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Development and Validation of an RP-HPLC Method for The Simultaneous Estimation of Benzthiazide and Triamterene in Pure and Pharmaceutical Dosage Forms

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Abstract: A new, simple and accurate, precise RP-HPLC method was developed for simultaneous determination of Benzthiazide and Triamterene in bulk and in combined pharmaceutical dosage form. The separation of Benzthiazide and Triamterene was achieved within 6 minutes on an Agilent Zorbax (C18) (150mm x 4.6mm, 5µm) column using Methanol: Acetate Buffer pH-3.8 (24:76v/v) as the mobile phase. Detection was carried out using wavelength at 262nm. The method showed adequate sensitivity concerning linearity, accuracy and precision over the range 100-500µg/ml and 30-70µg/ml for Benzthiazide and Triamterene, respectively. Careful validation proved advantages of high sensitivity, accuracy, precision, selectivity, robust and suitability for quality control laboratories. The developed method was robust as the %RSD was within the range and without effecting system suitability parameters. The proposed method is suitable for simultaneous determination of Benzthiazide and Triamterene in bulk and pharmaceutical dosage form.

Keywords: Benzthiazide and Triamterene, RP-HPLC, Validation, Precision, Robustness.

INTRODUCTION

Chromatography is a laboratory technique for the separation of a mixture. The mixture is dissolved in a fluid called the *mobile phase*, which carries it through a structure holding another material called the *stationary phase*. The various constituents of the mixture travel at different speeds, causing them to separate. The separation is based on differential partitioning between the mobile and stationary phases. Subtle differences in a compound's partition coefficient result in differential retention on the stationary phase and thus affect the separation.^[1]

Chromatography may be preparative or analytical. The purpose of preparative chromatography is to separate the components of a mixture for later use, and is thus a form of purification. Analytical chromatography is done normally with smaller amounts of material and is for establishing the presence or measuring the relative proportions of analytes in a mixture. The two are not mutually exclusive.^[2]

Chromatography is based on the principle where molecules in mixture applied onto the surface or into the solid, and fluid stationary phase (stable phase) is separating from each other while moving with the aid of a mobile phase. The factors effective on this separation process include molecular characteristics related to adsorption (liquid-solid), partition (liquid-solid), and affinity or differences among their molecular weights [1, 2]. Because of these differences, some components of the mixture stay longer in the

stationary phase, and they move slowly in the chromatography system, while others pass rapidly into mobile phase, and leave the system faster [3].

Based on this approach three components form the basis of the chromatography technique.

- Stationary phase: This phase is always composed of a “solid” phase or “a layer of a liquid adsorbed on the surface a solid support”.
- Mobile phase: This phase is always composed of “liquid” or a “gaseous component.”
- Separated molecules

The type of interaction between stationary phase, mobile phase, and substances contained in the mixture is the basic component effective on separation of molecules from each other. Chromatography methods based on partition are very effective on separation, and identification of small molecules as amino acids, carbohydrates, and fatty acids. However, affinity chromatographies (ie. ion-exchange chromatography) are more effective in the separation of macromolecules as nucleic acids, and proteins. Paper chromatography is used in the separation of proteins, and in studies related to protein synthesis; gas-liquid chromatography is utilized in the separation of alcohol, ester, lipid, and amino groups, and observation of enzymatic interactions, while molecular-sieve chromatography is employed especially for the determination of molecular weights of proteins. Agarose-gel chromatography is used for the purification of RNA, DNA particles, and viruses [4].

Stationary phase in chromatography, is a solid phase or a liquid phase coated on the surface of a solid phase. Mobile phase flowing over the stationary phase is a gaseous or liquid phase. If mobile phase is liquid it is termed as liquid chromatography (LC), and if it is gas then it is called gas chromatography (GC). Gas chromatography is applied for gases, and mixtures of volatile liquids, and solid material. Liquid chromatography is used especially for thermal unstable, and non-volatile samples [5].

The purpose of applying chromatography which is used as a method of quantitative analysis apart from its separation, is to achieve a satisfactory separation within a suitable time interval. Various chromatography methods have been developed to that end. Some of them include column chromatography, thin-layer chromatography (TLC), paper chromatography, gas chromatography, ion exchange chromatography, gel permeation chromatography, high-pressure liquid chromatography, and affinity chromatography [6].

- Column chromatography
- Ion-exchange chromatography
- Gel-permeation (molecular sieve) chromatography
- Affinity chromatography
- Paper chromatography
- Thin-layer chromatography
- Gas chromatography
- Dye-ligand chromatography
- Hydrophobic interaction chromatography
- Pseudoaffinity chromatography
- High-pressure liquid chromatography (HPLC)

High-pressure liquid chromatography (HPLC)

Using this chromatography technique it is possible to perform structural, and functional analysis, and purification of many molecules within a short time, This technique yields perfect results in the separation, and identification of amino acids, carbohydrates, lipids, nucleic acids, proteins, steroids, and other biologically active molecules, In HPLC, mobile phase passes through columns under 10–400 atmospheric pressure, and with a high (0.1–5 cm/sec) flow rate. In this technique, use of small particles, and application of high pressure on the rate of solvent flow increases separation power, of HPLC and the analysis is completed within a short time.

Essential components of a HPLC device are solvent depot, high- pressure pump, commercially prepared column, detector, and recorder. Duration of separation is controlled

Essential components of a HPLC device are solvent depot, high- pressure pump, commercially prepared column, detector, and recorder. Duration of separation is controlled with the aid of a computerized system, and material is accrued [25].

Types of HPLC

There are following variants of HPLC, depending upon the phase system (stationary) in the process:

1. Normal Phase HPLC

This method separates analytes on the basis of polarity. NP-HPLC uses polar stationary phase and non-polar mobile phase. Therefore, the stationary phase is usually silica and typical mobile phases are hexane, methylene chloride, chloroform, diethyl ether, and mixtures of these.

Polar samples are thus retained on the polar surface of the column packing longer than less polar materials.

2. Reverse Phase HPLC

The stationary phase is nonpolar (hydrophobic) in nature, while the mobile phase is a polar liquid, such as mixtures of water and methanol or acetonitrile. It works on the principle of hydrophobic interactions hence the more nonpolar the material is, the longer it will be retained.

3. Size-exclusion HPLC

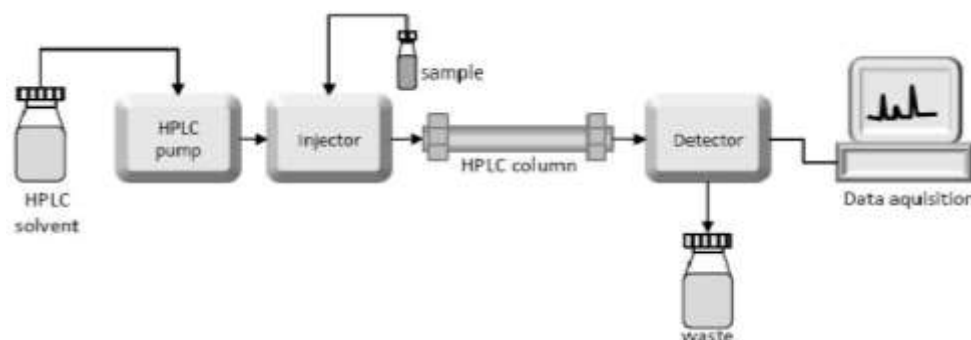
The column is filled with material having precisely controlled pore sizes, and the particles are separated according to its their molecular size. Larger molecules are rapidly washed through the column; smaller molecules penetrate inside the porous of the packing particles and elute later.

4. Ion-Exchange HPLC

The stationary phase has an ionically charged surface of opposite charge to the sample ions. This technique is used almost exclusively with ionic or ionizable samples.

The stronger the charge on the sample, the stronger it will be attracted to the ionic surface and thus, the longer it will take to elute. The mobile phase is an aqueous buffer, where both pH and ionic strength are used to control elution time.

Instrumentation of HPLC



As shown in the schematic diagram in Figure above, HPLC instrumentation includes a pump, injector, column, detector and integrator or acquisition and display system. The heart of the system is the column where separation occurs.

1. Solvent Reservoir

Mobile phase contents are contained in a glass resorvoir. The mobile phase, or solvent, in HPLC is usually a mixture of polar and non-polar liquid components whose respective concentrations are varied depending on the composition of the sample.

2. Pump

A pump aspirates the mobile phase from the solvent resorvoir and forces it through the system's column and detector. Depending on a number of factors including column dimensions, particle size of the

stationary phase, the flow rate and composition of the mobile phase, operating pressures of up to 42000 kPa (about 6000 psi) can be generated.

3. Sample Injector

The injector can be a single injection or an automated injection system. An injector for an HPLC system should provide injection of the liquid sample within the range of 0.1-100 mL of volume with high reproducibility and under high pressure (up to 4000 psi).

4. Columns

Columns are usually made of polished stainless steel, are between 50 and 300 mm long and have an internal diameter of between 2 and 5 mm. They are commonly filled with a stationary phase with a particle size of 3–10 μm .

Columns with internal diameters of less than 2 mm are often referred to as microbore columns. Ideally the temperature of the mobile phase and the column should be kept constant during an analysis.

5. Detector

The HPLC detector, located at the end of the column detects the analytes as they elute from the chromatographic column. Commonly used detectors are UV-spectroscopy, fluorescence, mass-spectrometric and electrochemical detectors.

6. Data Collection Devices

Signals from the detector may be collected on chart recorders or electronic integrators that vary in complexity and in their ability to process, store and reprocess chromatographic data. The computer integrates the response of the detector to each component and places it into a chromatograph that is easy to read and interpret.

Applications of HPLC

The information that can be obtained by HPLC includes resolution, identification and quantification of a compound. It also aids in chemical separation and purification. The other applications of HPLC include:

- **Pharmaceutical Applications**

1. To control drug stability.
2. Tablet dissolution study of pharmaceutical dosages form.
3. Pharmaceutical quality control.

- **Environmental Applications**

1. Detection of phenolic compounds in drinking water.
2. Bio-monitoring of pollutants.

- **Applications in Forensics**

1. Quantification of drugs in biological samples.
2. Identification of steroids in blood, urine etc.
3. Forensic analysis of textile dyes.
4. Determination of cocaine and other drugs of abuse in blood, urine etc.

- **Food and Flavour**

1. Measurement of Quality of soft drinks and water.
2. Sugar analysis in fruit juices.
3. Analysis of polycyclic compounds in vegetables.
4. Preservative analysis.

- **Applications in Clinical Tests**

1. Urine analysis, antibiotics analysis in blood.
2. Analysis of bilirubin, biliverdin in hepatic disorders.
3. Detection of endogenous Neuropeptides in extracellular fluid of brain etc.

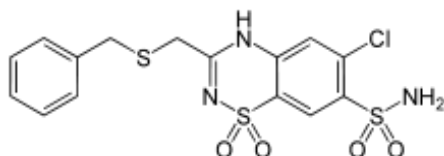
METHOD DEVELOPMENT

Analytical method development is considered as a critical process in pharmaceuticals. Availability of the different types of columns, operating parameters, mobile phase composition, diluent and pH values make it critical to develop an analytical method. A good analytic

MATERIALS AND METHODS

DRUG PROFILE FOR BENZTHIAZIDE:

Drug : Benzthiazide
Synonym : Benzthiazidum
Drug category : Diuretics
Structure :



Chemical name/ Nomenclature / IUPAC Name: 3-[(benzyl sulfanyl) methyl]-6-chloro-1,1-dioxo-4H-1lambda6,2,4-benzothiadiazine-7-sulfonamide

Molecular Formula: C₁₅H₁₄ClN₃O₄S₃

Molecular Weight: 431.937 gm/mole.

Official Pharmacopoeia: IP

PHYSICOCHEMICAL PROPERTIES:

Description (Physical State): Solid

Solubility: Benzthiazide is soluble in water, alcohol, chloroform or ether, and in alkaline solutions and soluble in dimethyl formamide, dimethyl sulfoxide, slightly soluble in methanol, ethanol.

Melting point: 210-211 °C

pKa (strongest acidic): 9.18

Log P: 1.73

PHARMACOKINETIC PROPERTIES:

Bioavailability : 60-70 %

Half-life : 5-15 hrs

Absorption : Absorbed in the digestive tract.

Protein binding : 30 %

Excretion : Renal

Adverse effects/Side effects:

- Hypokalemia
- Hyperglycemia
- Anorexia
- Gastric irritation
- Nausea
- Vomiting
- Cramps
- Diarrhea
- Jaundice
- Pancreatitis

PHARMACODYNAMICS:

Mechanism of action: As a diuretic, benzthiazide inhibits active chloride reabsorption at the early distal tubule via the Na-Cl cotransporter, resulting in an increase in the excretion of sodium, chloride, and water. Thiazides like benzthiazide also inhibit sodium ion transport across the renal tubular epithelium through binding to the thiazide sensitive sodium-chloride transporter. This results in an increase in potassium excretion via the sodium-potassium exchange mechanism. The antihypertensive mechanism of benzthiazide is less well understood although it may be mediated through its action on carbonic anhydrases in the smooth muscle or through its action on the large-conductance calcium-activated potassium (KCa) channel, also found in the smooth muscle.

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

Contraindications:

Hypersensitivity to Benzthiazide is a contraindication. In addition, Benzthiazide should not be used if you have the following conditions:

- Sensitivity to sulfur drugs
- Severe renal insufficiency

INTERACTIONS:

Drug interactions:

- The therapeutic efficacy of Benzthiazide can be decreased when used in combination with Acemetacin.
- The risk or severity of hyponatremia can be increased when Alaproclate is combined with Benzthiazide.
- Benzthiazide may increase the excretion rate of Acyclovir which could result in a lower serum level and potentially a reduction in efficacy.
- The therapeutic efficacy of Benzthiazide can be decreased when used in combination with Acetylsalicylic acid.

DRUG PROFILE²¹⁻²³

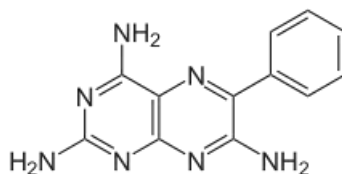
DRUG PROFILE:

Drug : Triamterene

Synonym : Triamteren
Triamterena
Triamtérène

Drug category : Cardiovascular Agents

Structure :



Chemical name/ Nomenclature / IUPAC Name: 6-phenylpteridine-2,4,7-triamine

Molecular Formula : C₁₂H₁₁N₇

Molecular Weight : 253.269 g/mol

Official Pharmacopoeia : USP

PHYSICOCHEMICAL PROPERTIES:

Description (Physical State) : Solid

Solubility: Triamterene is very slightly soluble in water, ethanol, and chloroform. It is practically insoluble in ether and soluble in formic acid. Very slightly soluble in

RESULTS AND DISCUSSION

METHOD DEVELOPMENT

7.1.1 Trials

Trial 1:

Mobile phase : Methanol: Water (60:40%v/v)
 Column : X-Bridge (4.6 ×150mm, 5µm particle size) Make; waters
 Flow rate : 1.0ml/min
 Wavelength : 262nm
 Column temp : 34°C
 Injection Volume : 10µl
 Run time : 10 minutes

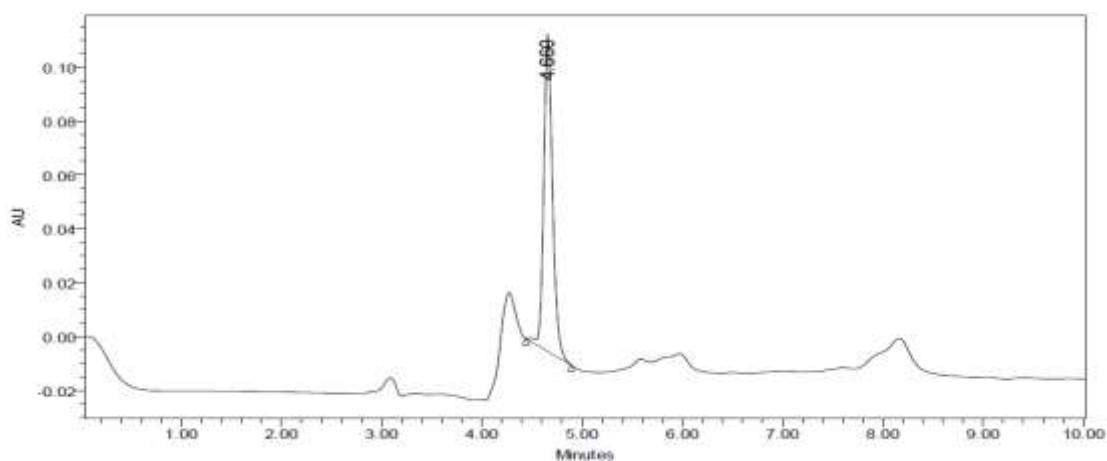


Fig 4: Chromatogram of trial 1

Table 6: Observation of trial 1 chromatogram

S. No	Peak Name	Retention Time	Area	Height	USP Tailing	USP Plate Count
1	Benzthiazide	4.660	721543	118542	1.4	12546.2

Inference:

The Retention Time observed from chromatogram was 4.66, only one peak was eluted.

Trial 2:

Mobile phase : Methanol: Water (80:20 v/v)
 Column : Inertsil C18 (4.6mm×250mm) 5µ Particle Size
 Flow rate : 0.8 ml/min
 Wavelength : 262nm
 Column temp : Ambient
 Injection Volume : 20µl
 Run time : 9 minutes

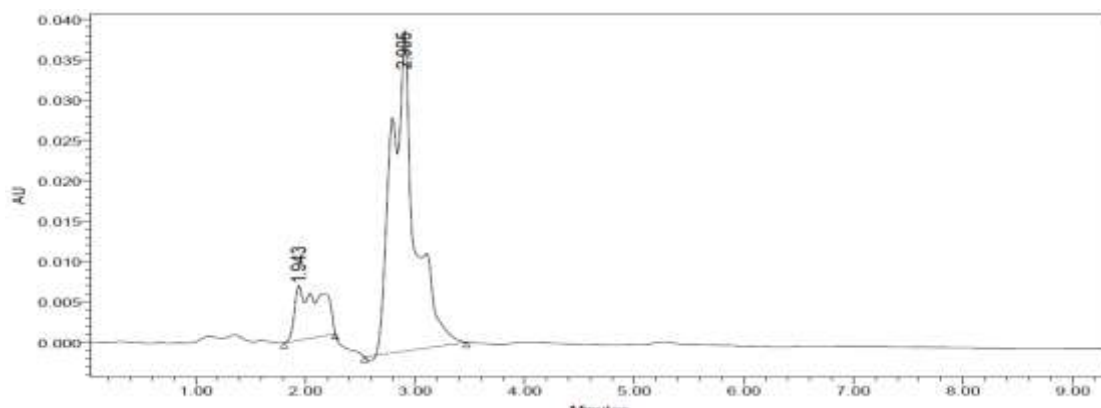


Fig 5: Chromatogram of trial 2

Table 7: Observation of trial 2 chromatogram

S.No	Peak Name	Retention Time	Area	Height	USP Tailing	USP Plate Count	USP Resolution
1	Benzthiazide	1.943	125461	6856	0.8	98.65	16
2	Triamterene	2.905	596532	39365	1.2	1521.9	

Inference:

The Retention Time, plate count decreased observed from the chromatogram by changing column.

Trial 3:

Mobile phase : Methanol: Acetonitrile (55:45 % v/v)

Column : Zorbax C18 (4.6mm×250mm 5µm)

Flow rate : 1.0 ml/min

Wavelength : 262 nm

Column temp : Ambient

Injection Volume : 10µl

Run time : 10 minutes

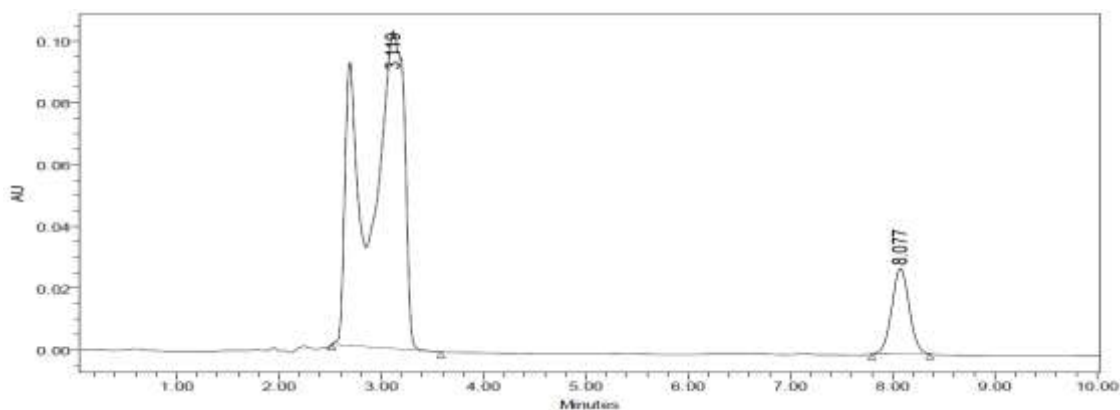


Fig 6: Chromatogram of trial 3

Table 8: Observation of trial 3 chromatogram

S. No	Peak Name	Retention Time	Area	USP Tailing	USP Plate Count	USP Resolution
1	Benzthiazide	3.119	2653265	0.9	854.0	9.0
2	Triamterene	8.077	345213	1.2	10256.5	

Inference:

1. The Retention Time is increased by decreased flow rate from 1.3 to 0.9ml /min.
2. Increased in the plate count observed from chromatogram.

Trial 4

Mobile phase : Acetonitrile: Phosphate Buffer (30:70 % v/v)
 Column : Symmetry C18 (4.6mm×250mm, 5µm)
 Flow rate : 1.0 ml/min
 Wavelength : 262 nm
 Column temp : 35°C
 Injection Volume : 20µl
 Run time : 8 minutes

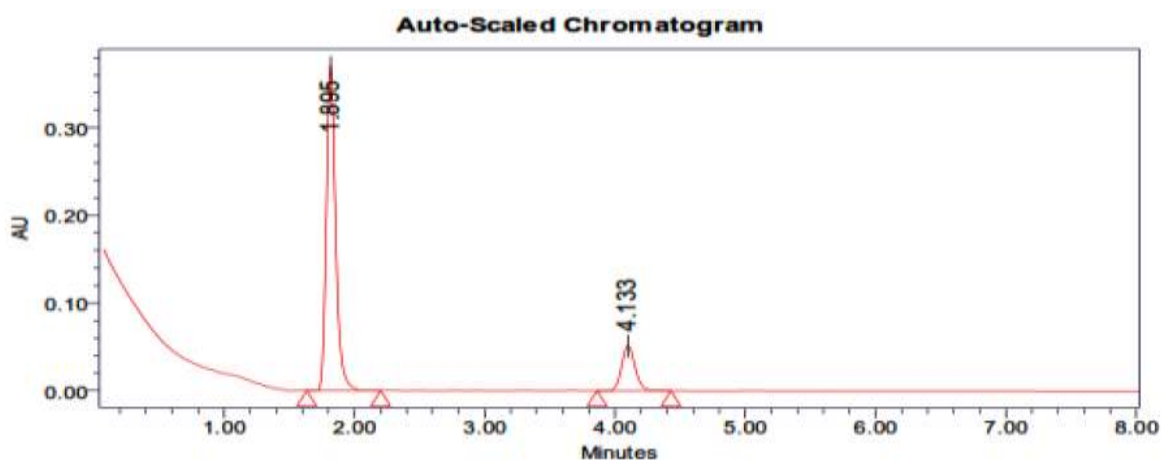


Fig 7: Chromatogram of trial 4

Table 9: Observation of trial 4 chromatogram

S. No	Peak Name	Retention Time	Area	USP Tailing	USP Plate Count	USP Resolution
1	Benzthiazide	1.895	1412532	1.2	9985	8.23
2	Triamterene	4.133	232564	1.5	6653	

Inference:

The Retention Time is decreased observed from chromatogram, for Benzthiazide 1.895 and Triamterene 4.133 min.

Trial 5

Mobile phase : Methanol: Acetate Buffer pH-4.6 (40:60v/v)
 Column : Agilent Zorbax (C18) (150mm x 4.6mm, 5µm) column
 Flow rate : 1.1 ml/min
 Wavelength : 262 nm
 Column temp : 40°C
 Injection Volume : 15µl
 Run time : 8 minutes

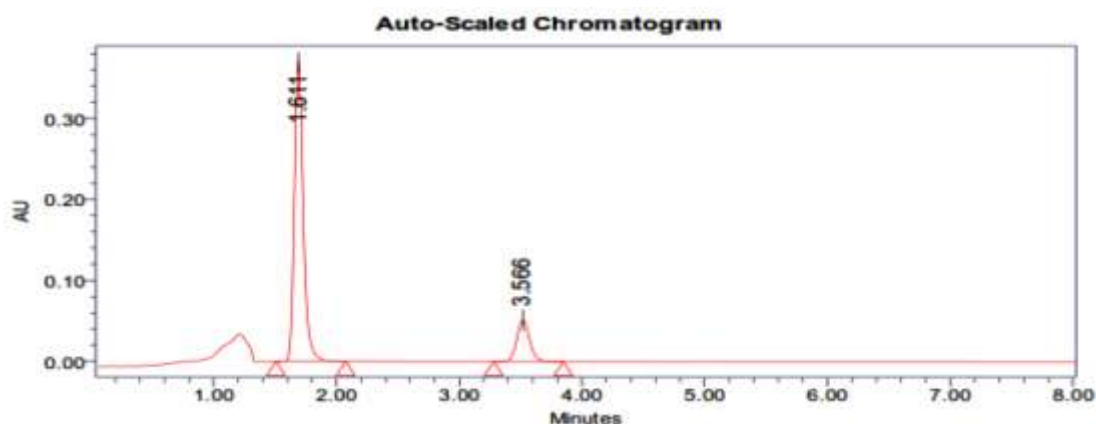


Fig 8: Chromatogram showing the trial 5

Table 10: Observation of trial 5 chromatogram

S. No	Peak Name	Retention Time	Area	USP Tailing	USP Plate Count	USP Resolution
1	Benzthiazide	1.611	1425685	1.39	6859	7.53
2	Triamterene	3.566	286598	1.25	8569	

Inference:

The Retention Time is decreased observed from chromatogram by increasing flow rate the retention time was Benzthiazide and Triamterene was found to be 1.611 and 3.566 min respectively.

Optimized Chromatogram

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

Temperature : 37°C

Column : Agilent Zorbax (C18) (150mm x 4.6mm, 5µm) column

Mobile phase : Methanol: Acetate Buffer pH-3.8 (24:76v/v)

Flow rate : 1ml/min

Wavelength : 262nm

Injection volume : 10 µl

Run time : 6 min

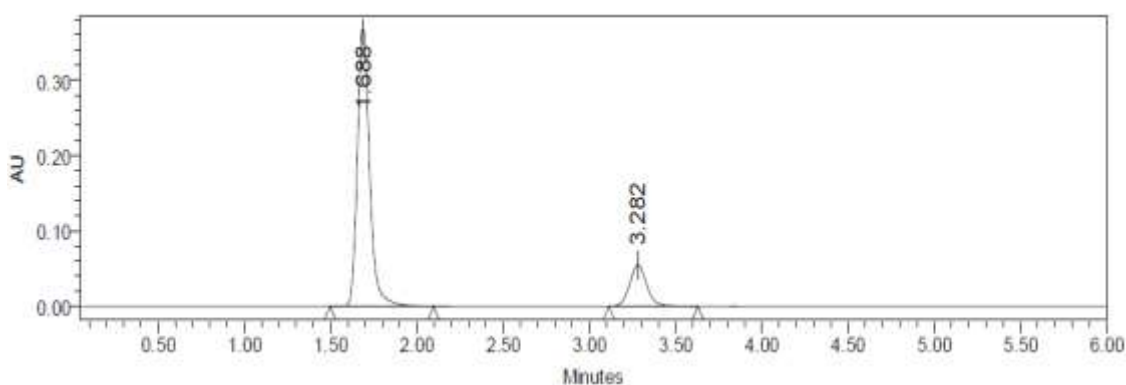


Fig 9: Optimized Chromatogram

Table 11: Observation of Optimized Chromatogram

S.No	Peak Name	Retention Time	Area	Height	USP Tailing	USP Plate Count	USP Resolution
1	Benzthiazide	1.688	1658785	385669	1.69	7586	10.85
2	Triamterene	3.282	425631	65245	1.58	6235	

Inference:

1. The Retention Time is decreased observed from chromatogram by increasing flow rate.
2. The retention time was Benzthiazide and Triamterene was found to be 1.688 and 3.282 respectively.
3. The tailing is not more than two and plate count observed is more than 2500. Pass all the system suitability parameters.
4. The peak shapes are good with good resolution and less Retention Time and more theoretical levels, pass the system suitability parameters.

Optimized Chromatographic Conditions

Table 12: Shows Optimized Chromatographic conditions

PARAMETER	OPTIMIZED CHROMATOGRAPHIC CONDITIONS
Mobile phase :	Methanol: Acetate Buffer pH-3.8 (24:76v/v)
Column :	Agilent Zorbax (C18) (150mm x 4.6mm, 5µm) column
Flow rate :	1ml/min
Diluent	Methanol: Acetate Buffer pH-3.8 (24:76v/v)
Injection Volume	10 µl
Wavelength:	262 nm
Column temp:	37°C
Run mode	Isocratic
Runtime	6minutes

- From the above experiment it was found that Benzthiazide and Triamterene can effectively be analyzed by using the RP-HPLC method with Mobile phase at a flow rate of 1 ml/min and detection wave length of 262nm.
- The retention time of Benzthiazide and Triamterene were found to be 1.688 and 3.282 minutes respectively.

Blank Chromatogram

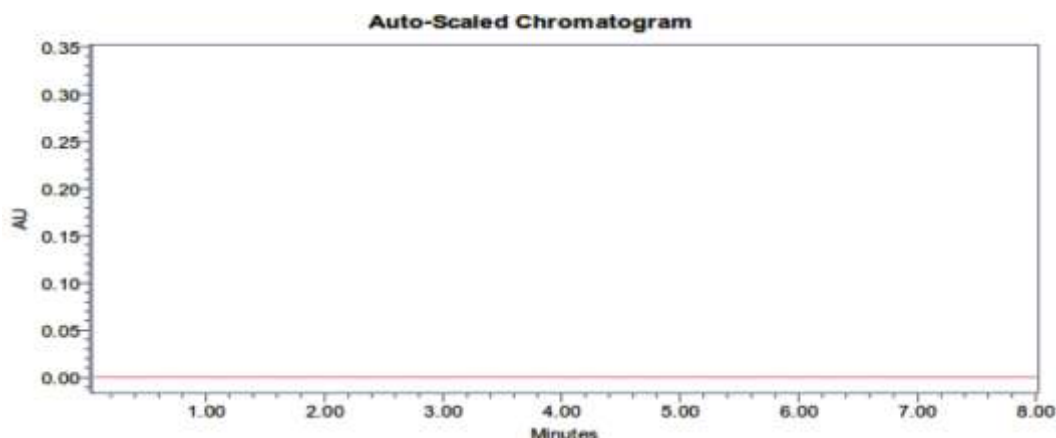
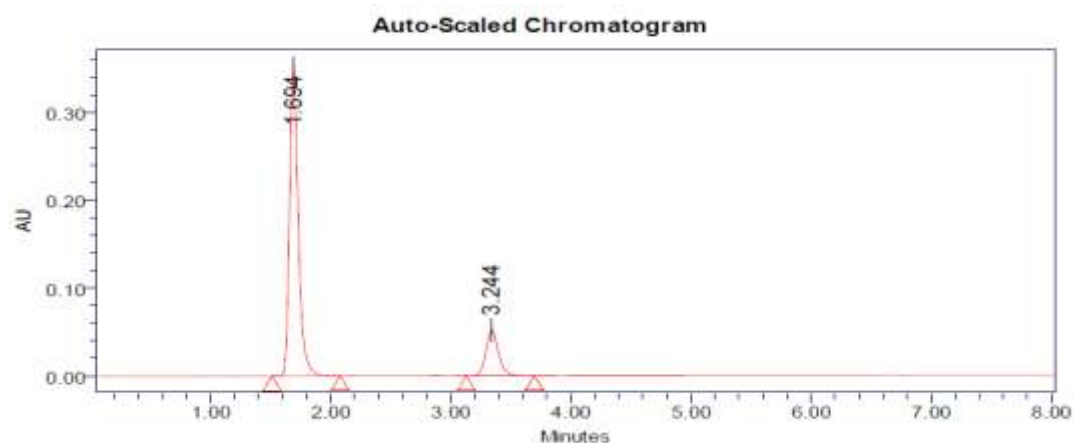
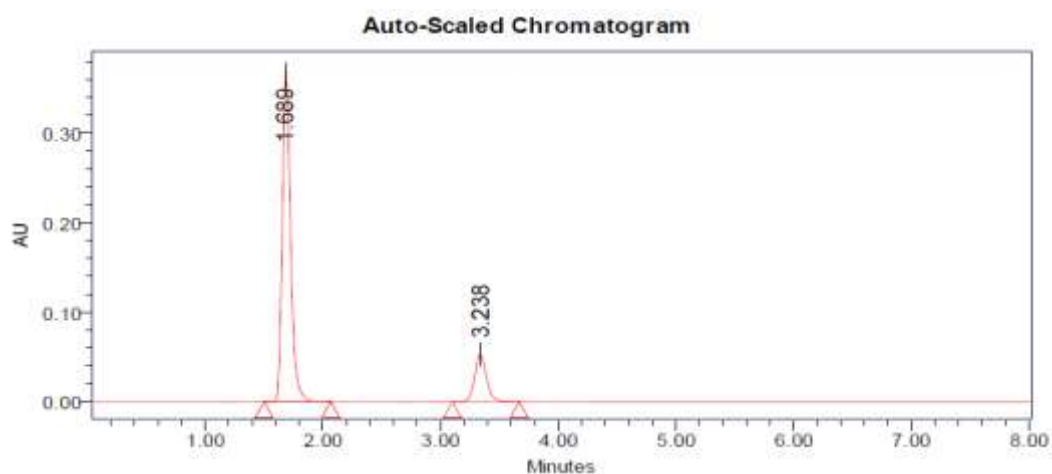


Fig 11: Blank Chromatogram

Standard Chromatogram**Fig 12: Standard Chromatogram-1****Table 13: Observation of Standard Chromatogram-1**

S.No	Peak Name	Retention Time (min)	Area	USP Tailing	USP Plate Count	USP Resolution
1	Benzthiazide	1.694	1659824	1.68	7595	10.89
2	Triamterene	3.244	426521	1.59	6274	

**Fig 13: standard Chromatogram-2****Table 14: Observation of standard Chromatogram-2**

S.No	Peak Name	Retention Time (min)	Area	USP Plate Count	USP Tailing	Resolution
1	Benzthiazide	1.689	1658598	7599	1.68	10.86
2	Triamterene	3.238	426598	6254	1.57	

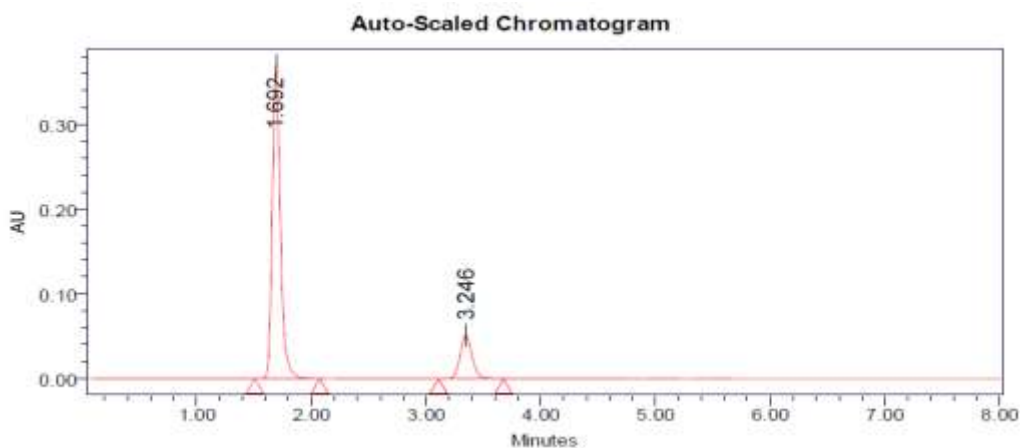


Fig 14: standard Chromatogram-3

Table 15: Observation of standard Chromatogram-3

S.No	Peak Name	Retention Time (min)	Area	USP Plate Count	USP Tailing
1	Benzthiazide	1.692	1658985	7659	1.70
2	Triamterene	3.246	425865	6359	1.59

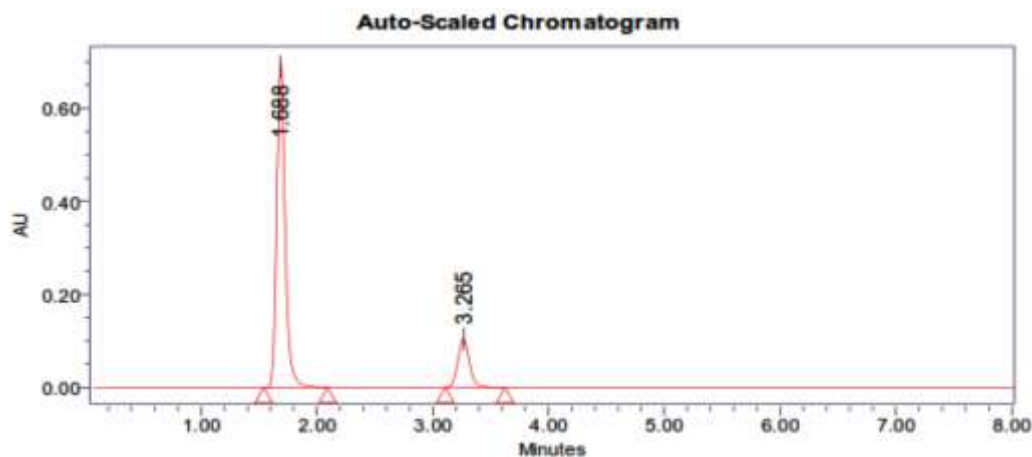


Fig 15: standard Chromatogram-4

Table 16: Observation of standard Chromatogram-4

S.No	Peak Name	Retention Time (min)	Area	USP Plate Count	USP Tailing
1	Benzthiazide	1.688	1658579	7672	1.71
2	Triamterene	3.265	435698	6295	1.60

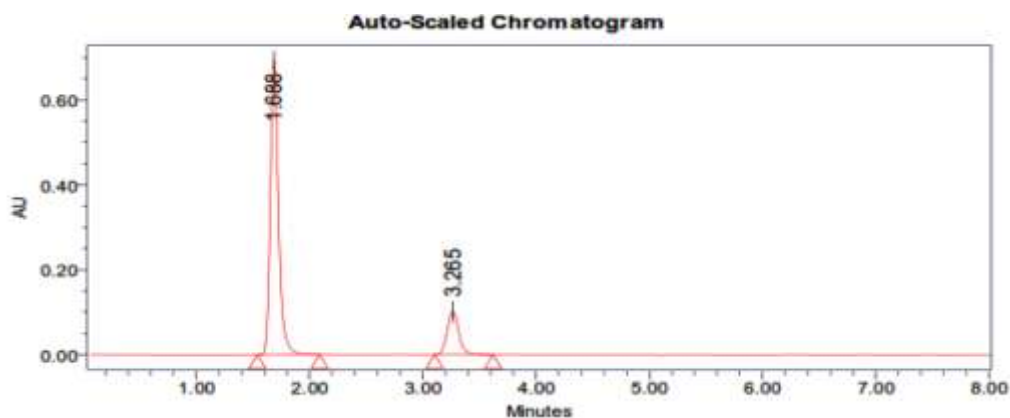
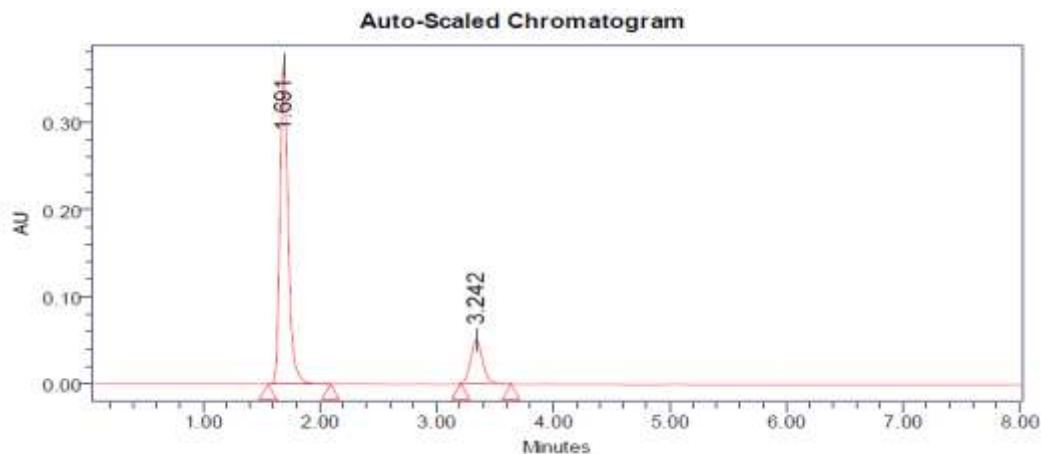


Fig 16: Standard Chromatogram-5

Table 17: Observation of standard Chromatogram-5

S.No	Peak Name	Retention Time (min)	Area	USP Plate Count	USP Tailing
1	Benzthiazide	1.688	1659852	7695	1.69
2	Triamterene	3.265	436598	6498	1.59

Assay (Sample):**CONCLUSION**

High performance liquid chromatography is at present one of the most sophisticated tool of the analysis. The estimation of Benzthiazide and Triamterene was done by RP-HPLC. The Phosphate buffer was p^H 3.8 and the mobile phase was optimized with consists of Methanol: acetate buffer (pH-3.8) mixed in the ratio of 24:76%v/v. An Agilent Zorbax (C18) (150mm x 4.6mm, 5 μ m) column or equivalent chemically bonded to porous silica particles were used as stationary phase. The solutions were chromatographed at a constant flow rate of 1.0 ml/min. The linearity range of Benzthiazide and Triamterene were found to be from 100-500 μ g/ml, 30-70 μ g/ml respectively. Linear regression coefficient was not more than 0.999, 0.999.

The values of % RSD are less than 2% indicating accuracy and precision of the method. The percentage recovery varies from 98-102% of Benzthiazide and Triamterene. LOD and LOQ were found to be within limits.

The results obtained on the validation parameters met ICH and USP requirements. It inferred the method found to be simple, accurate, precise and linear. The method was found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision.

BIBLIOGRAPHY

1. P.D. Sethi. HPLC: Quantitative analysis pharmaceutical formulations, CBS publishers and distributors, New Delhi (India), 2001, P.3-137.
2. Michael E, Scharzt IS, Krull. Analytical method development and validation. 2004, P. 25-46.
3. R. Snyder, J. Kirkland, L. Glajch. Practical HPLC method development, 2nd ed, A Wiley international publication, 1997, P.235, 266-268,351-353.653-600.686-695.
4. Basic education in analytical chemistry. Analytical science, 2001:17(1).
5. Method validation guidelines international Conference on harmonization; GENEVA; 1996
6. Berry RI, Nash AR. Pharmaceutical process validation, Analytical method validation, Marcel Dekker Inc. New work, 1993; 57:411-28
7. Anthony C Moffat, M David Osselton, Brian Widdop. Clarke's analysis of drugs and poisons, Pharmaceutical press, London, 2004, P.1109-1110, 1601-1602.
8. Klaus Florey, Analysis profile of drugs substances, Academic press, New York, 2005, P.406-435.
9. Sharma BK. Instrumental methods of chemical analysis, Introduction to analytical chemistry, 23th ed .Goel publishing house meerut, 2004, P12-23.

10. H.H. Willard, L.L. Merritt, J.A. Dean, F.A. Settle. Instrumental methods of analysis, 7th edition, CBS publishers and distributors, New Delhi. 1986, P.518-521, 580-610.
11. John Adamovics, Chromatographic analysis of pharmaceutical, Marcel Dekker Inc. New York, 2nd ed, P.74, 5-15.
12. Gurdeep Chatwal, Sahm K. Anand. Instrumental methods of chemical analysis, 5th edition, Himalaya publishing house, New Delhi, 2002, P.1.1-1.8, 2.566-2.570
13. D. A. Skoog, J. Holler, T.A. Nieman. Principle of instrumental analysis, 5th edition, Saunders college publishing, 1998, P.778-787.
14. Skoog, Holler, Nieman. Principals of instrumental analysis 5th ed, Harcourt publishers international company, 2001, P.543-554.
15. William Kemp. Organic spectroscopy, Palgrave, New York, 2005, P.7-10, 328-330.
16. P.N. Arora, P.K. Malhan. Biostatistics, Himalaya publishers house, India, P.113,139-140,154.
17. Doserge, Wilson and Gisvold's text book of organic medicinal and pharmaceutical chemistry, 8th ed, Lippincott Company, 1982, P.183-197.
18. <https://www.drugbank.ca/drugs/DB00562>
19. <https://en.wikipedia.org/wiki/Benzthiazide>
20. <https://www.tabletwise.com/medicine/benzthiazide/contraindications>
21. <https://www.drugbank.ca/drugs/DB00384>
22. <https://pubchem.ncbi.nlm.nih.gov/compound/triamterene#section=GC-MS>
23. <https://en.wikipedia.org/wiki/Triamterene>
24. VC Chauhan*, VN Shah, DA Shah, RR Parmar, Development and Validation of RP-HPLC Method for Simultaneous Estimation of Triamterene and Benzthiazide in Tablets, Pharma Tutor; 2014; 2(6); 115-122.
25. Rachita Jain*, Shivani Majithia, Patel Mayur, Reena Chaudhary and Kavita Pareek, RP-HPLC Method Development And Validation For Simultaneous Estimation Of Benzthiazide And Triamterene In Their Combined Dosage Form, World Journal of Pharmaceutical Research, Volume 6, Issue 4, 1635-1642.
26. Suresh Babu M*, Sai Lakshmi A D S, John Subhakar K And Bharat Kumar N, RP-HPLC Method For The Simultaneous Estimation Of Triamterene And Benzthiazide Pharmaceutical Dosage Forms, International Journal Of Pharmacy And Pharmaceutical Analysis, Ijppa, 2018; Vol-01(01); 13-19.
27. P. Hema, P. Chandra Kiran, B. Amalswara Rao, A. Shanta Kumari*, Chemometric assisted RP-HPLC quantitative estimation and validation of hydrochlorothiazide and Triamterene in tablet dosage form, Int. J. of Pharmacy and Analytical Research Vol-6(3) 2017 [442-448].
28. P.D. Sethi. HPLC: Quantitative analysis pharmaceutical formulations, CBS publishers and distributors, New Delhi (India), 2001, P.3-137.
29. Michael E, Scharz IS, Krull. Analytical method development and validation. 2004, P. 25-46.
30. R. Snyder, J. Kirkland, L. Glajch. Practical HPLC method development, 2nd ed, A Wiley international publication, 1997, P.235, 266-268,351-353.653-600.686-695.
31. Basic education in analytical chemistry. Analytical science, 2001:17(1).
32. Method validation guidelines international Conference on harmonization; GENEVA; 1996
33. Berry RI, Nash AR. Pharmaceutical process validation, Analytical method validation, Marcel Dekker Inc. New work, 1993; 57:411-28
34. Anthony C Moffat, M David Osselton, Brian Widdop. Clarke's analysis of drugs and poisons, Pharmaceutical press, London, 2004, P.1109-1110, 1601-1602.
35. Klaus Florey, Analysis profile of drugs substances, Academic press, New York, 2005, P.406-435.

36. Sharma BK. Instrumental methods of chemical analysis, Introduction to analytical chemistry, 23th ed .Goel publishing house meerut, 2004, P12-23.
37. H.H. Willard, L.L. Merritt, J.A. Dean, F.A. Settle. Instrumental methods of analysis, 7th edition, CBS publishers and distributors, New Delhi. 1986, P.518-521, 580-610.
38. John Adamovics, Chromatographic analysis of pharmaceutical, Marcel Dekker Inc. New York, 2nd ed, P.74, 5-15.
39. Gurdeep Chatwal, Sahm K. Anand. Instrumental methods of chemical analysis, 5th edition, Himalaya publishing house, New Delhi, 2002, P.1.1-1.8, 2.566-2.570
40. D. A. Skoog, J. Holler, T.A. Nieman. Principle of instrumental analysis, 5th edition, Saunders college publishing, 1998, P.778-787.
41. Skoog, Holler, Nieman. Principals of instrumental analysis 5th ed, Harcourt publishers international company, 2001, P.543-554.
42. William Kemp. Organic spectroscopy, Palgrave, New York, 2005, P.7-10, 328-330.
43. P.N. Arora, P.K. Malhan. Biostatistics, Himalaya publishers house, India, P.113,139-140,154.
44. Doserge, Wilson and Gisvold's text book of organic medicinal and pharmaceutical chemistry, 8th ed, Lippincott Company, 1982, P.183-197.
45. <https://www.drugbank.ca/drugs/DB00562>
46. <https://en.wikipedia.org/wiki/Benzthiazide>
47. <https://www.tabletwise.com/medicine/benzthiazide/contraindications>
48. <https://www.drugbank.ca/drugs/DB00384>
49. <https://pubchem.ncbi.nlm.nih.gov/compound/triamterene#section=GC-MS>
50. <https://en.wikipedia.org/wiki/Triamterene>
51. VC Chauhan*, VN Shah, DA Shah, RR Parmar, Development and Validation of RP-HPLC Method for Simultaneous Estimation of Triamterene and Benzthiazide in Tablets, Pharma Tutor; 2014; 2(6); 115-122.
52. Rachita Jain*, Shivani Majithia, Patel Mayur, Reena Chaudhary and Kavita Pareek, RP-HPLC Method Development And Validation For Simultaneous Estimation Of Benzthiazide And Triamterene In Their Combined Dosage Form, World Journal of Pharmaceutical Research, Volume 6, Issue 4, 1635-1642.
53. Suresh Babu M*, Sai Lakshmi A D S, John Subhakar K And Bharat Kumar N, RP-HPLC Method For The Simultaneous Estimation Of Triamterene And Benzthiazide Pharmaceutical Dosage Forms, International Journal Of Pharmacy And Pharmaceutical Analysis, Ijppa, 2018; Vol-01(01); 13-19.
54. P. Hema, P. Chandra Kiran, B. Amaleswara Rao, A. Shanta Kumari*, Chemometric assisted RP-HPLC quantitative estimation and validation of hydrochlorothiazide and Triamterene in tablet dosage form, Int. J. of Pharmacy and Analytical Research Vol-6(3) 2017 [442-448].