH 3.3	1541.268	1525.473	1550.477	1539.073	12.6457	0.82

Table: 5 Summary of Validation Parameter for RP-HPLC method.

Sr. No.	Validation Parameter	Benzthiazide	Triamterene
1.	**Linearity**		
	Regression Equation	y = 128.05x + 5.9254	y = 75.643x - 16.686
	Regression Coefficient	0.99	0.995
2.	**Range**	5-15	10-30
3.	**Accuracy(% Recovery)**	99.5	99.6
4.	**Precision(Range Of %RSD)**		
	Repeatability	0.4908	0.6490
	Intraday	1.29-1.34	1.133-0.80
	Interday	0.58-0.47	0.487-0.05
5.	**LOD (µg /ml)**	1.51	0.69
6.	**LOQ (µg /ml)**	4.58	2.10
7.	**Robustness (Range Of %RSD)**		
	Different Flow Rate	1.11-1.24	0.76-0.86
	Different Mobile Phase Ratio	0.99-1.39	1.11-0.90
	Different pH	1.01-1.14	0.82-0.94

Assay: The content of Benzthiazide and Triamterene found in the tablets by the proposed method is shown in Table 6.

Table: 6 Assay.

Drug	Label Claim (mg)	Amount Estimate(mg)	Assay (%estimated)
Benzthiazide	25	24.93	99.75
Triamterene	50	49.98	99.97

CONCLUSION

The proposed RP-HPLC method was accurate, precise and reliable for simultaneous estimation of Benzthiazide and Triamterene in their combined tablet dosage form. The method was validated for simultaneous estimation of Benzthiazide and triamterene using linearity, range, accuracy, precision and robustness. The %RSD for all parameter was found to be less than 2, which indicates validation of method. The development method can be used for the routine quantitative analysis for simultaneous estimation of Benzthiazide and Triamterene in their combined tablet dosage form.

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