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Simultaneous Determination and Validation of Triamterene and Benzthiazide by Zero Order Derivative and First Order Derivative Method in Bulk Drug and Pharmaceutical Formulation

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Abstract: The proposed method involves Zero order derivative and First order derivative spectroscopy method. A novel, simple and rapid UV Spectrophotometric determination method for Simultaneous estimation of Triamterene and Benzthiazide was successfully developed and validated in bulk and pharmaceutical formulation. First method is zero order derivative method where the solutions were scanned in the range from 400-200 nm and the peaks were observed at a λmax of 363 and 283nm for Triamterene and Benzthiazide respectively. Second method First order derivative spectroscopy method, which involved the measurement of absorbances of one drug at zero crossing point of other drug. 385 nm and 308 nm were selected for the estimation of Triamterene and Benzthiazide respectively in bulk drug and formulation. Both the methods showed linearity from 6 – 30 µg/ml and 3 -15 µg/ml for Triamterene and Benzthiazide respectively. Recovery studies showed that the method is accurate. Precision of the proposed methods were found to be within the acceptable limits. Thus the two proposed methods and results were validated according to ICH guidelines. So, the methods can be applied for routine analysis in bulk and pharmaceutical formulation.
Keywords: Benzthiazide, First order derivative method, Triamterene, Zero order derivative method.

INTRODUCTION:

Triamterene is a potassium-sparing diuretic (water pill) that prevents your body from absorbing too much salt and keeps your potassium levels from getting too low. Triamterene is used to treat fluid retention (edema) in people 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 too much aldosterone in your body. Aldosterone is a hormone produced by the adrenal glands to help regulate the salt and water balance 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. It is an official drug in British Pharmacopoeia[2] **Fig 1.**

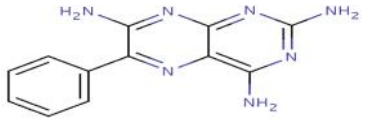

Fig.1.Chemical structure of Triamterene

Triamterene shows hyperkalemia as its major side effect. So, in order to counteract this effect it is used in combination with a thiazide diuretic which counteracts the side effect of Triamterene by its hypolkalemic effect[3,4].

Benzthiazide belong to thiazide diuretics, widely used in treatment of hypertension and edema associated with mild to moderate congestive heart failure. It increases the rate of urine excretion by the kidneys through decreased tubular reabsorption of sodium and chloride ions and by increasing osmotic transport of water to the renal tubules, which in turn lowers cardiac output and blood pressure. On prolonged thiazide treatment plasma volume and ECF return to normal, but their hypotensive effect continues. This is probably due to reduced sensitivity of the vascular bed to the circulating catecholamine and angiotensin[5,6].

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. It is an official drug in British Pharmacopoeia[7]. **Fig.2**

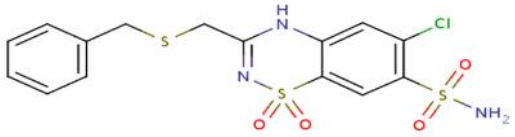

Fig.2.Chemical structure of Benzthiazide

Combination of Triamterene and Benzthiazide are used in treatment of edema and hypertension[8].

In the literature survey it was found that Triamterene and Benzthiazide were estimated independently and in combination with other drugs by several bioanalytical, HPLC and Spectrometric methods. But no method is found for simultaneous estimation of Triamterene and Benzthiazide even though this multi- ingredient's formulation is available in market from past 50 years. 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 analytical methods for simultaneous estimation of Triamterene and Benzthiazide and extend it for their determination in formulation.

EXPERIMENTAL

Instrument used:

For both the methods, Shimadzu model 1700 double beam UV-VIS spectrophotometer with spectral bandwidth of 1.8nm, wavelength accuracy of 2nm and a pair of 1 cm matched quartz cells of 10 mm optical path length was used as an instrument for spectral measurements. An analytically pure sample of Triamterene and Benzthiazide was procured as gift sample from Remedix Pharma Pvt Ltd, Bangalore, India. Tablet formulation containing Triamterene (50 mg) and Benzthiazide (25 mg) was purchased from local market.

Solvent Used:

Ethanol AR grade was used as solvent.

Preparation of standard stock solution:

100 mg each of Triamterene and Benzthiazide were weighed separately and transferred into two different 100 ml volumetric flasks. Both the drugs were dissolved in 25 ml of ethanol by ultrasonication and then volume was made upto the mark with ethanol to obtain final concentration of 1000 µg/ml of each component (stock 'A' solution). From the above stock 'A' of each solution, 5 ml of aliquot was pipetted out in a 50 ml volumetric flask and the volume was made upto the mark with ethanol to obtain the final concentration of 100 µg/ml of each component (stock 'B' solution).

Preparation of sample stock solution using formulation:

From the powder of twenty tablets, a quantity equivalent to 100mg of Triamterene was weighed accurately and transferred to a flask containing 50ml of ethanol, ultrasonicated for 15mins, solution was filtered through whatmann filter paper no.41 into a 100ml volumetric flask, volume was made upto mark with ethanol to get 1000 µg/ml (stock I). Aliquots were further prepared by diluting stock II (100 µg/ml) in ethanol to get a concentration of 6-30 µg/ml.

Methodology:

Calibration curve

Method A: Zero order derivative method[9,10]

Zero order derivative method involved the normal absorption spectrum referred to as the fundamental, zero order or D^0 spectrum. The solution was scanned in the range from 400-200 nm and the wavelength selected for the analysis were 363 nm and 283 nm for Triamterene and Benzthiazide respectively (Fig.3 and 4). Beer's law is obeyed in the concentration range of 6-30 µg/ml and 3-15 µg/ml for Triamterene and Benzthiazide respectively and the calibration curve was plotted. (Fig.5 and 6). Similarly absorbances of sample solutions were measured and amount of Triamterene and Benzthiazide was

determined from standard calibration curve (Fig.7 and 8).

Method B: First order derivative method[11-19]

Using appropriate dilutions of the standard stock solution, the solution was scanned in the wavelength region of 400 – 200 nm. The absorbance spectrum, thus obtained was derivatized to remove the interference of absorbing species. From the examination of first derivative spectra zero crossing point of one drug was selected for estimation of another drug. 385 nm and 308 nm were selected as analytical wavelength for the estimation of Triamterene and Benzthiazide (Fig.9 and 10). The calibration curve was plotted (Fig.11 and 12). Similarly absorbances of samples solution were measured and amount of Triamterene and Benzthiazide was determined from standard calibration curve (Fig.13, 14 and 15).

Validation of methods[20,21]

The above methods were satisfactory in accordance to the ICH guidelines. Accuracy studies were carried out at three different levels i.e. 80%, 100% and 120% by adding the pure drug to previously analyzed tablet sample and the percentage recovery was calculated.

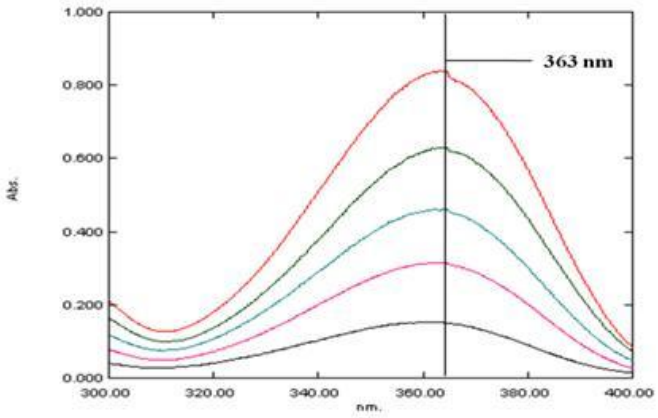

Fig.3. Linearity overlain spectra of Triamterene from 6 to 30 µg/ml at 363 nm

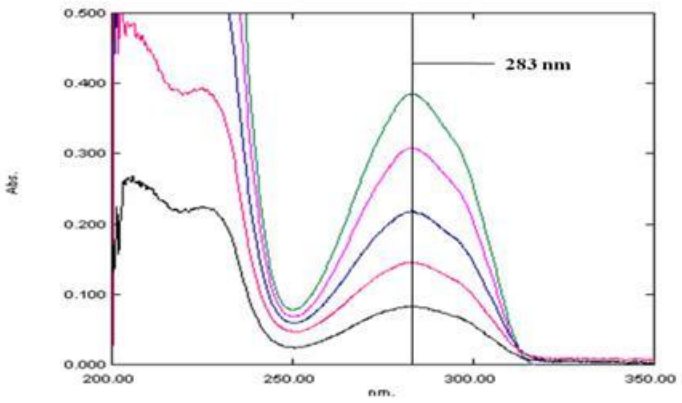

Fig.4. Linearity overlain spectra of Benzthiazide from 3 to 15 µg/ml at 283 nm

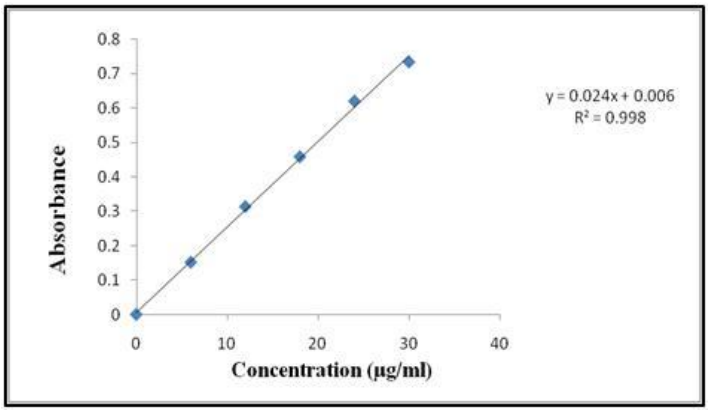

Fig.5. calibration curve of Triamterene at 363 nm in ethanol by Zero order derivative method

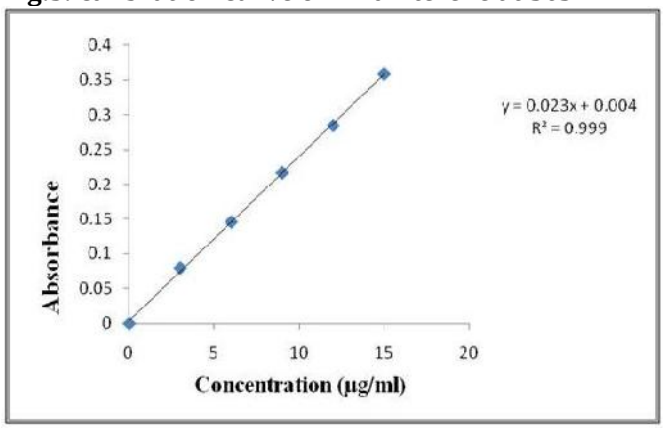

Fig.6. calibration curve of Benzthiazide at 363 nm in ethanol by Zero order derivative method

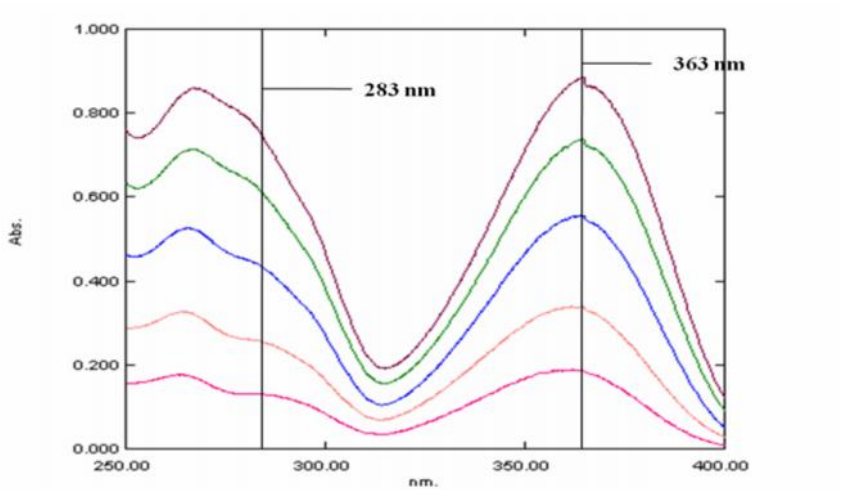

Fig.7. Linearity overlain spectra of Formulation from 6 to 30 µg/ml at 363 and 283 nm

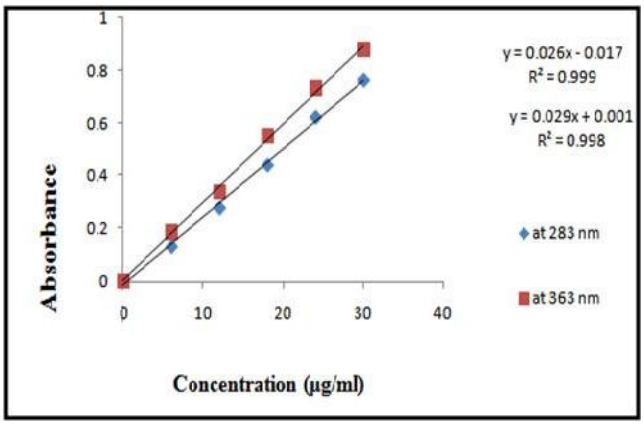

Fig.8. calibration curve of formulation at 363 and 283 nm in ethanol by Zero order derivative method

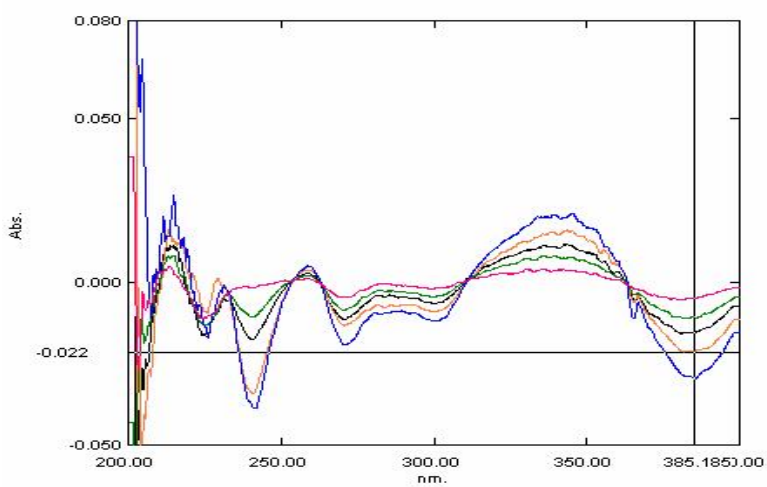

Fig.9. Derivatized overlain spectra of Triamterene at 385 nm, which is zero crossing point of Benzthiazide in ethanol

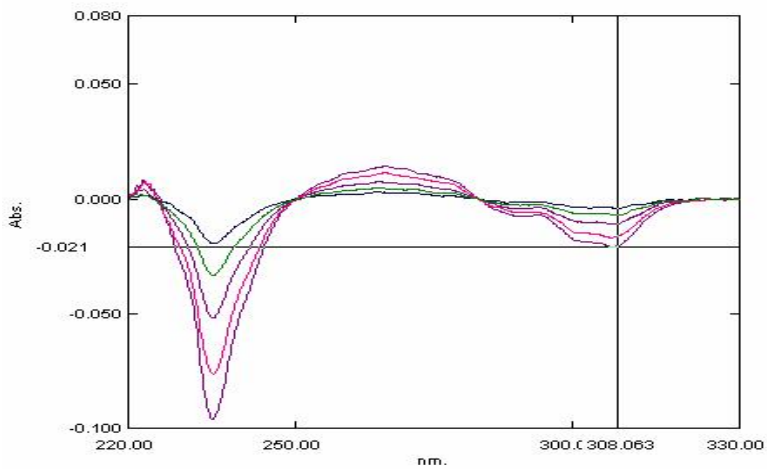

Fig.10. Derivatized overlain spectra of Benzthiazide at 308 nm, which is zero crossing point of Triamterene in ethanol

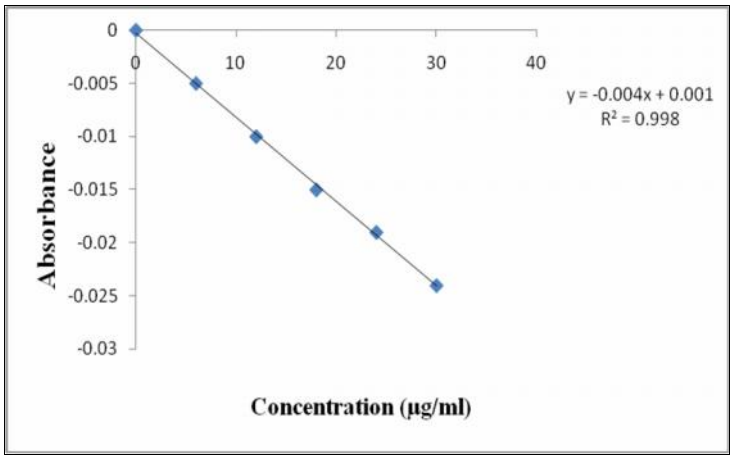

Fig.11. Calibration curve for Triamterene at 385 nm in ethanol by First order derivative method

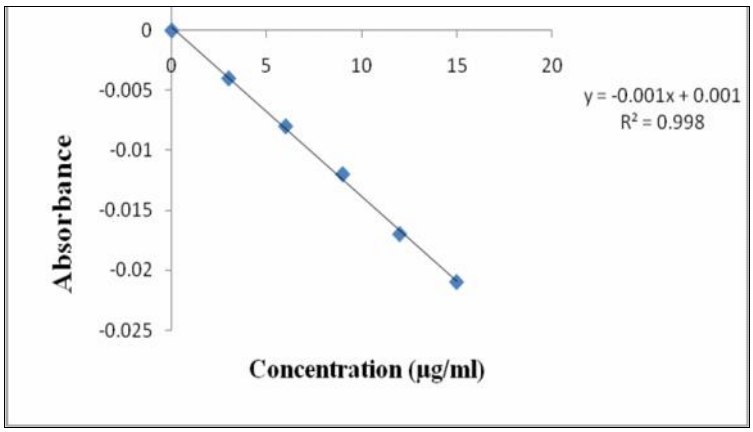

Fig.12. Calibration curve for Benzthiazide at 308 nm in ethanol by first order derivative method

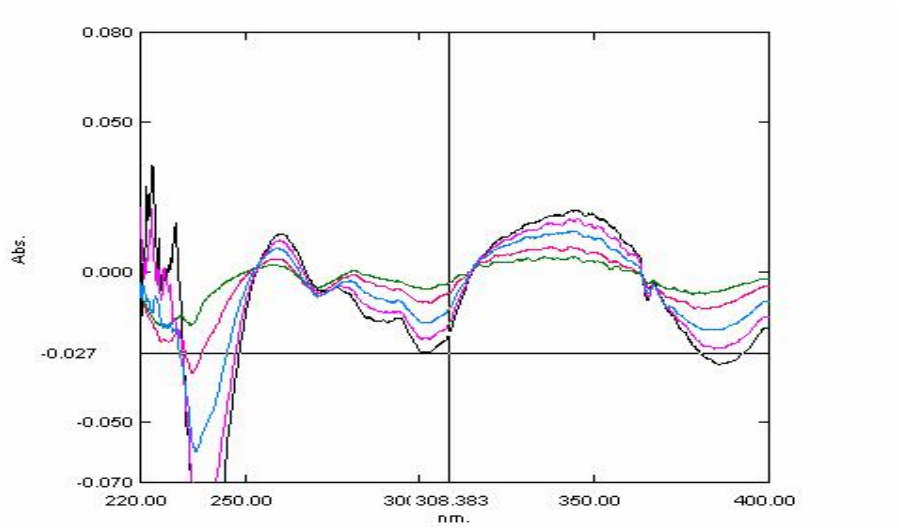

Fig.13. Derivatized overlain spectra of formulation at 308 nm

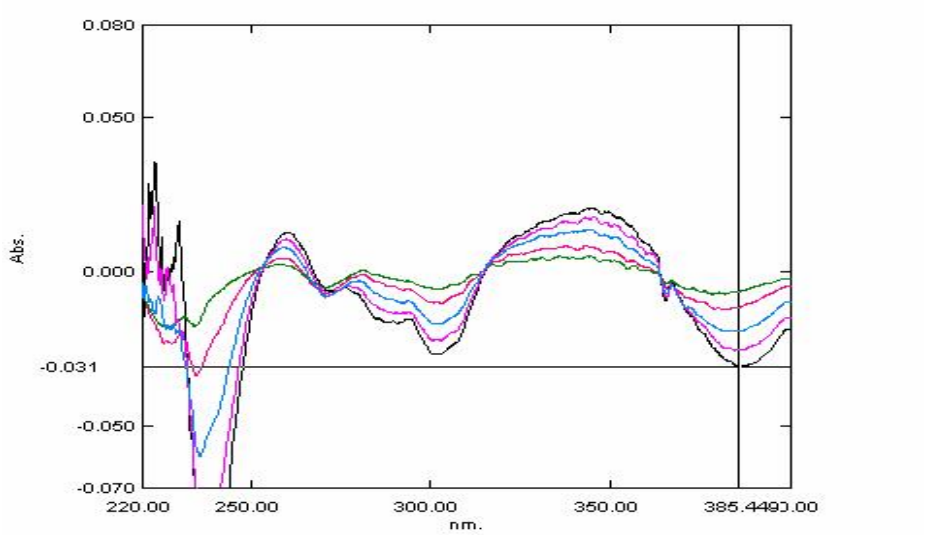

Fig.14. Derivatized overlain spectra of formulation at 385 n

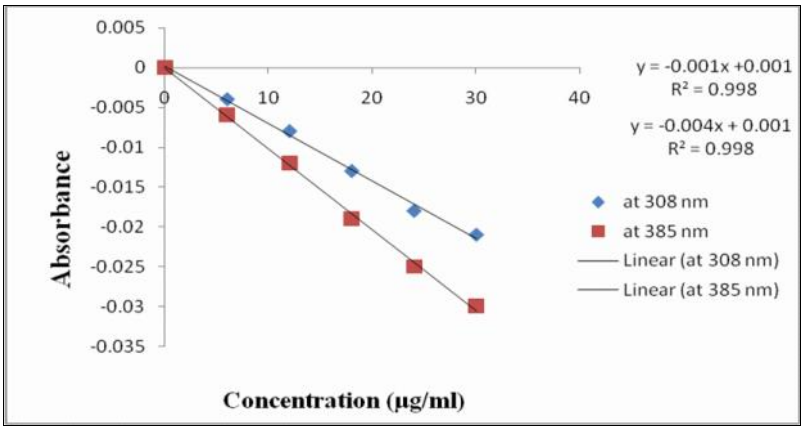

Fig.15. Calibration curve for Formulation at 308 nm and 385 nm in ethanol by first order derivative method

Table.1. Spectral characteristics of Triamterene, Benzthiazide and formulation at 383 nm and 283 nm by zero order derivative method.

Parameter	**Triamterene at 363 nm**	**Benzthiazide at 283 nm**	**Formulation at 283 nm**	***Formulation at 363 nm***
Linear Range (µg/ml)	6-30	3-15	6-30	*6-30*
Slope	0.024x	0.023x	0.026x	*0.029x*
Intercept	0.006	0.004	0.017	*0.001*
Regression co-efficient	0.998	0.999	0.999	*0.998*
Standard deviation	0.00122	0.00112	0.00107	*0.00105*
Sandell's sensitivity	0.0392	0.0407	-	-
Limit of Detection (µg/ml)	0.16	0.17	0.13	*0.11*
Limit of Quantification (µg/ml)	*0.5*	*0.53*	*0.41*	*0.363*

Table.2. Determination of Accuracy of Triamterene and Benzthiazide.

Level of % recovery	Amount of formulation (mg/tab)		Amount of standard drug added (mg)		Total amount recovered (mg)		% Recovery	
	TRM	BNZ	TRM	BNZ	TRM	BNZ	TRM	BNZ
80%	50	25	40	20	90.36	45.7	99.6	98.45
	50	25	40	20	90.27	45.5	99.7	98.9
	50	25	40	20	90.49	45.4	99.45	99.1
100%	50	25	50	25	99.33	49.79	100.67	100.42
	50	25	50	25	98.61	49.72	101.4	100.56
	50	25	50	25	98.43	49.8	101.59	100.4
120%	50	25	60	30	110.29	55.16	99.73	99.68
	50	25	60	30	111.26	55.01	98.86	99.98
	50	25	60	30	110.89	55.62	99.19	98.87

Statistical validation Data for Recovery studies.

Components	Mean* (%)	Standard Deviation*	% Relative standard deviation*	Standard Error*
TRM	100.0211	0.350243	0.349559	0.143015
BNZ	99.59556	0.33143	0.333568	0.135333

*n = 3

Table.3. Statistical validation Data for Intra-day Precision.

Components	Mean*	Standard Deviation*	% Relative standard deviation*	Standard Error*
TRM	0.4566	0.001226	0.268511	0.000502
BNZ	0.216867	0.001122	0.517146	0.00046
FORM at 283 nm	0.447633	0.001079	0.241128	0.000442
FORM at 363 nm	0.537467	0.001054	0.196025	0.000432

***n = 6**

Table.4. Spectral characteristics of Triamterene, Benzthiazide and formulation at 385 nm and 308 nm by first order derivative method.

Parameter	Triamterene at 385 nm	Benzthiazide at 308 nm	Formulation At 308 nm	Formulation at 385 nm
Linear Range (µg/ml)	6-30	3-15	6-30	6-30
Slope	-0.004x	-0.001x	-0.004x	-0.001x
Intercept	0.001	0.001	0.001	0.001
Regression co-efficient	0.998	0.998	0.998	0.998
Standard deviation	0.000512	0.0006837	0.0005564	0.000606
Sandell's sensitivity	-0.7335	-1.222	-	-
Limit of Detection (µg/ml)	0.422	2.256	0.45	1.99
Limit of Quantification (µg/ml)	1.28	6.837	1.39	6.06

Table: 5. Determination of Accuracy of Triamterene and Benzthiazide.

Level of % recovery	Amount of formulation (mg/tab)		Amount of standard drug added (mg)		Total amount recovered (mg)		% Recovery	
	TRM	BNZ	TRM	BNZ	TRM	BNZ	TRM	BNZ
80%	50	25	40	20	90.9	44.8	101	99.5
	50	25	40	20	89.6	45.5	99.5	101.1
	50	25	40	20	89.3	44.2	99.2	98.2
100%	50	25	50	25	99.76	50.2	99.76	100.26
	50	25	50	25	99.9	50.7	99.99	101.4
	50	25	50	25	99.4	50.3	99.4	100.6
120%	50	25	60	30	109.2	55.3	99.2	100.5
	50	25	60	30	109.8	54.6	99.81	99.27
	50	25	60	30	110.17	54.9	100.15	99.28

Statistical validation Data for Recovery studies.

Components	Mean* (%)	Standard Deviation*	% Relative standard deviation*	Standard Error*
TRM	99.77889	0.581032	0.582086	0.237253
BNZ	100.0122	0.91504	0.916275	0.373638

*n = 3

Table.6. Statistical validation Data for Intra-day Precision.

Components	Mean*	Standard Deviation*	% Relative standard deviation*	Standard Error*
TRM	-0.01443	0.000512	-3.544073	0.00021
BNZ	-0.01223	0.0006837	-5.58907	0.00028
FORM at 385 nm	-0.01817	0.000606	-3.3363	0.000248
FORM at 308 nm	-0.01253	0.0005564	-4.43912	0.000609

***n = 6**

RESULTS AND DISCUSSION:

The absorption spectra for Triamterene and Benzthiazide were recorded in the wavelength region of 200-400 nm for both method A and method B. The spectra were reported . These methods found to be economic, simple, and accurate. Beer-Lambert's Law was obeyed in the concentration range of 6-30 µg/ml and 3-15 µg/ml for Triamterene and Benzthiazide respectively. The accuracy was found by recovery studies at three different levels i.e.80%, 100% and 120%. The %RSD values are less than 2 for both the methods. The optical characteristics such as Beer's law limit, sandell's sensitivity, % relative standard deviation, limit of detection, limit of quantitation and range of errors in each method were calculated and the results were reported in Table 1 and Table 4. Also the regression characteristics like slope (m), intercept (c), and correlation coefficient (r) were calculated and are presented in same tables mentioned above. The results showed that the methods have reasonable precision and the results were repoted in Table 3 and Table 6. The accuracy of the methods was confirmed by the recovery studies by adding known amount of the pure drug to the pharmaceutical formulation previously analyzed by this method and the results were reported in Table 2 and Table 5.

CONCLUSION

For routine analytical purpose it is always necessary to establish methods capable of analysing huge number of samples in a short time period with due accuracy and precision. Chromatopgraphic technique coupled with multivariate algorithms can generate large amount of quality data which serve as highly powerful and convenient analytical tool. In

view of the need for a suitable method for routine analysis of Triamterene and Benzthiazide in bulk and formulation, in the present work, an attempt was made to develop a newer, simple, accurate, precise and economic two spectrophotometric methods.

The methods was developed and validated as per ICH guidelines. The standard deviation and % RSD calculated for the proposed methods are within limits, indicating high degree of precision of the methods. The results of the recovery studies performed indicate the methods to be accurate. Hence, it can be concluded that the developed two spectrophotometric methods, zero order derivative and first order derivative methods are new, simple, accurate, precise and economic and can be employed successfully for the estimation of Triamterene and Benzthiazide in bulk and formulation.

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