ISSN No:-2456-2165

Analysis of Hydrocholorothiazide by Using Ultra Performance Liquid Chromatography

Anupama Maurya*, Urmila Nishad, Meraj Ali, Manoj Kumar Yadav, Vijay Kumar Yadav
*Faculty of J.P. Verma College of Pharmacy Terwa, Gausgunj, Hardoi (U.P)
Faculty of Gaya Prasad Institute of Human Excellence for Pharmacy, Malihabad
Faulty of Department of Pharmacy Dr. B. R. Ambedkar University, Agra

Abstract:- A new, simple, precise and stability-indicating UPLC (Ultra Performance Liquid Chromatography) method was developed and validated for the simultaneous determination of Diuretic Drug of thiazide class of Hydrochlorothiazide in urine sample.

It involved 150 mm × 2.1 mm, Acclaim column. The separation was achieved on simple gradient method. The mobile phase was composed 0.2% TFA in water and 0.2% TFA in acetonitrile. The flow rate of the mobile phase was set at 0.5 ml/min^{-1} for gradient elution and column temperature was maintained at 40˚C. The detector wavelength was 271 nm for Hydrochlorothiazide. The retention times of Hydrochlorothiazide are 2.9 and 3.4 minutes; respectively. The total run time was 6.0 minutes within which two compounds and was separated. The described method was validated with respect to system linearity, precision and accuracy. The precision of the assay method was evaluated by carrying out six independent assays Hydrochlorothiazide. The described method was linear over the range, 0 .625 to 10 µg·mL^{-1} for Hydrochlorothiazide. The detection limit for HCTZ 0.4602580, LOQ for HCTZ 1.534194 The method was validated in terms of linearity, accuracy and reproducibility. Recovery was in the range of 93 – 108%. The method is fast and is suitable for analysis of hydrochlorothiazide in urine samples.

I. INTRODUCTION

Hydrochlorothiazide and Chlorothiazide are thiazide diuretic. All thiazides have a similar dual-ring structure. These are drug belongs to diuretic class, are drug mainly used in the treatment of hypertension, used as a antihypertensive, and also used in the other treatment such as heart failure, lung disease, kidney failure.

In 1937 sulphonamide was found to cause metabolic acidosis in patients. Carbonic anhydrase (CA) had been found in 1932. The first modern orally active of Chlorothiazide was discovered in 1957 and other thiazide drugs were discovered in 1960. The searching more potent class of diuretic drug continued, led to the development of furosemide and ethacrynic acid (Jackson and Chabbers, 2001).

➢ *Types of Drug*

- High efficacy of diuretics Sulphamoyl derivatives (Inhibitors of cotransport Na+-K+ -2Cr) Furosemide, Bumetanide, Torasemide
- Medium efficacy of diuretics (Inhibitors of symport Na+-Cl) Hydrochlorothiazide, Chlorothiazide, Benzthiazide, Hydroflumethiazide.
- Weak and adjunctive diuretics

✓ Inhibitors of Carbonic anhydrase
Acetazolamide
✓ Diuretics of Potassium sparing
Spironolactone
✓ Osmotic diuretics
Manitol, Isosorbide, Glycerol

➢ *Renal Anatomy and Physiology*

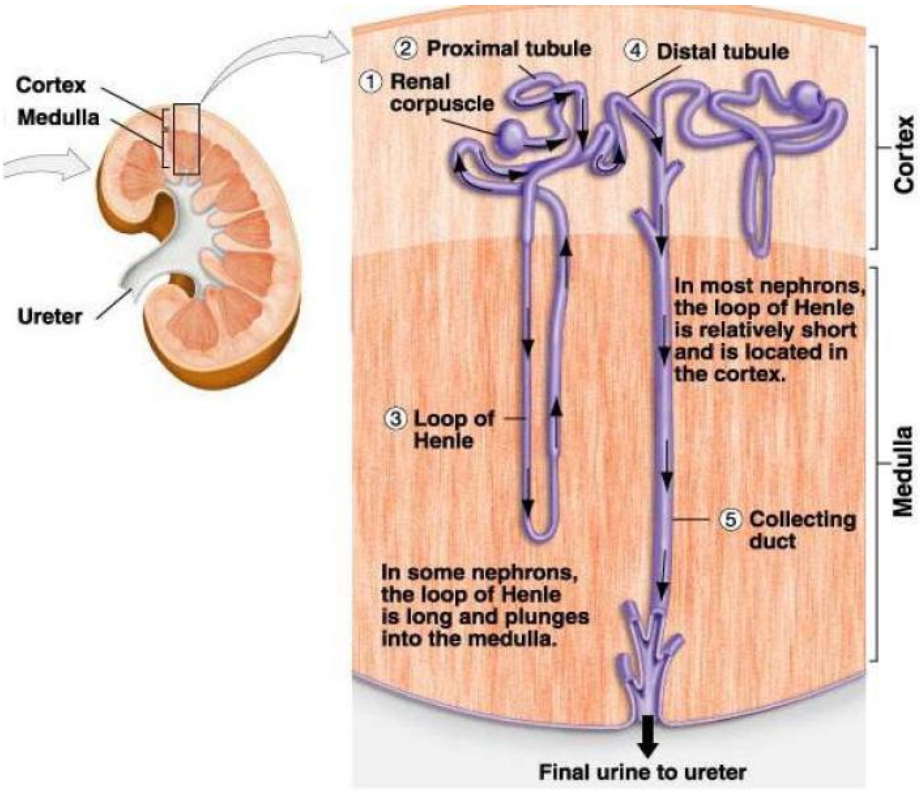


Fig 1:- The Anatomy of Kidney and Nephron (Guyton, 2006)

A pair of bean shaped kidneys organs found along the posterior wall of the abdominal cavity. The left kidney is found to be somewhat higher than the kidney in light of the fact that the right part of the liver is much larger than the left. The kidneys, not at all like the alternate organs of abdominal depression, are found back in the peritoneum and contact with the muscles of the back. The kidneys are surrounded by a layer of fat that establishes them and protects them from physical harm. The kidneys filter metabolic wastes, excess ions and chemicals from the blood to make urine (Sands and verlander, 2010).

In sagittal section, the kidneys have three main regions: the outer region called cortex, the central region called medulla and the innermost tip of the inner medulla called papilla (Guyton and Hall, 2006; Saladin, 2008).

Blood enters each kidney via a renal artery and leaves through a small veins into the renal vein, which drains into the inferior venacava (Saladin, 2008).

➢ *Mechanism of Action Diuretics*

Many diuretics exerting theirs effects on specific membrane transport proteins in renal tubular epithelial cells.

Different diuretics, apply Osmotic impacts that avert water reabsorption of (Manitol), hinder compounds (acetazolamide), or meddle with hormone receptors in renal epithelial cell (aldosterone receptor blockers).

Loop diuretics - these are drugs Prevent the Na^+ - K^+ - $2Cl^-$ Cotransporter present in the thick ascending loop of Henle.

Uses –Edema; Acute pulmonary Edema, cerebral Edema, hypertension hypercalcaemia and kidney stone

Thiazide Diuretics- these are drugs prevent the Na+ - 2Cl- Cortical segment of loop of Henle and early of portion of duct

Uses -Edema, hypertension, in diabetes insipidus.

Carbonic anhydrase inhibitors- These agents inhibit to carbonic anhydrase enzyme which is responsible for the reabsorption of Na^+ ion in the exchange of H^+ ion in the Proximal Convoluted Tube. Inhibition of this enzyme prevents the reabsorption of Na^+ ion from PCT.

Carbonic anhydrase inhibitors- These agents inhibit to carbonic anhydrase enzyme which is responsible for the reabsorption of Na^+ ion in the exchange of H^+ ion in the Proximal Convoluted Tube. Inhibition of this enzyme prevents the reabsorption of Na^+ ion from PCT.

Uses- used for the treatment of glaucoma. They may likewise be utilized to treat seizure issue and intense mountain infection. Since they energize solubilisation and discharge of uric corrosive, they can be utilized in the treatment of gout.

ISSN No:-2456-2165

Potassium sparing diuretics act primarily at the collecting tubules and DCT either by direct blockage of mineralocorticoid receptors (e.g., Spironolactone and triamterene) or blocking epithelial sodium channels in the luminal membrane (e.g., Amiloride and triamterene).

Uses- Hypertension and Edema

Osmotic diuretics- osmotic diuretic is a diuretic that inhibits reabsorption of water and sodium (Na).

Uses- Such agents can be used to reduce intracranial pressure and to promote prompt removal of renal toxins

➢ *Drug Profile of Hydrochlorothiazide*

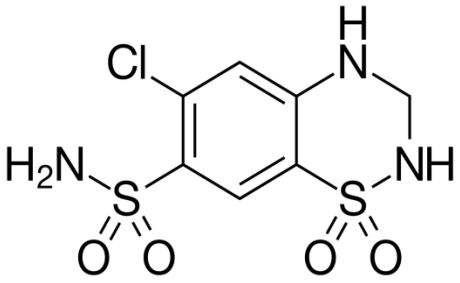
Chemical structure	
IUPC Name	6-chloro-1,1-dioxo-3,4-dihydro-2H-1,2,4-benzthiadiazine-7-sulfonamide
Chemical Names	Hydrochlorothiazide; 58-93-5; Hypothiazide; Esidrix; HCTZ;
Formula of Molecule	$C_7H_8ClN_3O_4S_2$
Molecular Weights	297.73912 g/mol
Appearance	White powder
Melting points	273-275oC
Solubiliteis	These are soluble in dilute ammonia or sodium hydroxide and also soluble in methanol, ethanol, acetone
Storage Condirion	Its storage condition at Room temp
Therapeutics category	Thiazide diuretic Antihypertensive agent
Mechanism of actions	Hydrochlorothiazide, is also known as thiazide diuretic, its inhibitions of water reabsorption in the nephron through inhibiting the Na-Cl Symporter in the distal convoluted tubule, which was responcible for 5% of Na reabsorbtion.
Indication	Treatment for the hypertension and administration in edema heart congestive failure
pKa	7.9
Bioavability	60-80
Half life	4-6 hrs
Elimination	Renal

Table 1

ISSN No:-2456-2165

II. DEVELOPMENT OF METHOD AND VALIDATION

➢ *Method Development:-*

Analyticals chemistry include with method for identification, extraction, separations and quantifications of the chemical component of natural and synthetic ingredients.

Initiatory procedure which has to be applied in method development is the sample preparation.

➢ *Sample Preparation*

The sample preparation involves several techniques like dissolution, extraction, reaction with chemical species, treatment with chelating agents (EDTA), masking, filtering, dilution, sub-sampling, pulverizing (grinding, crushing) etc. Sample preparation is an important step since it nullifies the effect of interfering species present in analyte by removing it through extraction.

Facilitate extraction is performed for the recognizable proof of the analyte of intrest. This includes arrangement of methodologies.

EXTRACTION - It is a process through which desired substance (analyte) is separated from a mixture. In this mixture is brought into contact with a solvent in which analyte of interest is soluble, while others are insoluble. This extraction can be carried out by two methodologies-s

➢ *Classical Method*

- *LLE (Liquid-Liquid Extraction)-*

It is an extraction of an analyte in liquid phase into another liquid phase. The two liquid phases which are taken are of different polarities as a result, more polar solute dissolve in more polar solvent and less polar solute dissolve in less polar solvent. Example: Extraction of caffeine from water by taking chloroform. Here water and chloroform are of different polaritie

- *SPE (Solid Phase Extraction)-*

This technique can be used to extract analytes from wide variety of matrices including urine, blood, water, beverages, soil and animal tissue (Hennion 1999),(Augusto et al. 2013),(Whang and Pawliszyn 1998). Here cartridges are in the form of stationary phase in which desired analyte are retained on it. The cartridges used in this are C-18, C-8, Alumina etc.

- *Soxhlet Extraction*

This type of sample preparation is carried out with the help of an assembly known as soxhlet extractor. This apparatus was invented by Franz Von Soxhlet.

This type of extraction is used when the analyte has a limited solubility in solvent, and the impurity is insoluble in the solvent. In this the source compound from which analyte has to be extracting is kept inside the thimble and then this thimble is loaded into the main chamber of Soxhlet extractor. The extraction of solvents is placed in distillations flask which is given heat continuously. Here the extraction solvent used (acetone, chloroform, ethyl acetate, hexane and methanol) is not eco-friendly. As a result, the experiment has to be performed carefully.

- *Quechers*

This type of extraction is a streamlined approach which makes it easy and cheap for examining pesticides residues in food. This type of extraction is confined to the pesticide extraction only. The name is formed by the blends of words quick, easy, cheap, effective, rugged and safe. In this extraction process the analyst first homogenizes the sample through blender and then is placed in tarsons with reagent which is agitated by centrifugation for 1 min.

- *PLE (Pressurized Liquid Extraction)*

The extraction process is involve extraction with solvents at the higher pressures and temperature e.g. foods and biologicals sample. In general; higher temperatures will cause and increases of the PLE efficiency.

- *MAE (Microwave Assisted Extraction)-*

The MAE are usually high extraction of rates due to the very rapids heating and the elevatedting temperatures, and the ease of instruments operation.

III. MICROEXTRACTION

A. SPME (Solid-phase microextraction) –

In 1990, solid-phase microextraction (SPME) was introduced through the Arthur and Pawliszyn an free-from organic-solvents extraction techniques. Mostly,the techniques of enables the trace enrichment of analytes through the exposure of a fused-silica fibers coated with a suitable sorbent layers, Selected time of a gas or liquids sample, with the subsequents (rapid) desorption of the targeted analytes by the heating exposed fiber in the injection ports of a GC. A number of fibers coating, which offer a wide ranges of analyte solubilities and porositiy are commercially available.

B. DLLME (Dispersive Liquid-Liquid Microextraction)-

This microextraction technique was introduced by Reazee.et.al in 2006. In this process of microextraction there are three phases-(1) extraction phase (2) aqueous phase (3) dispersion phase.

The solvent used in dispersing phase are methanol, acetone and acetonitrile. Whereas, solvents used in extraction phase are chlorinated solvents.

Dispersive solvent- acetone, acetonitrile and methanol(dispersive phase miscible with both extraction solvent and aqueous phase), Extraction solvent- hexane, xylene heptane Dichloromethane Chloroform, Chlorobenzene(immiscible with aqueous phase).

ISSN No:-2456-2165

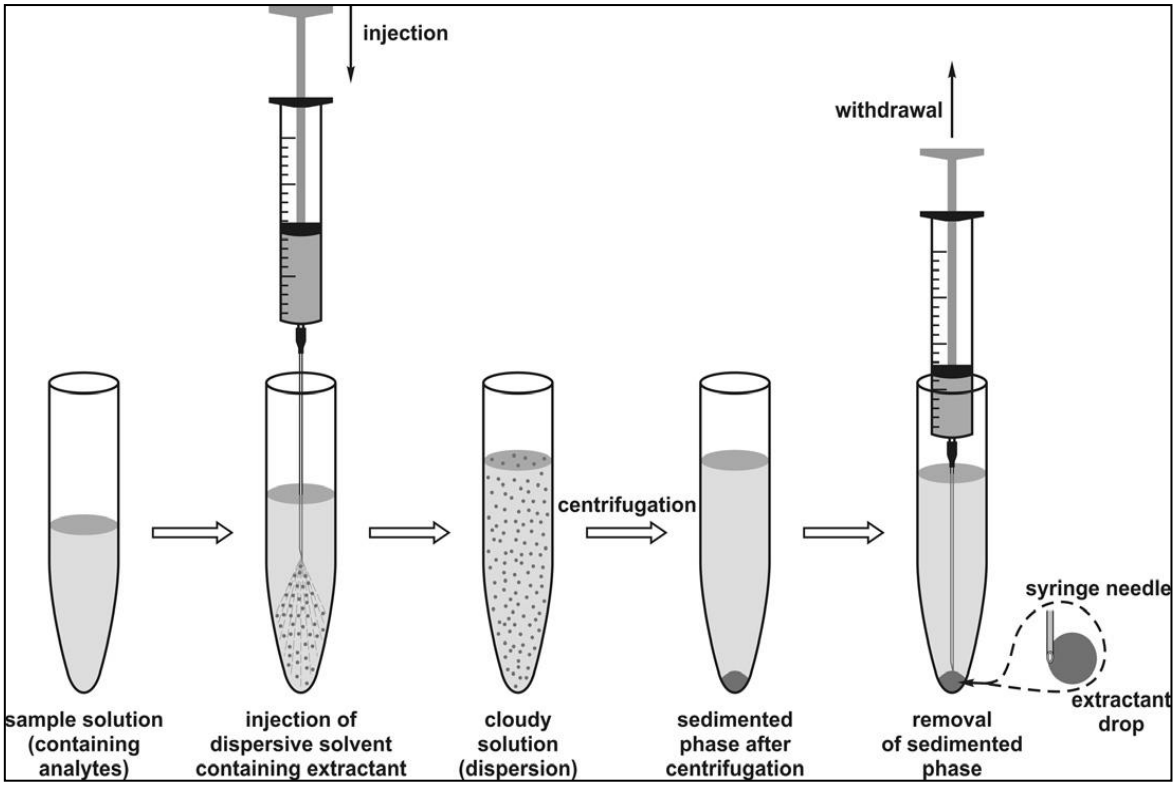


Fig 2:- Dispersive liquid-liquid microextraction. (Agnieszka Zgola-Grzes'kowiak)

In DLLME SFO the analyte along with extraction solvent floats on the surface of sample which is further solidified by freezing it. As a result, solidified organic droplet is formed which is injected in chromatographic instrument for analysis.

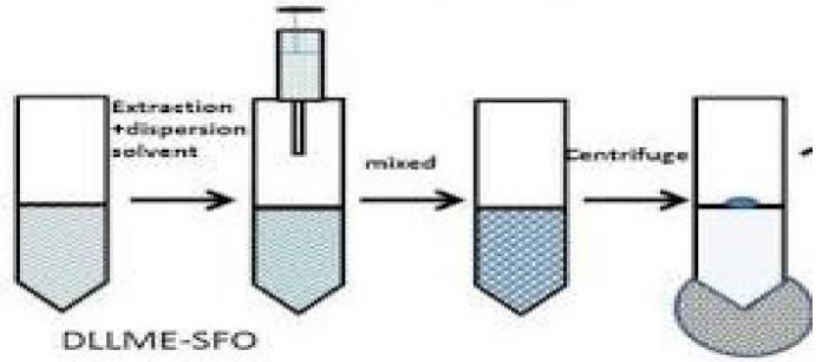


Fig 3:- DLLME Solidification of floating organic drop

➢ *Application of DLLME*

- The novel samplw – preparation method
- The DLLME can be combined with GC, HPLC, AAS, UV- VIS Spectophotometer.
- It most usely for the analysis of both organic and inorganic compounds.
- This is used as green chemistry since it reduced consumption of hazardous organic solvent

C. VALLME (Vortex Assisted Liquid-Liquid Microextraction)-

This microextraction technique was introduced by Yiantchi.et.al in 2010. In this process of microextraction there are 2 phases- (1) extraction phase and (2) aqueous phase. The main difference between DLLME and VALLME is that dispersion solvent is replaced by vortex agitation and extraction solvents used here are low density solvents like Dodecanol, 2-dodecanol, 2-undecanone etc. or ionic liquids like 1-butyl-3- methylimidazolium hexafluorophosphate ([BMIM]PF6) etc. The extraction process involves two steps- (1) The extraction solvent is rapidly injected in water sample and is agitated by

vortexing it. Dispersion formed and facilitates fast extraction of analytes from the water sample. (2) The dispersion is removed by micro syringe.

In comparison to vortex assisted liquid-liquid microextraction (VALLME), liquid-liquid extraction (LLE), solid phase microextraction (SPME) and solid phase extraction (SPE) are more tedious, laborious and time consuming process. In this we are doing simultaneously deivatization and extraction. So VALLME is a rapid and sensitive technique for the detection of minor quantity of sample. This technique is also useful in the detection of pharmaceutical product, in forensic laboratory for the detection of explosive, suicidal cases, arson cases, narcotics drugs, pesticides, poisons, metals etc.

➢ *Method Validation*

The technique was approved through the International Conferencess on Harmonization rules for the validation of analyticals pocedure. Approval is requried for some compounds or corrected compounds of guarantee that is cabable for giving reproducible and solid outcomes. when used by the differents operator employing the same equipments in the same different laboratory. The types of validation program required depend entirely on particularly methods and its proposed applications.

➢ *Component Method validation*

- *Accuracy*

Agreement between the test method obtained by the proposed method and the true value. This expresses the purity of the method.

Should be reported as:

✓ Percentage of recovery to known amount added or
✓ The difference the mean assay result and the accepted value
✓ The absolute error is ameasure of the accuracy of the measurement, it is is then calculated as , Absolute error = Mean error / true value X100

- *Precision:*

Precision refer to the agreement among the individual test results when a method is applied repeatedly to the same sample. It measure of degrre of repeatbility of a method. The presion of analytical precdure is expressed by :

$$\textbf{RSD} = \frac{\textbf{standard deviation} \times 100}{\textbf{mean}}$$

- *Specificity:*

Specificity is the ability to judge obviously the analyte within the sight of constituents which might be evaluated to be available.

- *Range:*

The range of analytical technique is the gaping betwwen the upper and lower amount of the analyte. Focus bend ought to be direct no less than 5-6 point in the range. Concentration curve should be linear at least 5-6 point in the range.

- *Limit of Detection:*

The range of detection 20 is the lowest concentration of analyte in a sample that can be detected but not quantitatively determined using a specific method under the required experimental conditions.

$$\textbf{LOD} = \frac{\textbf{3.3 x standard deviation of the regression line}}{\textbf{Slope}}$$

S= slope of the calibration curve of the analyte.

- *Limit of Quantitation:*

The limit of quantization is the lowest concentration of analysis in a sample that can be determined with acceptable accuracy and precision under determined experimental conditions. The limit of the volume is expressed as the concentration of the sample analyzed (eg, percent, parts per million).

$$\text{LOQ} = \frac{\textbf{10 x standard deviation of the regression line}}{\textbf{Slope}}$$

IV. CHROMATOGRAPHY

The chromatography word derived from two Greek words –“ colour writing”. This is technique used in biochemical lab ,analytical ,for separation and identification. everal types of chromatography used such as – paper chromatography , thin layer chromatography , gas chromatography , and high performance liquid chromatography, or HPLC.

This is for the separation of mixture. It involving passing of liquid the sample. Chromatography is in most scientific laborities around the word . for example – forensic lab, analytical lab and in forensic crime laboratories.

Chromatography is allow a mixtre of different chemical labs to be distrbuted or partion betwwen a stationary phase(eluent or solvent) the mobile phase may be liquid or gas , the stationary phase is solid. The mobile phase flow over the stationary phase.

A. Principles of Chromatography

Great difference in the retention of various components on the material filling the column, a small column can be used to separate and isolate a rapidly growing component from a highly maintained material. This is the basis of a useful sample preparation technique called solid phase extraction (SPE). Real instrumental chromatography employs highly engineered materials for the stationary phase past, with a continuous division of analytics between the two phases, a mobile phase fluid carrying a mixture of analytics. The column must be sufficiently long and slow enough to process.

B. Types of Chromatography

The chromatography is mainly divided into two parts :
1) Gas Chromatography 2) Liquid Chromatography

➢ *Gas Chromatography*

In gas chromatography sample is vaporized and injected on to the head of the chromatographic column .

- *Gas Liquid Chromatoraphy*

In gas liquid chromatography the mobile phase is gas and stationary phase is a liquid that is retained on the surface of inert solid by adssorption or chemical bonding.

- *Gas Solid Chromatoraphy*

In gas solid chromatography the mobile phase is gas and stationary phase is a solid that retains the analytee by physical adsorption. This chromatpgraohy allows the separation of and determination of low mass gases such as air component, carbon monooxide and nitrogen oxides.

➢ *Liquid Chromatography*

The separation occurs on based the in intraction of sample with mobile phases. There are many mobile phase and stationary phase combination that can be employed while seperating a mixture. types of liquid chromatography:

- *Thin Layer Chromatography*

It is discovered by E. Stahl in 1956, the chromatography separation process in which the stationaary phase consist of a thin layer apply to a solid support .

- *Paper Chromatography*

Paper chromatography was Developed by condenson as a technique for the analysis of amino acid. the compound can be located after separation is one of most important feautre of paper chromatography. It detecd to small as 0.1 µg of the given sample.

- *Normal Phase Chromatography*

In this chromatography the stationary is polar and mobile phase is non polar . For example, – water and treithylene glycol and versatile stage, for example, hexane or n-propyl ether. In typical pase minimum polar segment are eluated first and most polar segment are eluated finally. when polarity of mobile phase increased then eluation time is also increased.

- *Reverse Phase Chromatography*

In reversed phase, the C_{18} C_8 and C_4 are used as stationary phase and acetontrile, Trifluoric acid , Amino silica, methanol are used as mobile phase. In turned around pase more polar part are eluated first and slightest polar is eluated finally.When polarity of mobile phase increased then eluation time is also increases.

- *Ion Exchange Chromatography*

Ion exchange chromatography is a liquid chromatography in which the analyte are ions dissolved in aqueous solution mobile phase . the stationary phase is finely grouped ion exchange resins serve as the stationary phase in ion – exchange chromatography . it is separation process in which ions charge are separated by elution from a column packed with finely divided resins.

- *Size Exclusion Chromatography*

In Size Exclusion Chromatography separation differ from chromatoghraphic procrdures because in this no physical and no chemical properties inteaction between analyte and the stationary phase is required .the separation is made on effective suzes of the molecules. There are two types
1). Geel permeation 2). Gel filtration

✓ **Gel permeation-** It is type of size – exclusion liquid chromatography in which packing is hydrophobic. It is used to separate non polar species.
✓ **Gel filtration-** It is a type size exclusion Chromatoghraphy in which packing is hydrophilic . It is used to separate polar species.

V. MATERIALS AND METHODS

➢ *Reagents and Chemicals*

S. N.vc	Name	Grade	Manufacturer
1.	Hydrochorothiazide	-	Sigma
2.	Acetonitrile	HPLC	Merck
3.	Acetone	HPLC	Merck
4.	Methanol	HPLC	Merck
5.	Trifluoro acetic acid	HPLC	Merck
6.	2-dodecanone	HPLC	Sigma
7.	Ethyle acetate	HPLC	Sigma
8	Carbon tetrachloride	HPLC	Sigma
9.	Mili Q Water	-	-

Table 2:- Chemicals and Reagents

ISSN No:-2456-2165

➢ *Preparation of Standard Stock Solutions*

Hydrochlorothiazide (5 mg) were accurately weighed and transferred to 10 ml volumetric flasks separately.

They were dissolved and diluted to 10 mL with acetonitrile to obtain a stock solution of Hydrochlorothiazide with a final concentration of 0.5 mg/mL (500 µg/mL).

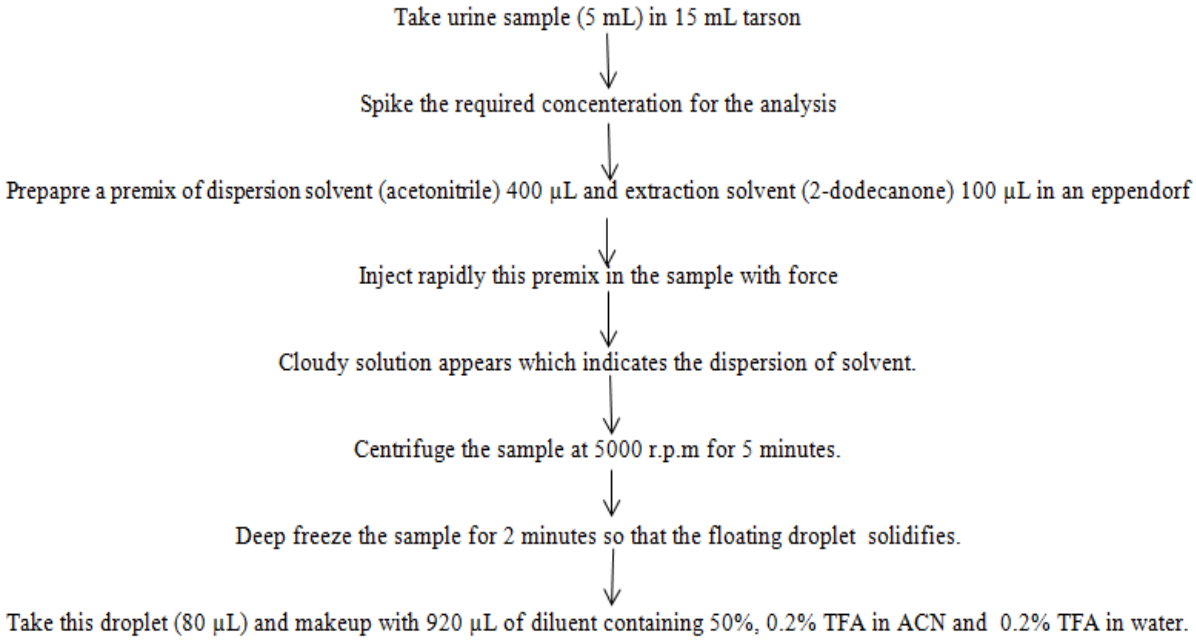
➢ *Preparation of Working Standard Solutions*

Aliquots (0.5mL) of standards solution of HCTZ were pipette out, and transfer to 10 ml-volumetric flask separately and dilute to 10 ml with acetonitrile to obtained the working standards solutions of HCTZ with a final concentration of 50 µg /ml.

Further (0.2 mL) of working standard solution of HCTZ were pipetted out. And transferred to 2 ml eppondrof and diluted with 0.2% mL trifluoric acid in acetonirile to obtain working standard solutions of HCTZ with a final concentration of 10 µg /mL.

➢ *Dllme SFO Procedure:*

Extraction procedure which was selected for the analysis of diuretic drugs (HCTZ) is DLLME-SFO. This method includes following steps:



➢ *Validation Assay:*

Validation assay of the proposed study was performed with respect to the LOD, LOQ, precision, linearity. A 5 points of calibration curve ranging from 10 – 0.625 µgmL^{-1}. The intra-day (repeatability) and inter-day (reproducibility) precision for Hydrochlorothiazide were calculated by analyzing 6 replicates at 5µgml^{-1}, 1µgmL^{-1} levels of calibration graph on a same day and 2 different days. The limit of detection and limit of quantitation were calculated. Stock solutions were systematically prepared to achieve desired concentrations.

VI. RESULTS AND DISSCUSSION

➢ *Method Develpoment*

In the this work a method development and validation was carried out for the estimation of hydrochlorothiazide and chorothiazide by UPLC technique. The wavelength selection was made at 271 nm.

➢ *Liquid Chromatographic Conditions*

Instrumentation for Hydrochlorothiazide analysis was shimadzu UPLC (japan) It consist of quaternary solvent manager, sample manager and column manager to achieve reverse-phase liquid chromatographic conditions. HCTZ were chromatographically separated on Acclaim C_8 (150 mm × 2.1 mm) column with 5 µm particle size and was maintained at 40ºC with an alarm band of in the column oven. The mobile phase was composed 0.2% TFA in water and 0.2% TFA in acetonitrile. The flow rate of the mobile phase was set at 0.5 ml/min for gradient elution. The auto sampler temperature was maintained at 10ºC for uniform temperature o f samples while inject. The total LC run time was 1ml/10 min.

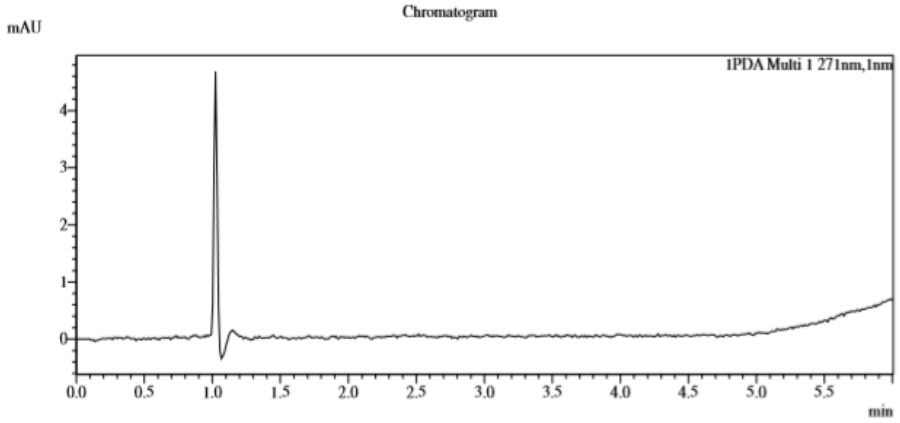


Fig 4:- Chromatogram Standard Preparation

[Mobile Phase 0.2% TFA in water: 0.2 TFA Acetonitrile (50:50)]

➢ *Method Validation*

After method development, the validation method was carried out according to the guidelines of ICH, including accuracy, precision, selectivity, linearity and range, robustness and roughness.

- *Linearity*

The linearity of the method was determined at five concentration levels ranging from 0.625–10 µg / mL for each drug. Calibration curves were constructed based on concentrations of peak areas vs. hydrochlorothiazide and Chlorothiazide.

Hydrochlorothiazide	
Conc.(µg/mL)	**Area**
0.625	5095.5
1.25	11495
2.5	20766
5	42162
10	84590.5
Mean	32821.8
SE	578.6849
SD	1293.939
LOD	0.460258
LOQ	1.534194

Table 3:- Results of LOD and LOQ

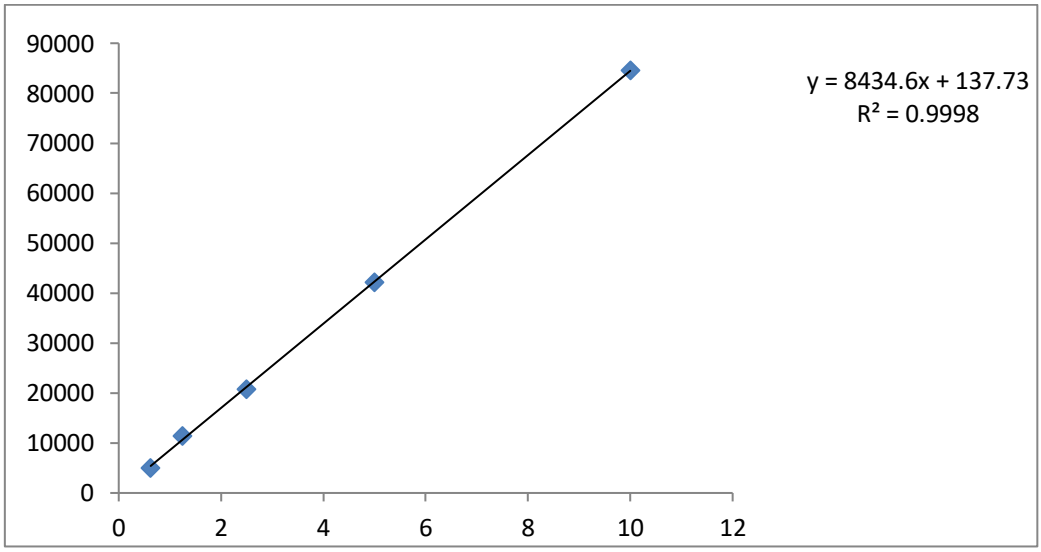


Fig 5:- Calibration Curve of Hydrochlorothiazide at five concentrations

➢ *LOD and LOQ*

The value of detection of limit and quatification limit of hydrochlorothiazide obtained from table 4 & 5.

Compound	LOD	LOQ
HCTZ	0.4602580	1.534194

Table 4:- Results of LOD &LOQ

- *Precision*

The accuracy, intra-day repeatability of the method was evaluated by performing six independent assays of test sample preparation and calculating% RSD. Intermediate purity of the method was checked by performing the same procedure on different days. The RSD value for intraday precision study and interceded precision studies was <5.0% for hydrochlorothiazide. Who confirm that the method was accurate.

INJ	HCTZ (Area)
1	18635
2	18355
3	17989
4	18668
5	18735
6	18544
Mean	18487.67
SD	277.4323
RSD%	1.500634

Table 5:- Results of Precision Study of HCTZ at 1.25 µg/mL

INJ	HCTZ (Area)
1	45527
2	47950
3	49275
4	48898
5	46186
6	48208
Mean	47674
SD	1499.764
RSD%	3.145874

Table 6:- Results of Precision Study of HCTZ at 5 µg/mL

INJ	HCTZ (Area)
1	18988
2	18645
3	18268
4	18759
5	17988
6	18865
Mean	18585.5
SD	382.5131
RSD%	2.058127

Table 7:- Results of Precision Study of HCTZ at 1.25 µg/mL

INJ	HCTZ (Area)
1	48527
2	49950
3	49275
4	46898
5	45186
6	47208
Mean	47840.67
SD	1750.074
RSD%	3.65813

Table 8:- Results of Precision Study of HCTZ at 5 µg/mL

- *Recovery (%)*

Recovery was performed in Hydrochlorothiazide as the patients consuming. Recovery was in the range of 93 -108 %.After analysis gave the best extraction recovery (%) which is represented by the bar graph given below figure 11 and 12.

Non Spiked (Real Sample) 1mL	HCTZ (Area)
1 INJ	6205
2 INJ	6206
mean	6205.5

Table 9:- Recovery (%) Study

Conc.	INJ	Spiked (area)	STD (area)	Non spiked (area)	Recovery (%)
1.25 µg/mL	1	18635	11495	6205.5	108
	2	18355	11495	6205.5	105
	3	17989	11495	6205.5	102
	4	18668	11495	6205.5	108
	5	18735	11495	6205.5	108
	6	18544	11495	6205.5	107

Table 10:- Results of Recovery (%) Study of HCTZ at 1.25µg/mL

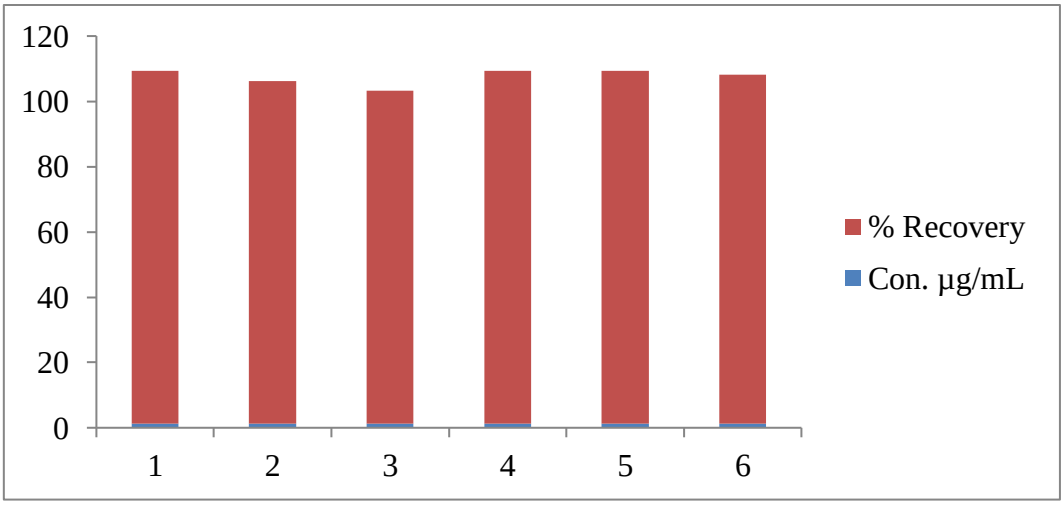


Fig 6:- Recovery (%) of HCTZ at 1.25µg/mL

Conc.	INJ	Spiked area	STD (area)	Non (spiked area)	Recovery (%)
5 µg/mL	1	45527	42162	6205.5	93
	2	47950	42162	6205.5	99
	3	49275	42162	6205.5	102
	4	48898	42162	6205.5	101
	5	46186	42162	6205.5	95
	6	48208	42162	6205.5	100

Table 11:- Results of Recovery (%) Study of HCTZ at 5µg/mL

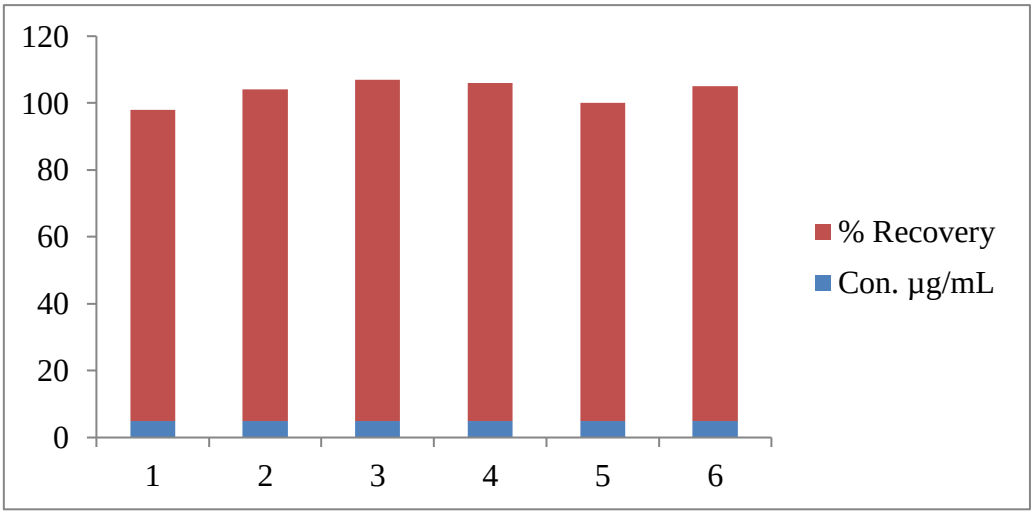


Fig 7:- Recovery (%) of HCTZ at 5µg/mL

➢ *Chromatograms*

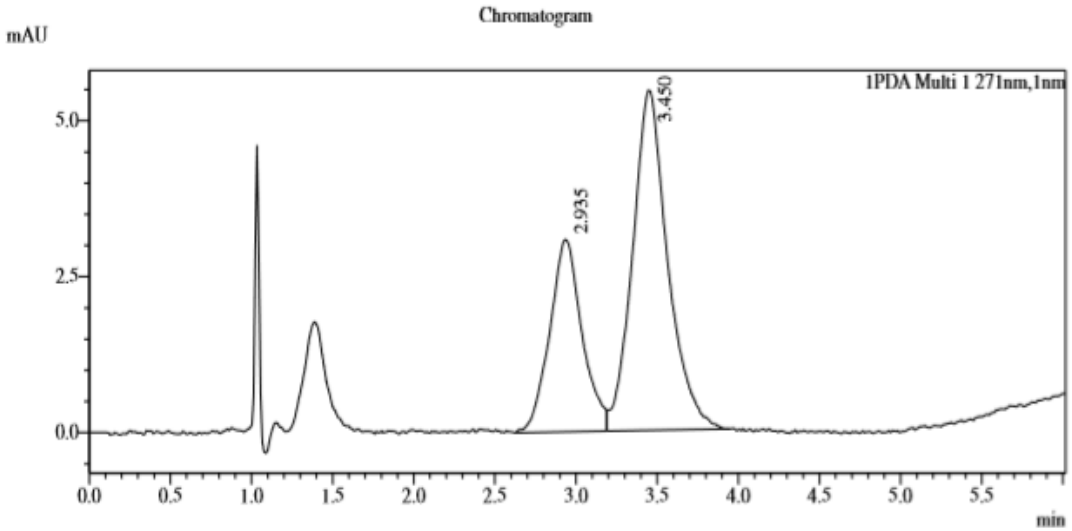


Fig 8:- Chromtogram of Standard of 5µg/mL (mix of Hydrochlorothiazide and Chlorothiazide)

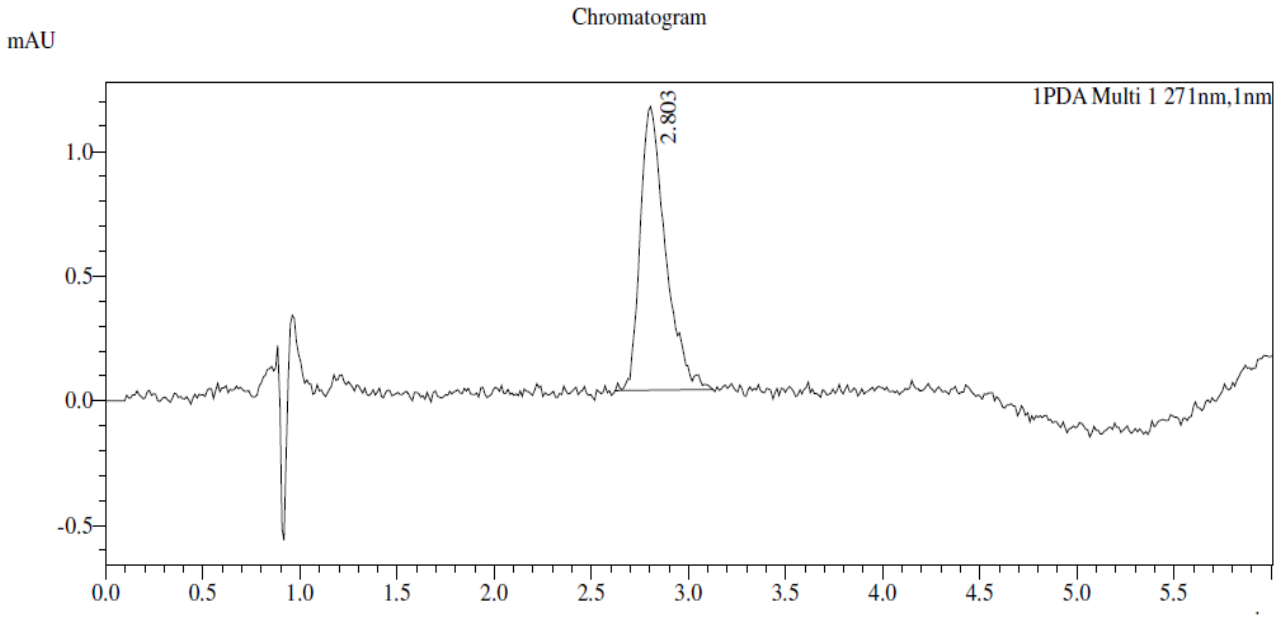


Fig 9:- Chromatogram of Non Spiked (1mL Real sample analysis of Hydrochlorothiazide)

VII. CONCLUSIONS

A DLLME-SFO based methods is developed for simultaneous identification and quantification of Chlorothiazide and Hydrochlorothiazide by RP-UPLC methods. Sample preparation is simple and analysis time is short. The analytical process is validated according to ICH Eurechem guidelines and is shown to be accurate, precise and specific and economic and stability indicating. This method represents an economically benifitted, eco friendly and useful for medical identification of Hydrochlorothiazide from urine samples of patients suffering from Hypertension (high blood pressure) . The method is responsible for routine analysis of large numbers of samples with good accuracy and precision.

REFERENCES

[1]. Agnieszka Zgola-Grzes'kowiak Dispersive Liquid–Liquid Microextraction Article in TrAC Trends in Analytical Chemistry, October 2011.
[2]. A. Zgoła-Grzes´kowiak, Chromatographia 72 (2010) 671.
[3]. Beevers DG, Hamilton M and Harpur JE (1971). The long-term treatment of hypertension with thiazide diuretics. Postgraduate Medical Journal 47: 639-643
[4]. Beyer KH (1982).Chlorothiazide.British journal of clinical pharmacology 13: 15-24.
[5]. Bonet Domingo, E.; Medina Hernandez, M. J.; Ramis Ramos, G.; Garcia Alvarez-Coque, M. C. High-performance Liquid Chromatographic Determination of Diuretics in Urine by Micellar Liquid Chromatography. J. Chromatogr. B 1992, 582, 189–194.
[6]. Brater DC. Diuretic therapy. N Engl J Med 1998;339:387 – 95.
[7]. Chlorothiazide drug bank.
[8]. Dandan Han; Baokun Tang & Kyung Ho Row(2013)., Journal of Liquid Chromatography & Related Technologies, 36:15,2069-2081.
[9]. D. Jothieswari, K. A. Kumar, K. Vijaya, D. S. Vijayar, B. kumar, D. Priya, Stephen Rathnaraj. Validated revesed-phase high performance liquid chromatography method for the simultaneous determination of amlodipine, valsartan and hydrochlorothiazide. J. Pharma. Biomed. Sci. 5(12) (2010)1-7.
[10]. Duarte, Cooper – Dehoff , RM ." Mechanism for blood pressure lowering and metabolic effects of thiazide & thiazide like diuretics". Expert review of cardiovascular therapy 8(6):793-802.
[11]. Donald a. PaviaT. Sunil Kumar Reddy et al. / Vol 2 / Issue 1 / 2012 / 24-31.
[12]. From Wikipedia, the free encyclopaedia
[13]. Gordon J. Murraya,b, Jonathan P. Danaceaub., Journal of Chromatography B, 877 (2009) 3857–3864.
[14]. Hydrochlorothiazide Drug bank.
[15]. ICH Harmonized Tripartite Guideline Q2B: Text on Validation of Analytical Procedures: Methodology, ICH Steering Committee, 1994.
[16]. International conference on Harmonization, Validation of Analytical Procedure: Methodology, Federal Register; 1995, 11260-11262.
[17]. John-Olof Tho¨ rngren, Fredrik O¨ stervall and Mats Garle. J. Mass Spectrom. 2008; 43: 980–992.
[18]. Jackson EK. Diuretics. In: Hardman JG, Limbird LE, Gilman AG, eds. Goodman & 5 Gilman's the Pharmacological Basis of Therapeutics. 10th edn. New York: McGraw-Hill, 2001: 757
[19]. KD Tripathi; Essentials of Medical Pharmacology; 6 th edition.
[20]. Monroy, A., Plata, C., Hebert, S.C., and Gamba, G. Characterization of the thiazide-sensitive Na+-Cl- cotransporter: A new model for ions and diuretics interaction. Am. J. Physiol. Renal Physiol., 2000, 279:F161-F169.

[21]. Rang HP, Dale MM, Ritter JM, Flower RJ and Henderson G (2012). Drugs affecting major organ systems; In Rang & Dale's Pharmacology 7th edition.Elsevier.347- 359.
[22]. Snigdha M, Kumar SS, Jaya Y and Kasana B (2013).A review on "how exactly diuretic drugs are working in our body".Journal of Drug Delivery & Therapeutics 3(5): 115-120.
[23]. S. J. Varghesea, T. K. Ravi, Quantitave simultaneous determination of amlodipine, valsartan and hydrochlorothiazide in tablets using high performance liquid chromatography and high performance thin layer chromatography. J. chroma. Tech. 34 (2011) 984-994.
[24]. Saladin KS (2008). The urinary system. In: Human Anatomy 2nd edition. Boston, McGraw-Hill: 717-135.
[25]. Yadav R, Kharya MD, Yadav N and Savadi R (2011). Diuretic activity of Spilanthes acmella, Murr. Leaves extract in rats. International journal of research in pharmacy and chemistry 1(1): 57-61.