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Full Length Research Paper

Analytical method development and validation of simultaneous estimation of hydrochlorothiazide and triamterene in combined tablet dosage form by RP-HPLC

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Abstract

A new simple, accurate, rapid and precise isocratic high performance liquid chromatographic method was developed and validated for the determination of Hydrochlorothiazide (HTZ), and Triamterene (TMT) in tablet formulation. The optimized conditions comprises of column Symmetry C_{18} 250 mm × 4.6 mm I.D; 5 µm with a flow rate of 1.0 mL/min, 0.05 M Phosphate buffer, methanol and acetonitrile mixture was used as mobile phase in the ratio 55:35:10 v/v at a detection wavelength 270 nm. Retention times of HTZ and TMT were found to be 3.49 min, and 4.68 min with a tailing factor 1.25, 1.27 and 4704, 4841 as theoretical plates respectively which are within the limits. All the parameters were validated according to the ICH guidelines and found to be within limits. The LOD values of HTZ and TMT were found to be 0.089 and 0.251 µg/mL respectively. HTZ and TMT LOQ's were found to be 0.27, and 0.78µg/mL respectively. Linearity ranges for HTZ, and TMT were 2-10 µg/mL, and 3-15 µg/mL respectively. Percent recovery study values of HTZ and TMT were found to be within 98-102%.

Keywords: Hydrochlorothiazide, triamterene, RP-HPLC, validation, simultaneous estimation.

INTRODUCTION

HPLC is also called as high pressure liquid chromatography. Its has high specificity and wide range of sensitivity make it ideal for the analysis of many drugs in both dosage forms and biological fluids. This technique is based on the same methods of separation as that of classical column chromatography but it differs from the column chromatography in the fact that the mobile phase is passed through the packed column under high pressure.

Types of HPLC techniques

- *Based on the mode of separation*
  - Normal phase chromatography
  - Reverse phase chromatography
- *Based On Principle Of Separation*
  - Adsorption chromatography
  - Ion exchange chromatography
  - Size exclusion chromatography
  - Affinity chromatography
  - Chiral phase chromatography
- *Based on Elution technique*
  - Isocratic separation
  - Gradient separation
- *Based on the scale of operation*
  - Analytical HPLC
  - Preparative HPLC
- *Based on the type of analysis*
  - Qualitative analysis
  - Quantitative analysis.

PLAN OF STUDY

1. Solubility determination of Hydrochlorothiazide and Triamterene in various solvents and buffers.

2. Determine the absorption maxima of both the drugs in UV-Visible region in different solvents/buffers and selecting the solvents for HPLC method development.

3. Optimize the mobile phase and flow rates for proper resolution and retention times.

LITERATURE REVIEW

Jonczyk and Nowakowska (2001) have determined pharmaceutical preparation containing mixture of Hydrochlorothiazide, Triamterene, proponalol hydrochloride by the spectrophotometric method and high performance liquid chromatographic method. The Spectrophotometric method was performed by using 1 M HCl: Water in the ratio of 1:9 as solvent and absorption maxima was found to be 272 nm, 360 nm and 290 nm for Hydrochlorothiazide, Triamterene, and proponalol hydrochloride respectively. Another by chromatographic method, they developed chromatogram by using a Nucelosil100 C_{18} [150 x 4.6 mm] column, and the mobile phase was Acetonitrile ,0.05M Phosphate buffer of pH 3.5 in the ratio of 17:83 with a flow rate of 1.5 mL/min and detection wavelength was 270 nm. The observed retention times for Hydrochlorothiazide and Triamterene and Proponalol hydrochloride was 2.4, 3.6, and 20 mins respectively. The linearity range was found to be 0.00045 mg/mL to 0.2 mg/mL for Hydrochlorothiazide, 0.00006 mg/mL to 0.2mg/mL for Triamterene and 0.013 mg/mL to 0.25 mg/mL for propranolol hydrochloride. The detection limits for the compounds was 0.05 µg/mL, 0.1 µg/mL, 0.01 µg/mL respectively. The % RSD was 2.17 %, 1.31 % and 0.65 %. An accurate and quick method for simultaneous determination of Triamterene and Hydrochlorothiazide in tablets by using first derivative (D_1) and second derivative (D_2) spectroscopy was developed by Apola et. al., 2008. The zero crossing technique was employed in measurements using D_1 at wavelength of 240.9 nm and D_2 at 278.2 nm for determining Triamterene and D_1 at wavelength of 255.7 nm and D_2 at 283.2 nm for Hydrochlorothiazide. The linearity of derivatives and analyte concentrations were maintained for concentrations from 2.40 µg/mL to12.0 µg/mL for Triamterene and from 1.25 µg/mL to 6.25 µg/mL for Hydrochlorothiazide. LOD for Triamterene was 0.90 µg/mL or 1.02 µg/mL while LOQ was 2.73 µg/mL or 3.08 µg/mL. The corresponding values for Hydrochlorothiazide were LOD was 0.25µg/mL or 0.17 µg/mL and LOQ was 0.77 µg/mL or 0.51 µg/mL depending on the derivative used. The determination results of drugs were shown high accuracy, selectivity and sensitivity. Hence the developed method was satisfactory. Raja et al. 2011 developed a simple reversed phase HPLC method for the Simultaneous determination of Olmesartan medoxomil and Hydrochlorothiazide in combined dosage forms. The method was based on reversed phase HPLC using Xterra C18 column 150 × 4.6 mm; 5 µm with detection at 230 nm. The mobile phase consisting of acetonitrile and potassium dihydrogen phophate (45:55 v/v) and at a flow rate 0.7 mL/min. Retention time of Olmesartan medoxomil was 4.385 min and Hydrochlorothiazide was 3.06 min. The method was linear over the concentration range for Olmesartan medoxomil 20-60 µg/mL and for Hydrochlorothiazide 20-60 µg/mL. The recoveries of Olmesartan medoxomil and Hydrochlorothiazide were found to be in the range of 98-102 % and 98-102 % respectively. The LOD and LOQ were found to be 0.02 µg/mL and 0.07 µg/mL for Olmesartan medoxomil and 0.07 µg/mL and 0.025 µg/mL for Hydrochlorothiazide. The values shown that the method was sensitive (Table 1).

MATERIALS AND METHOD

Table 1: Materials and Methods.

Material	Instruments
Water	HPLC
Methanol	UV-Visible Spectrophotometer
Acetonitrile	Sonicator
Potassium Phosphate Buffer	Column
Hydrochloric Acid	pH meter
Hydrochlorothiazide, Triamterene	Micropipette

DRUG PROFILE

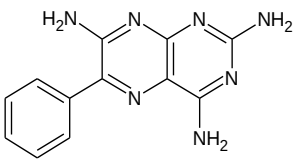


Figure 1: Structure of Triamterene.

IUPAC name: 6 – phenylpteridine - 2, 4, 7- triamine
Molecular formula: $C_{12}H_{11}N_7$
Molecular weight: 253.27
Category: Diuretic.
Description: Yellow, crystalline powder, odourless.

Solubility: Soluble in methanol. Very slightly in water, chloroform and ethanol
pKa: 6.2
Storage: Store protected from light and moisture (figure 1).

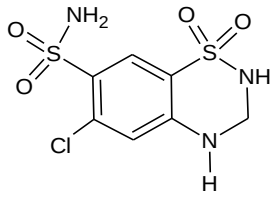


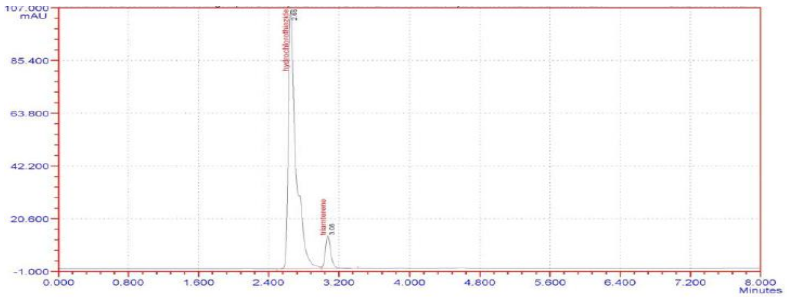
Figure 2: Structure of Hydrochlorothiazide.

IUPAC name: 6 – chloro - 3,4 – dihydro - 2 *H* - 1,2,4-benzothiadiazine-7-sulphonamide 1,1dioxide
Molecular formula: $C_7H_8ClN_3O_4S_2$
Molecular weight: 297.7
Category: Diuretic
Description: A white or almost white, crystalline powder, odourless.
Solubility: Slightly soluble in water, Soluble in aqueous alkaline solutions, Sparingly soluble in methanol.
pKa: 7
Storage: Store protected from moisture (figure 2).

METHOD DEVELOPMENT TRIALS

Trial-1: Chromatographic conditions

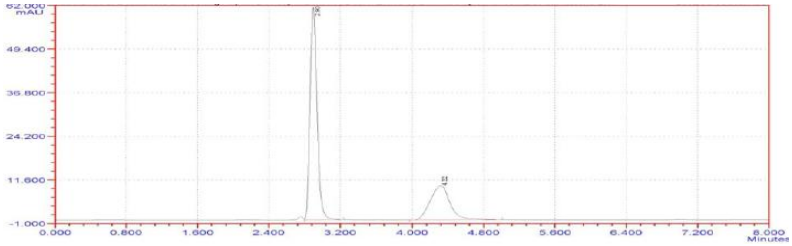
Column : Symmetry 150mm x 4.6mm, 5µ
Flow rate : 1.0 m L/min
Wavelength : 270 nm
Mobile phase : methonal and water 90:10
Injection volume : 20 µL
Column Temperature : Ambient
Retention time : for HTZ 2.65min and TMT 3.07 (Trial 1).



Trial 1: Chromatogram of HTZ and TMT

Trial 2: Chromatographic conditions

Column : SymmetryC_{18} 150 mm x 4.6 mm, 5 µ
Flow rate : 1.0 m L/min
Wavelength : 270 nm
Mobile phase : Methonal and water 60:40
Injection volume : 20 µL
Column Temperature : Ambient
Retention time : for HTZ 2.89min and TMT 4.31 min (Trial 2).

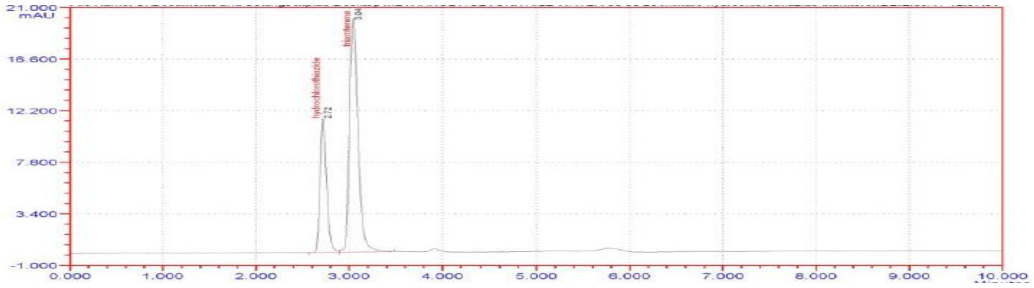


Trial 2: chromatogram of HTZ and TMT

Trial-3: Chromatographic conditions

Column : SymmetryC_{18} 150mm x 4.6mm, 5µ

Flow rate : 1.0 m L/min
Wavelength : 270 nm
Mobile phase : Methonal and ACN andwater 50:30:20
Injection volume : 20 µL
Column Temperature : Ambient
Retention time : for HTZ 2.717min and TMT 3.042 min (Trial 3).



Trial 3 chromatogram of HTZ and TMT.

OPTIMIZED CHROMATOGRAPHIC CONDITIONS

Preparation of 0.01 M HCl

0.01 M HCL was prepared by taking 0.08 mL of HCl (37 %) dissolved in few mL of HPLC grade water and made up to 100mL with HPLC grade water.

Preparation of 0.05M Phosphate buffer

0.68045 grams of KH_2PO_4 was accurately weighed and transferred into a 1000 mL beaker, dissolved and made up to the volume with HPLC grade water and the pH was adjusted with 0.01 M HCl.

Preparation of mobile phase

A Combination of 0.05M Phosphate buffer-pH 3.8 (65 %), Methanol (35 %), Acetonitrile (10 %) was mixed and degassed in ultrasonic water bath for 5 minutes, finally filtered through 0.45 µ membrane filter. This prepared solution was used as mobile phase. This solution was also used for specificity blank solution.

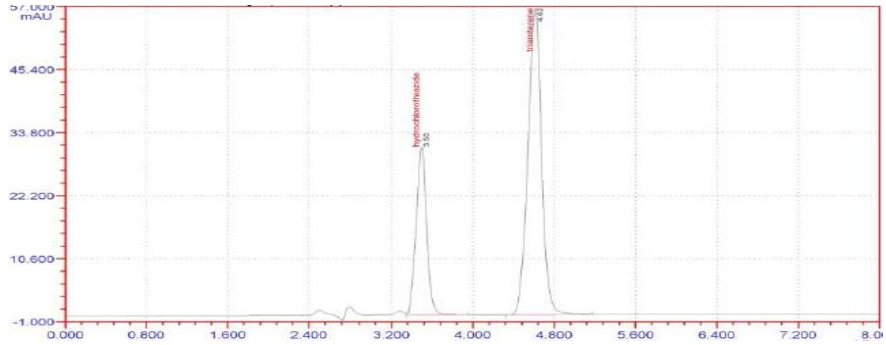
Sample solution

Ten tablets were weighed and titurated to a fine powder, was weighed accurately weight equivalent to 10 mg from the powdered sample and dissolved in few mL of methanol and diluted to 10 mL with methanol. The solution was shaken well and allowed to stand for 15 min with intermittent sonication to ensure complete solubility of drug and filtered through a 0.45 µm membrane filter. From the filtrate, further dilution was made in a 10 mL volumetric flask by taking 0.3 mL of above solution and diluted to 10 mL with diluent (table 2).

Table 2: Optimized chromatographic conditions.

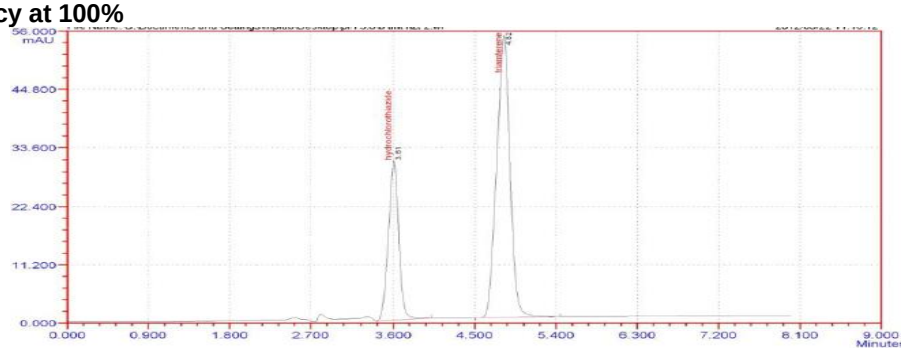
Parameters	Method
Stationary phase (column)	0.05M Phosphate buffer: Methanol: Acetonitrile (pH 3.8) in the ratio of 55:35:10v/v.
Mobile Phase	CemetryC18,250×4.6mm ID, 5µm Particle size
Flow rate (ml/min)	1 mL/min
Run time (minutes)	Room temperature(20-25°C)
Column temperature (°C)	Room temperature(20-25°C)
Volume of injection loop (ml)	270 nm
Detection wavelength (nm)	20 µL
Drug RT (min)	10min

Observation: There is no tailing and fronting good peaks are observed.
Accuracy:
Accuracy at 80%



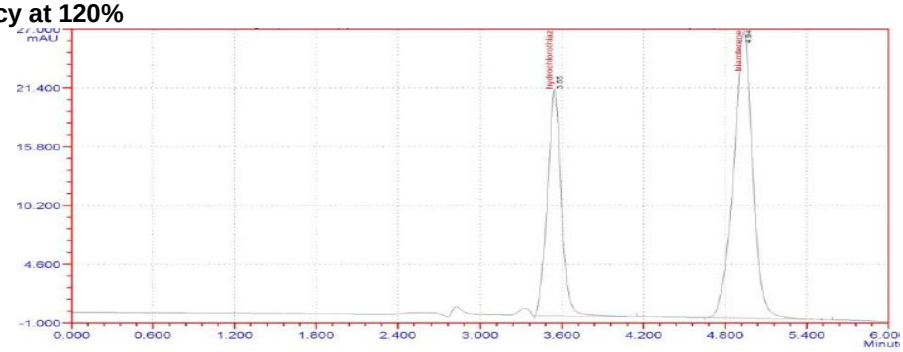
Chromatogram for Accuracy 80 %

Accuracy at 100%



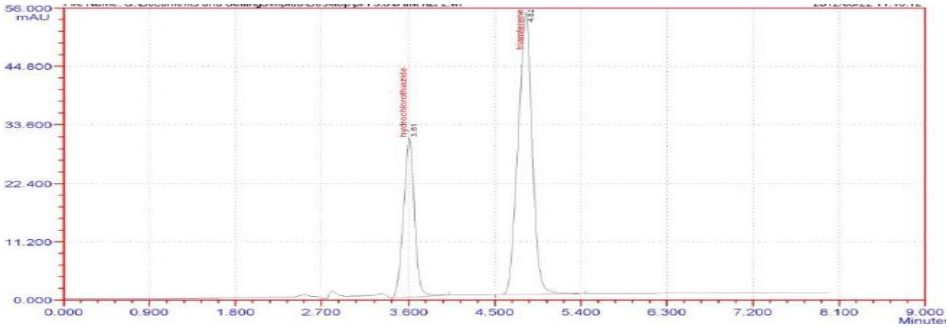
Chromatogram for Accuracy 100 %.

Accuracy at 120%



Chromatogram for Accuracy 120 %.

Precision:



Chromatogram for intraday precision

RESULT AND CONCLUSION

Simple, precise, rapid and accurate RP-HPLC method was developed for the simultaneous estimation of HTZ and TMT in pharmaceutical dosage forms.

In RP-HPLC method, optimization of chromatographic parameters was done. Parameters optimized were, selection of wavelength, effect of nature of mobile phase, ratio of mobile phase, P^H of the Buffer and effect of flow rate.

A wavelength 270 nm was selected and the mobile phase consists of 0.05M phosphate buffer (pH 3.8 adjusted with 0.01 M HCl), methanol, and Acetonitrile in 55:35:10 % v/v ratios at a flow rate of 1 mL/min were found to be optimum conditions for analysis. The peaks were well resolved with C_{18} column. System suitability studies were also carried out which includes theoretical plates, resolution and tailing factors etc.

Using the optimized chromatographic conditions, chromatograms of mixed standard solutions which contained HTZ and TMT were recorded. Retention times were found to be 3.49 and 4.68 min. for HTZ and TMT

respectively. Calibration curves were obtained by using peak area vs. concentration and correlation coefficient value was found to be > 0.999 for HTZ and TMT.
Precision of the method was studied by making the replicate injections of the standard solutions and standard deviation was determined. The reliability and sensitivity of the method could be seen from recovery studies. There is no interference due to excipients. The proposed method is simple, accurate and rapid.

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Instrumentation of chromatography.