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Review Article

Development and Validation of RP-HPLC Method for Simultaneous Estimation of Triamterene and Benzthiazide in Tablets

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ABSTRACT
A specific, accurate, precise and reproducible RP-HPLC method has been developed and subsequently validated for the simultaneous determination of Triamterene and Benzthiazide in tablets. The proposed HPLC method utilizes BDS hypersil (Thermo scientific) C18 column (250 mm × 4.6 mm id, 5 µm particle size), and mobile phase consisting of phosphate buffer: methanol (70:30) and pH adjusted to 3.5 with sodium hydroxide and flow rate of 1.0 ml/min. Quantitation was achieved with UV detection at 245 nm based on peak area with linear calibration curves at concentration ranges 10-30 µg/ml for Triamterene and 5-15 µg/ml for Benzthiazide. The retention time of Triamterene and Benzthiazide were found to be 5.960 min and 3.493 min respectively. The method was validated in terms of accuracy, precision, linearity, limits of detection, limits of quantitation and robustness. This method has been successively applied to tablet formulation and no interference from the formulation excipients was found.

Keywords: Triamterene, Benzthiazide, RP-HPLC

INTRODUCTION
Triamterene is a potassium-sparing diuretic (water pill) that prevents human body from absorbing excessive salt and keeps potassium levels from getting too low. Triamterene is used to treat fluid retention (edema) in individuals with congestive heart failure, cirrhosis of the liver, or a kidney condition called nephrotic syndrome. Triamterene is also used to treat edema caused by having excessive aldosterone in your body[1].

Triamterene chemically is 2,4,7 – triamino, 6-phenylpteridine with a molecular formula $C_{12}H_{11}N_7$ and molecular weight of 253.27 gm/mol[2]. It is an official drug in Indian Pharmacopeia[3].

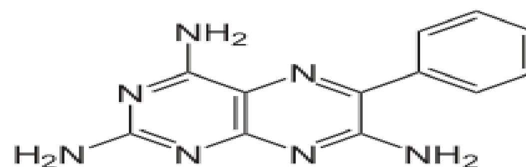

Figure.1.Chemical structure of Triamterene[2]

Triamterene shows hyperkalemia as its major side effect[4]. So, in order to neutralize this effect it is used in combination with a thiazide diuretic which counteracts the side effect of Triamterene by its hypolkalemic effect[5]. Benzthiazide belong to thiazide class of diuretics, extensively used in treatment of hypertension and edema associated with mild to moderate congestive heart failure. It

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increases the rate of urine excretion by the kidneys via decreased tubular reabsorption of sodium and chloride ions and by increasing osmotic transport of water to the renal tubules, which in turn decreases cardiac output and blood pressure[6]. On long-lasting thiazide treatment plasma volume and ECF return to normal, but their hypotensive effect continues. This is possibly due to reduced sensitivity of the vascular bed to the circulating catecholamine and angiotensin. Benzthiazide chemically is 6-chloro-3- [(phenylmethyl) thio]methyl]- 2H-1,2,4- benzthiadiazine-7-sulfonamide-1,1 dioxide with a molecular formula $C_{15}H_{14}ClN_3O_4S_3$ and molecular weight of 431.94 gm/mol[7]. Fig.2

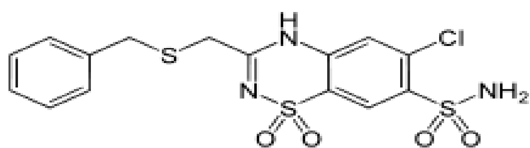

Figure.2.Chemical structure of Benzthiazide[2]

Combination of Triamterene and Benzthiazide are used in treatment of edema and hypertension. In the literature survey it was found that Triamterene and Benzthiazide were estimated individually or in combination with other drugs by UV, HPLC, Spectrofluori methods[7-21] and both together estimated by UV spectroscopic method[22]. But no method has been found for simultaneous estimation of Triamterene and Benzthiazide by chromatographic method. In the view of the need in the industry for routine analysis of Triamterene and Benzthiazide in formulation, attempts are being made to develop simple and accurate RP-HPLC method for simultaneous estimation of Triamterene and Benzthiazide and extend it for their determination in formulation.

MATERIAL AND METHOD

Equipment

RP–HPLC instrument equipped with SPD-20 AT UV-Visible detector, (LC-20AT, Shimadzu), Rheodyne injector (20 µl Capacity), BDS hypersil (Thermo scientific) C_{18} column (250 mm × 4.6 mm, 5 µ particle size) and Spinchrom software was used.

Chemicals and reagents

Reference standard of TRM and BNZ were obtained from Remedix pharma, Bangalore. Methanol used was of HPLC grade and phosphate buffer of (pH 3.5) and all other reagent were of AR grade.

Preparation of standard and test solutions

Preparation of mobile phase

Mobile phase was prepared by mixing of 700 ml of methanol with 300 ml of phosphate buffer, whose pH was previously adjusted to pH 3.5 by addition of sodium hydroxide. The mobile phase prepared was degassed by ultrasonication for 20 min, so as to avoid the disturbances caused by dissolved gases. The degassed mobile phase was filtered through 0.45 µ filters to avoid the column clogging due to smaller particles.

Preparation of standard stock solutions

An accurately weighed quantity of TRM (20 mg) and BNZ (10 mg) were transferred to a 100 ml volumetric flask and dissolved and diluted to the mark with mobile phase to obtain standard solution having concentration of TRM (200 µg/ml) and BNZ (100 µg/ml)

Preparation of solutions for calibration curve

The calibration curves were plotted over the concentration range 10-30 µg/ml for TRM and 5-15 µg/ml for BNZ. From the stock solution 200 µg/ml of TRM, the quantity of (0.5 ml, 0.75 ml, 1.0 ml, 1.25 ml, 1.5 ml), and from the stock solution 100 µg/ml of BNZ, the quantity of (0.5 ml, 0.75 ml, 1.0 ml, 1.25 ml, 1.5 ml) were transferred to a series of 10 ml of volumetric flasks and diluted to the mark with mobile phase. Aliquots (20 µl) of each solution were injected under the operating chromatographic conditions described above.

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Preparation of sample solution

Take quantity equivalent to 10 mg TRM and 5 mg BNZ was transferred to 100 ml volumetric flask in mobile phase. The solution was filtered through whatman filter paper No. 41 and the volume was adjusted up to the mark with mobile phase. From the above solution 1 ml of solution is taken in 10 ml volumetric flask and suitably diluted with mobile phase to get a final concentration of 10 µg/ml of TRM and 5 µg/ml of BNZ.

METHOD VALIDATION[23-24]

The developed method was validated according to ICH guidelines. To check the system performance, the system suitability parameters were measured. System precision was determined on six replicate injections of standard preparations. Number of theoretical plates and asymmetry were measured.

Linearity

Linearity was performed with five concentrations ranging from 10-30 µg/ml and 5-15 µg/ml for TRM and BNZ respectively. The peak areas versus concentration of drug were plotted and a linear least-square regression analysis was conducted to determine the slope, intercept and correlation coefficient (r) to demonstrate the linearity of the method.

The limit of detection (LOD) and limit of quantitation (LOQ)

LOD and LOQ of TRM and BNZ were calculated using the following equations as per International Conference on Harmonization (ICH) guidelines.

$LOD = 3.3 \times \sigma/S$

$LOQ = 10 \times \sigma/S$

Where σ = the standard deviation of the response

S = Slope of calibration curve.

Precision

The intraday and interday precision of the proposed method was determined by analyzing the corresponding responses 3 times on the same day and on 3 different days 3 different concentrations of sample solutions of TRM (10 µg/ml, 20 µg/ml and 30 µg/ml) and BNZ (5 µg/ml, 10 µg/ml and 15 µg/ml). Percentage relative standard deviation (RSD) was calculated

Accuracy

Accuracy was performed by adding known amounts of TRM and BNZ to the pre-analysed tablet formulation and then comparing the added concentration with the found concentration. Three levels of solutions were made which correspond to 80, 100 and 120% of the nominal analytical concentration (10 µg/ml for TRM and 5 µg/ml for BNZ). Each level was prepared in triplicate. The percentage recoveries of TRM and BNZ at each level were determined. The mean recoveries and the relative standard deviation were then calculated.

Robustness

The robustness of the method was evaluated by assaying the test solutions after slight but deliberate changes in the analytical conditions i.e. flow rate (± 0.2 ml/ min), proportion of buffer and methanol (72:28 and 68:32 v/v), and pH of buffer (± 0.2).

RESULT AND DISCUSSION

System Suitability

The chromatogram of TRM and BNZ show retention time 5.960 min and 3.493 min respectively. Mobile phase used for separation was phosphate buffer (pH 3.5) : methanol (70:30). pH of buffer adjusted with sodium hydroxide. Standard chromatogram was given in Figure 3. System suitability parameters were shown in table 1.

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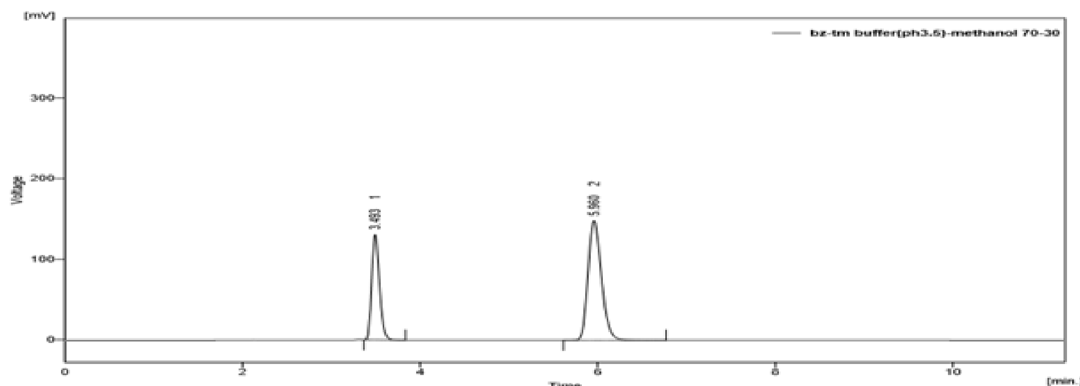

Figure 3: Standard Chromatogram of Triamterene and Benzthiazide

Table 1: System suitability parameters of chromatogram for TRM & BNZ

Parameters	**TRM ± %RSD (n = 6)**	**BNZ ± %RSD (n = 6)**	**Specification**
Retention time (min)	5.960 ± 0.1855	3.493 ± 0.1587	-
Tailing factor	1.395 ± 1.6587	1.409 ± 1.3557	Not more than2
Theoretical plates	7084± 0.3513	7235 ± 0.2836	>2000
Resolution	11.024 ± 0.6357		>2

Method validation

The calibration curves were plotted over the concentration range 10-30 µg/ml for TRM and 5-15 µg/ml for BNZ are shown in figure 4 and figure 5 respectively. The data for linearity are shown in table 2. Intraday and interday precision for TRM and BNZ are shown in table 3. Statistical analysis of recovery data is shown in table 4. Results of robustness study of TRM and BNZ are recorded in table 5. It suggests that the developed method is robust. Summary of validation parameter is shown in table 6.

Table 2: Linearity Data for TRM and BNZ

TRM		**BNZ**	
Conc. µg/ml	**Area* ± SD**	**Conc. µg/ml**	**Area* ± SD**
10	1159.443±3.73	5	825.345±2.01
15	1712.512± 4.37	7.5	1218.817± 2.45
20	2341.769±11.15	10	1666.832±2.31
25	2886.713± 8.65	12.5	2052.491± 2.89
30	3512.547± 5.54	15	2497.857±2.82

*Average of three determination

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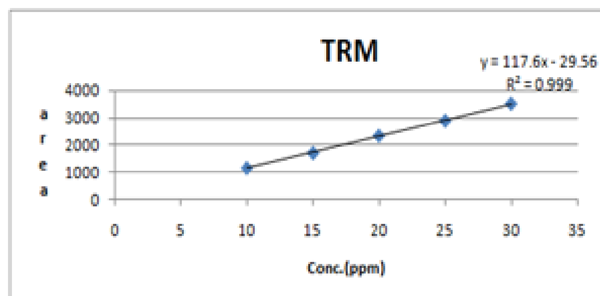

Figure 4: Calibration curve of TRM

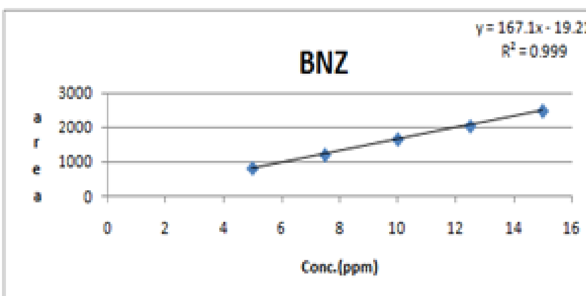

Figure 5: Calibration curve of BNZ

Table 3: Intraday and Interday precision data for estimation of TRM and BNZ

TRM			BNZ		
	Intraday	Interday		Intraday	Interday
Conc. µg/ml	Peak Area* ± SD	Peak Area* ± SD	Conc. µg/ml	Peak Area* ± SD	Peak Area* ±SD
10	1149.995 ± 7.89	1149.737 ± 4.786	5	820.061 ±4.306	817.993 ± 5.62
20	2320.579 ± 21.97	2321.195 ± 15.45	10	1655.922 ± 9.148	1653.849 ± 6.67
30	3473.003 ± 29.08	3471.336 ±34.94	15	2475.065 ± 15.70	2472.599 ± 22.22

*average of three determination

Table 4: Recovery

Level of %recovery	Amount of pure drug added (µg/ml)		HPLC Method % recovery	
	TRM	BNZ	TRM	BNZ
80	8	4	98.55	100.04
100	10	5	99.86	99.45
120	12	6	100.12	100.74
Mean % recovery			99.51	100.07
Standard Deviation			0.8414	0.6457
Relative Standard Deviation			0.8455	0.6452

Table 5: Robustness

Condition		Peak Area	
		TRM	BNZ
Flow Rate	1.2 ml/ min	2286.389	1624.919
	0.8 ml/min	2419.734	1725.205
Mobile phase ratio	A B 72:28	2271.842	1623.087
	A B 68:32	2396.663	1703.603
pH	3.7	2235.02	1588.323
	3.3	2397.628	1707.533
Average		2334.546	1662.112
S.D		79.05867	56.77426

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Assay of the tablet formulation

The proposed validated method was successfully applied to determine TRM and BNZ in their tablet formulation shown in figure 6. The result obtained for TRM and BNZ were comparable with the corresponding labelled amounts shown in table 6. No interference of the excipients with the peak of interest appeared.

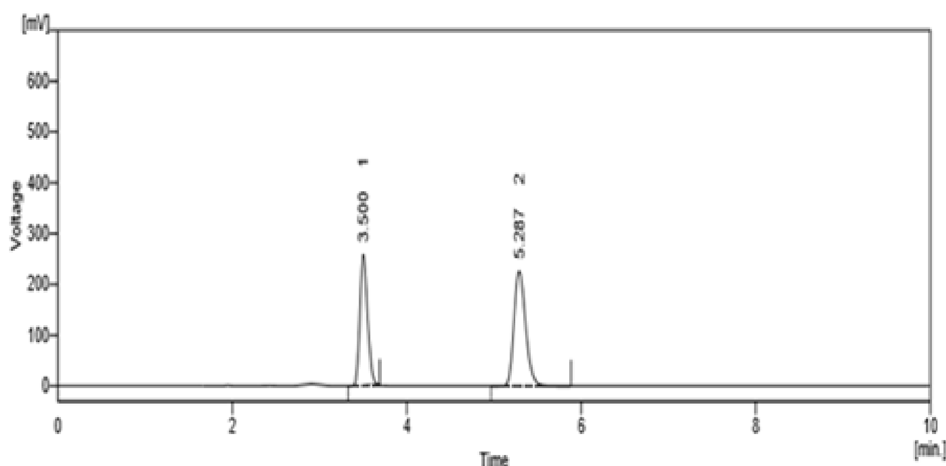

Figure 6: Chromatogram of sample solution of TRM and BNZ at 245 nm

Table 6: Assay of formulation

Tablet	Mg per Tablet		Assay (content in mg)*		(% of label claim*) ± % RSD	
DITIDE	TRM	50	TRM	49.35	TRM	98.7± 1.156
	BNZ	25	BNZ	25.27	BNZ	101.08±1.01

*average of six determination

Table 7: Summary of Validation Parameters

PARAMETERS	RP-HPLC method	
	TRM	BNZ
Concentration range (µg/ml)	10-30 µg/ml	5-15 µg/ml
Regression equation	y = 117.6x - 29.56	y = 167.1x - 19.21
Correlation coefficient	0.9994	0.9995
LOD(µg/ml)	0.2890	0.2075
LOQ(µg/ml)	0.8758	0.6289
Repeatability (% RSD, n= 6)	0.714994	0.934420
Precision (%RSD) Interday (*n* = 9) Intraday (*n* = 3)	0.6960 0.8213	0.6634 0.5707
Accuracy (% recovery), (n=3)	99.51 ± 0.8455	100.07 ± 0.6452
% Assay	98.7 ± 1.156	101.08 ± 1.01

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CONCLUSION

The RP-HPLC method developed for analysis of Triamterene and Benzthiazide in their tablet dosage form is precise, accurate and with short run time. The method was fully validated showing satisfactory data for all the method validation parameters tested. The developed method is suitable for the quality control of the raw material, formulation and dissolution studies.

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