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Original Research Paper

Pharmacy

AN RP- HPLC METHOD DEVELOPMENT AND VALIDATION OF TRIAMTERENE AND BENZTHIAZIDE IN BULK AND PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT A rapid and precise reverse phase high performance liquid chromatographic method has been developed for the validated of Triamterene and Benzthiazide, in its pure form as well as in tablet dosage form. Chromatography was carried out on a Symmetry C18 (4.6 x 150mm, 5µm) column using a mixture of Methanol: Phosphate Buffer pH 3.5 (75:25) as the mobile phase at a flow rate of 1.0ml/min, the detection was carried out at 271 nm. The retention time of the Triamterene and Benzthiazide was 2.344, 3.282 ±0.02min respectively. The method produce linear responses in the concentration range of 10-50mg/ml of Benzthiazide and 20-100mg/ml of Triamterene. The method precision for the determination of assay was below 2.0%RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.

KEYWORDS : Triamterene, Benzthiazide, RP-HPLC, validation.

INTRODUCTION:

Analytical techniques There are numerous chemical or physico-chemical processes that can be used to provide analytical information. The processes are related to a wide range of atomic and molecular properties and phenomena that enable elements and compounds to be detected and/or quantitatively measured under controlled conditions.

Analytical methods

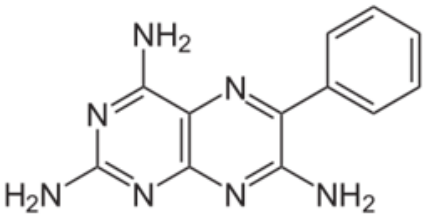
An analytical method consists of a detailed, stepwise list of instructions to be followed in the qualitative, quantitative or structural analysis of a sample for one or more analytes and using a specified technique. It will include a summary and lists of chemicals and reagents to be used, laboratory apparatus and glassware, and appropriate instrumentation. The quality and sources of chemicals, including solvents, and the required performance characteristics of instruments will also be specified as will the procedure for obtaining a representative sample of the material to be analyzed. This is of crucial importance in obtaining meaningful results. The preparation or pre-treatment of the sample will be followed by any necessary standardization of reagents and/or calibration of instruments under specified conditions. Qualitative tests for the analyte(s) or quantitative measurements under the same conditions as those used for standards complete the practical part of the method. The remaining steps will be concerned with data processing, computational methods for quantitative analysis and the formatting of the analytical report. The statistical assessment of quantitative data is vital in establishing the reliability and value of the data, and the use of various statistical parameters and tests is widespread.

DRUG PROFILE:

Triamterene:

Drug category : Potassium-sparing diuretic

Structure



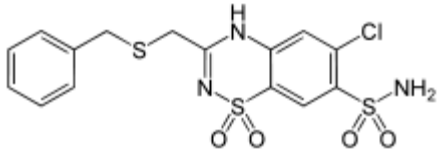
Chemical name/ Nomenclature / IUPAC Name : 6-phenylpteridine-2,4,7-triamine
Molecular Formula : C12H11N7
Molecular Weight : 253.263 gm/mole.

Therapeutic efficacy/ Indications: For the treatment of edema associated with congestive heart failure, cirrhosis of the liver, and the nephrotic syndrome; also in steroid-induced edema, idiopathic edema, and edema due to secondary hyper aldosteronism.

BENZTHAZIDE

Drug category : Antihypertensive Agent, Diuretic, Stimulant

Structure



IUPAC Name : 6-chloro-1,1-dioxo-3 -(phenylmethylsulfanylmethyl)- 4H-benzo[e][1,2,4]thiadiazine-7-sulfonamide
Molecular Formula : C15H14ClN3O4S3
Molecular Weight : 431.94 gm/mole.

Therapeutic efficacy/ Indications: For the treatment of high blood pressure and management of edema.

MATERIALS & METHODS:

Preparation of standard solution:

Accurately weigh and transfer 10 mg of Triamterene and Benzthiazide working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 0.6ml of Triamterene and 0.3ml of Benzthiazide from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

PREPARATION OF BUFFER AND MOBILE PHASE:

Preparation of Phosphate buffer pH 3.5:

Accurately weighed 6.8 grams of KH2PO4 was taken in a 1000ml volumetric flask, dissolved and diluted to 1000ml with HPLC water and the volume was adjusted to pH 3.5.

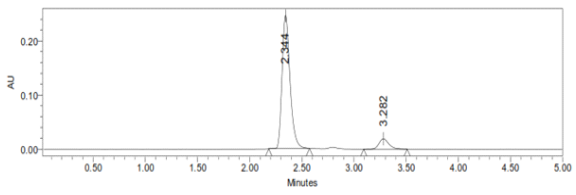
Preparation of mobile phase:

Accurately measured 750 ml (75%) of Methanol and 250 ml of Phosphate buffer (25%) a were mixed and degassed in digital ultrasonicater for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

OPTIMIZED CHROMATOGRAPHIC CONDITIONS:

HPLC:Waters HPLC with auto sampler and PDA Detector 996 model.

Temperature :40°C
Column : Symmetry C18 (4.6×150mm, 5µ)
pH : 3.5
Mobile phase:Methanol : Phosphate buffer (75:25v/v)
Flow rate : 1ml/min
Wavelength :271 nm
Injection volume : 10 l
Run time : 5 min



Optimized Chromatogram

Peak results for Optimized Chromatogram

S. No	Peak name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Triamterene	2.344	1128848	247861		1.3	4558
2	Benzthiazide	3.282	124391	19413	6.0	1.2	6031

Observation: From the above chromatogram it was observed that the Triamterene and Benzthiazide peaks are well separated and they shows proper retention time, resolution, peak tail and plate count. So it's optimized trial.

ASSAY:

Results for Assay sample

S.No	Injection	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count
1	1	Triamterene_1	2.344	1107139	246586		1.3	4642.5
2	1	Benzthiazide_1	3.282	124452	19117	6.0	1.2	6036.3
3	2	Triamterene_2	2.342	1108903	248422		1.3	4721.5
4	2	Benzthiazide_2	3.282	124632	19178	6.0	1.2	6127.3
5	3	Triamterene_3	2.342	1125993	248924		1.3	4701.2
6	3	Benzthiazide_3	3.282	126697	19237	6.0	1.3	6090.3

Results: The % purity of Triamterene and Benzthiazide in pharmaceutical dosage form was found to be 100.45 %.

VALIDATION:

Accuracy:

Accuracy Results for Triamterene

%Concentration(at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	606659.3	30	29.9	100	99.9%
100%	1192925	60	59.8	100.08	
150%	1774609	90	89.7	99.8	

Accuracy results for Benzthiazide

%Concentration(at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	63467	15	14.9	100	99.8%
100%	125020	30	29.7	99.3	
150%	187274.3	45	44.7	100.1	

System Suitability:

Results of system suitability for Triamterene

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Triamterene	2.343	1102519	248455	4506	1.3
2	Triamterene	2.343	1102945	249526	4674	1.2
3	Triamterene	2.342	1103237	250012	4298	1.2
4	Triamterene	2.344	1104076	246695	4032	1.0
5	Triamterene	2.342	1109958	248699	4812	1.3
Mean			1104547			
Std. Dev			3077.988			
% RSD			0.27			

Results of system suitability for Benzthiazide

S no	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Benzthiazide	3.281	123450	19573	6387.9	1.2	6.0
2	Benzthiazide	3.285	123699	19280	6152.4	1.2	6.0
3	Benzthiazide	3.282	124301	19530	6280.3	1.2	6.0
4	Benzthiazide	3.282	123996	19623	6325.7	1.2	6.0
5	Benzthiazide	3.282	124979	19489	6178.5	1.2	6.0
Mean			124085				
Std. Dev			592.8815				
% RSD			0.47				

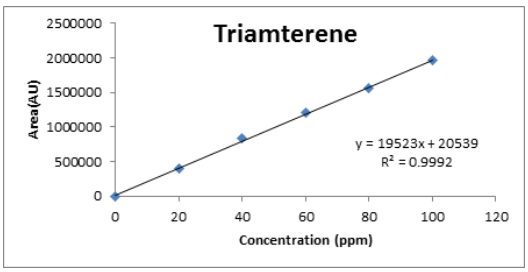
LINEARITY:

CHROMATOGRAPHIC DATA FOR LINEARITY STUDY:

Triamterene:

Concentration Level (%)	Concentration g/ml	Average Peak Area
33.3	20	408934
66.6	40	836781
100	60	1203873

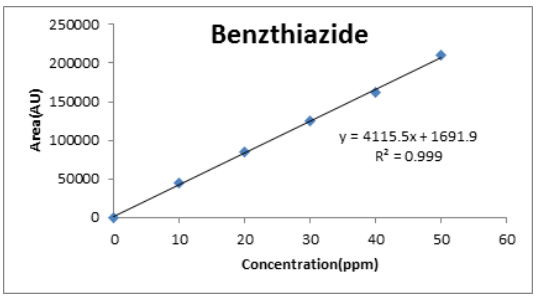
133.3	80	1563458
166.6	100	1967084



Calibration graph for Linearity of Triamterene

Benzthiazide:

Concentration Level (%)	Concentration g/ml	Average Peak Area
33	10	45510
66	20	84701
100	30	124802
133	40	162731
166	50	209732



Calibration graph for Linearity of Benzthiazide

Method Precession:

Results of method precession for Triamterene:

S.No	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Triamterene	2.345	1102729	248455	4755.2	1.3
2	Triamterene	2.344	1102947	249526	4814.8	1.3
3	Triamterene	2.343	1103236	250012	4822.2	1.3
4	Triamterene	2.344	1103977	246695	4709.2	1.3
5	Triamterene	2.345	1109759	248699	4704.8	1.3
Mean			1104530			
Std. Dev			2961.088			
% RSD			0.26			

Results of method precession for Benzthiazide:

S.No	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Benzthiazide	3.287	123149	19573	6387.9	1.2	6.0
2	Benzthiazide	3.287	123766	19280	6152.4	1.2	6.0
3	Benzthiazide	3.288	124271	19530	6280.3	1.2	6.0
4	Benzthiazide	3.285	124691	19623	6325.7	1.2	6.0
5	Benzthiazide	3.288	124956	19489	6178.5	1.2	6.0
Mean			124166.6				
Std. Dev			725.4373				
% RSD			0.5				

Intermediate precision:

Results of Intermediate precision for Triamterene

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Triamterene	2.344	1100148	247140	4703.7	1.3
2	Triamterene	2.343	1104520	245696	4645.7	1.3
3	Triamterene	2.345	1105937	247870	4707.5	1.3
4	Triamterene	2.344	1106476	246764	4639.2	1.3
5	Triamterene	2.342	1108271	247280	4642.8	1.3
6	Triamterene	2.343	1106582	247166	4631.4	1.3
Mean			1105322			
Std. Dev			2807.405			
% RSD			0.25			

Results of Intermediate precision for Benzthiazide

S no	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Benzthiazide	3.281	122487	19115	6076.6	1.2	6.0
2	Benzthiazide	3.281	122626	19003	6040.0	1.2	6.0
3	Benzthiazide	3.283	122632	19073	6120.1	1.2	6.0
4	Benzthiazide	3.281	122702	19123	6114.0	1.2	6.0
5	Benzthiazide	3.278	122962	19165	6118.5	1.2	6.0
6	Benzthiazide	3.281	122972	19145	6130.3	1.2	6.0
Mean			122730.2				
Std. Dev			196.2859				
% RSD			0.15				

Robustness:

Results for Robustness of Triamterene

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	1128848	2.344	4558	1.3
Less Flow rate of 0.9 mL/min	1569971	2.911	7036.3	1.3
More Flow rate of 1.1 mL/min	1114875	2.014	4389	1.4
Less organic phase	1120197	2.361	4508.4	1.4
More organic phase	1107845	2.038	4417	1.4

Results for Robustness Benzthiazide

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	124391	3.282	6031	1.2
Less Flow rate of 0.9 mL/min	156550	4.075	7036.3	1.3
More Flow rate of 1.1 mL/min	120951	3.089	6215	1.2
Less organic phase	122406	4.422	6387.7	1.2
More organic phase	121589	3.015	6285	1.2

DISCUSSION:

The analytical method was developed by studying different parameters.

First of all, maximum absorbance was found to be at 271 nm and the peak purity was excellent.

Injection volume was selected to be 10µl which gave a good peak area.

The column used for study was Symmetry C18 because it was giving good peak.

Ambient temperature was found to be suitable for the nature of drug solution. The flow rate was fixed at 1.0ml/min because of good peak area and satisfactory retention time.

Mobile phase is Methanol: Phosphate Buffer pH 3.5 (75:25) was fixed due to good symmetrical peak. So this mobile phase was used for the proposed study.

Run time was selected to be 5 min because analyze gave peak around 2.344, 3.282 ±0.02min respectively and also to reduce the total run time.

The percent recovery was found to be 98.0-102 was linear and precise over the same range. Both system and method precision was found to be accurate and well within range.

The analytical method was found linearity over the range 20-100mg/ml of Triamterene and 10-50mg/ml of Benzthiazide of the target concentration.

The analytical passed both robustness and ruggedness tests. On both cases, relative standard deviation was well satisfactory.

CONCLUSION:

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Triamterene and Benzthiazide in bulk drug and pharmaceutical dosage forms. This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps. Triamterene and Benzthiazide was freely soluble in ethanol, methanol and sparingly soluble in water. Methanol: Phosphate Buffer pH 3.5 (75:25) was chosen as the mobile phase. The solvent system used in this method was economical. The %RSD values were within 2 and the method was found to be precise. The results expressed in Tables for RP-HPLC method was promising the RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods. This method can be used for the routine determination of Triamterene and Benzthiazide in bulk drug and in Pharmaceutical dosage forms.

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