Journal of Pharmaceutical Research, Volume 17, Issue 1, Jan-Mar, 2018

ISSN (Online) : 2454-8405
DOI: 10.18579/jpcrkc/2018/17/1/117904

UPLC QUANTITATIVE ANALYSIS OF HYDROCHLOROTHIAZIDE IN ITS DIFFERENT COMBINATIONS

Raja sundararajan*, Christopher vasanth kumar, Jayaveera

GITAM Institute of Pharmacy, GITAM University, Visakhapatnam- 530 045, Andhra Pradesh, India

Research Article

Received on: 8/28/2018 **Revised on:** 2/17/2018 **Accepted on:** 2/29/2018

Abstract

Purpose: The present work describes analytical method development and validation for the estimation of Hydrochlorothiazide in different combinations using UPLC. **Methodology:** The separation was achieved on column ACE C18 (2µ x 3mm x 50mm), using mobile phases method A, B, C (0.5% Ammonium Acetate buffer solution: Methanol (50:50v/v)), D, E (0.005M KH_2PO_4 Buffer: Methanol: ACN (40:52:8) final P^H-3 with orthophosporic acid.) respectively. The retention time of Hydrochlorothiazide were found to be 0.59, 0.60, 0.58, 0.61, 0.69 mins for method A, B, C, D, E, respectively with combination. **Finding:** The method was validated as per ICH, for Accuracy, Precision, Linearity and Robustness, specisity, LOD and LOQ. **Conclusion:** The developed and validated method was used to estimated the Hydrochlorothiazide in its different combinations. In all combinations % of assay were same, this method was used to routine quality control analysis.

Keywords: Hydrochlorothiazide, UPLC method

1. INTRODUCTION

Hydrochlorothiazide: Hydrochlorothiazide is chemically 6-chloro-1, 1-dioxo-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide. It belongs to the thiazide class of diuretics and acts on kidneys to reduce sodium reabsorption in the distal convoluted tubule [2]. This increases the osmolarity in the lumen causing less water to be reabsorbed from the collecting ducts, finally increasing urinary output. It is often used in the treatment of hypertension, congestive heart failure, symptomatic edema and the prevention of kidney stones. It is effective for nephrogenic diabetes insipidus and is also sometimes used for hypercalciuria, Dent's disease. Thiazides are also used in the treatment of osteoporosis. Thiazides decrease mineral bone loss by promoting calcium retention in the kidney and by directly stimulating osteoblast differentiation and bone mineral formation. Hydrochlorothiazide is official in BP and IP

**Author for correspondence:* Raja Sundararajan, GITAM Institute of Pharmacy, GITAM University, Visakhapatnam- 530 045, Andhra Pradesh, India, sraja61@gmail.com

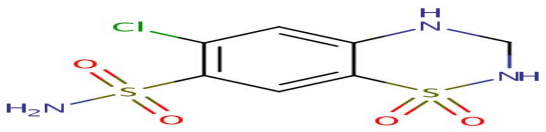
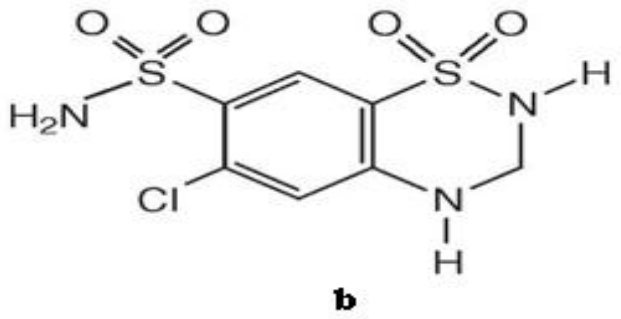


Figure 1. Structure of Hydrochlorothiazide



Losartan: Losartan potassium (LOS), chemically, is 2b utyl4chloro1[p(o1Htetrazol5ylphenyl)benzyl]imidazole-5methanol monopotassium salt. It is an angiotensin II receptor blocker and chemically is used as an antihypertensive agent. Losartan is official in IP and USP,

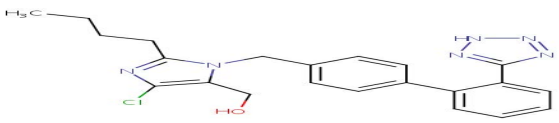


Figure 2. Structure of Losartan

Amlodipine: Amlodipine besilate (AMLO), chemically is [3ethyl5methyl(4RS)2[(2aminoethoxy) methyl]4(2chlorophenyl)methyl1dihydropyridine3,5-dicarboxylate benzenesulfonate. It is a long acting calcium channel blocker used as an antihypertensive agent. AMLO is official in BP.

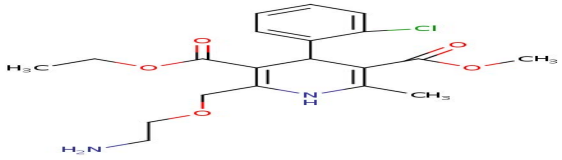


Figure 3. Structure of Amlodipine

Metoprolol: Metoprolol competes with adrenergic neurotransmitters such as catecholamines for binding at beta (1) adrenergic receptors in the heart.

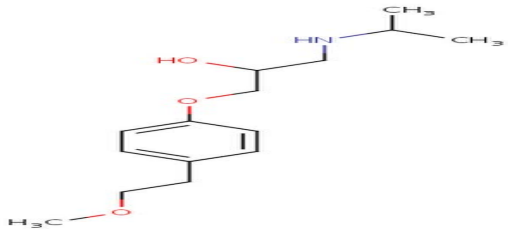


Figure 4. Structure of metoprolol

Ramipril: Ramipril is chemically (1S,5S,7S)-8-[(2S)-2-[[(1S)-1-ethoxycarbonyl-3-phenyl-propyl]amino] propanoyl]-8-azabicyclo[3.3.0]octane-7-carboxylic acid (Figure. 1A), is an angiotensin-converting enzyme (ACE) inhibitor, used to treat hypertension and congestive heart failure. Hydrochlorothiazide. Ramipril is a potent long acting ACE inhibitor and Ramipril blocks the counter regulatory rise in Angiotensin-II triggered by diuretic theory.

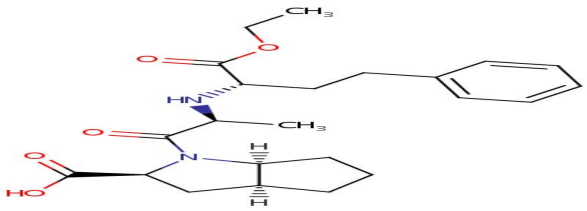


Figure 5. Structure of Ramipril

Telmisartan: Telmisartan is chemically 2-(4-{[4-methyl-6-(1-methyl-1H-1,3-benzodiazol-2-yl)-2-propyl-1H-1,3- benzodiazol-1-yl]methyl}phenyl) benzoic acid. It is an angiotensin receptor blocker (ARB) that shows high affinity for the angiotensin II type 1 (AT1) receptors, has a long duration of action, and has the longest half-life of any ARB [1]. In addition to blocking the renin-angiotensin system (RAS), telmisartan acts as a selective modulator of peroxisome proliferator-activated receptor gamma (PPAR-γ), a central regulator of insulin and glucose metabolism. It is believed that telmisartan's dual mode of action may provide protective benefits against the vascular and renal damage caused by diabetes and cardiovascular disease (CVD). Telmisartan has binding affinity 3000 times with AT-2 receptor than AT-1

receptor. Telmisartan is also having maximum half-life in sartans - 24 hrs.

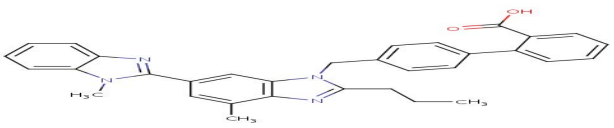


Figure 6. structure of Telmisartan

Marketed Formulations

S.No	BRAND NAME	COMBINATION
1	Losartar-H	Hydrochlorothiazide and Losartan (working standard 99.10 and 99.70)
2	Amlong-H	Hydrochlorothiazide and Amlodipine (working standard 99.10 and 99.70)
3	Betaloc H	Hydrochlorothiazide and Metoprolol (working standard 99.10 and 99.70)
4	cardace H 2.5	Hydrochlorothiazide and Ramipril (working standard 99.10 and 99.70)
5	Telemar-40H	Hydrochlorothiazide and Telmisartan (working standard 99.10 and 99.70)

2. MATERIALS AND METHODS

Chemicals & solvents: Methanol (HPLC Grade; Merck), 0.5% Ammonium Acetate buffer, 0.005M KH2Po4 Buffer, Ortho phosphoric acid.

Instrumentation: All UPLC experiments were carried out on a Waters Alliance 2695 separation module, with waters 2996 photodiode array detector in isocratic mode using Auto sampler. Data collection and processing was done using Empower PDA 2 software. The analytical column used for the separation was ACE C18 2µ x 3mm x 50mm., Other equipment's used were ultra-sonicator (model 3210, Branson Ultrasonics Corporation, Connecticut, USA), Analytical balance (contech balance).

The literature survey revealed that there are a very few spectroscopic and HPLC methods available for the determination of Hydrochlorothiazide individual and combined dosage forms. The present study was aimed to develop a new UPLC method for simultaneous estimation of Hydrochlorothiazide in Bulk and its combined pharmaceutical dosage forms using more economical chromatographic conditions.

2.1 Preparation of solutions:

Diluents: Methanol

Preparation of buffer solutions:

Preparation of 0.5% Ammonium Acetate buffer solution

5gm of Ammonium Acetate dissolve in 1000ml HPLC grade water. Then finally ph was adjusted.

Table 1. Preparation of Used Buffer solution

Method	Used Buffer solution
A	0.5% Ammonium Acetate buffer solution
B	0.5% Ammonium Acetate buffer solution
C	0.5% Ammonium Acetate buffer solution
D	0.005M KH_2PO_4 buffer solution
E	0.005M KH_2PO_4 buffer solution

Table 2. Mobile phase composition

Method	Mobile phase composition
A	0.5% Ammonuim Acetate buffer solution: Methanol (50:50v/v)
B	0.5% Ammonuim Acetate buffer solution: Methanol (50:50v/v)
C	0.5% Ammonuim Acetate buffer solution: Methanol (50:50v/v)
D	0.005M KH_2PO_4 Buffer: Methanol: ACN (40:52:8) final P^H-3 with orthophosporic acid.
E	0.005M KH_2PO_4 Buffer: Methanol: ACN (40:52:8) final P^H-3 with orthophosporic acid.

All mobile phases (method A,B,C,D,E,) were prepared and it was filter to 0.22µ membrane filter to remove the impurities otherwise they may interfere in the final chromatogram and it was sonicated for 15min to remove the undissovable gases and air bubbles.

2.2 Preparation of Standard solution:

Standard solutions of Hydrochlorothiazide and its combination drugs

were prepared by dissolving 10mg of Hydrochlorothiazide and its related combination drugs are taken in separate 10 ml volumetric flasks contain

Table 3. Chromatographic conditions

S.No	Parameter	Method -1	Method B	Method C	Method D	Method E
1	Column	ACE C18 2µ x 3mm x 50mm	ACE C18 2µ x 3mm x 50mm	ACE C18 2µ x 3mm x 50mm	ACE C18 2µ x 3mm x 50mm	ACE C18 2µ x 3mm x 50mm
2	Retention times	0.59 mins (Hydrochlorothiazide) 0.99 mins (Losartan)	0.600 mins (Hydrochlor-othiazide) 1.067 mins (Amlodipine)	0.250ml /min	0.61 mins (Hydrochl-orothiazide) 1.25mins (Ramipril)	0.69 mins (Hydrochlor-othiazide) 1.12mins (Telmisartan)
3	Flow rate	0.250ml /min	0.250ml /min	0.588 mins (Hydrochlor-othiazide) 1.001 mins (Metoprolol)	0.250ml /min	0.250ml /min
4	Wavelength	254nm	238nm	275nm	224nm	276nm
5	Column temperature	30°c	30°c	30°c	30°c	30°c
6	Injection volume	10µl	10µl	10µl	10µl	10µl
7	Run time	3min	3min	3min	3min	3min
8	Diluents	methanol	Methanol	methanol	methanol	methanol
9	Elution	Isocratic	Isocratic	Isocratic	Isocratic	Isocratic
10	Mobile phase	Buffer Methanol(50:50)	0.5% Ammonuim Acetate Buffer: Methanol(50:50)	0.5% Ammonuim Acetate Buffer: Methanol(50:50)	Buffer : Methanol: ACN (40:52:8) final PH-3 with orthopho-sporicacid	0 Buffer : Methanol: ACN (40:52:8) final PH-3 with orthophos-poricacid

10ml of methanol in each flask, sonicate for 5min and final volume were made up to the mark with methanol to get the concentrations of 1000 µg/ml.

2.3 Method development

To saturate the column, the mobile phase was pumped for about 30 minutes in each method thereby to get the base line corrected. The separate standard calibration lines were constructed for each drug. A series of aliquots were prepared from the above stock solutions using mobile phases to get the concentrations 6-10µg/ml Hydrochlorothiazide and 26-39 µg/ml Losartan. Hydrochlorothiazide (6-10µg/ml), Losartan (26-39µg/ml), Amlodipine (21-32µg/ml), Metoprolol (26-39µg/ml), Ramipril (1.2-2.4µg/ml), Telmisartan (21-32 µg/ml). Each concentration 6 times was injected in to chromatographic system. Each time peak area and retention time were recorded separately for Hydrochlorothiazide in different combinations. Calibration curves were constructed by taking average peak area on Y-axis and concentration on X-axis separately for both the drugs. From the calibration curves regression equations were calculated as shown in the figure 7 to 12. This equation were used to estimate the Hydrochlorothiazide drug content in different combined pharmaceutical formulations.

2.4 Estimation of Hydrochlorothiazide in different marketed formulation

For the analysis of Hydrochlorothiazide in different combination take 20 tablets from each combination transferred to 5 glass mortar, triturated well to get fine powder. From glass mortar accurately weighed powder equivalent to 25mg of Hydrochlorothiazide transferred in to 25 ml five volumetric flasks and dissolved in sufficient

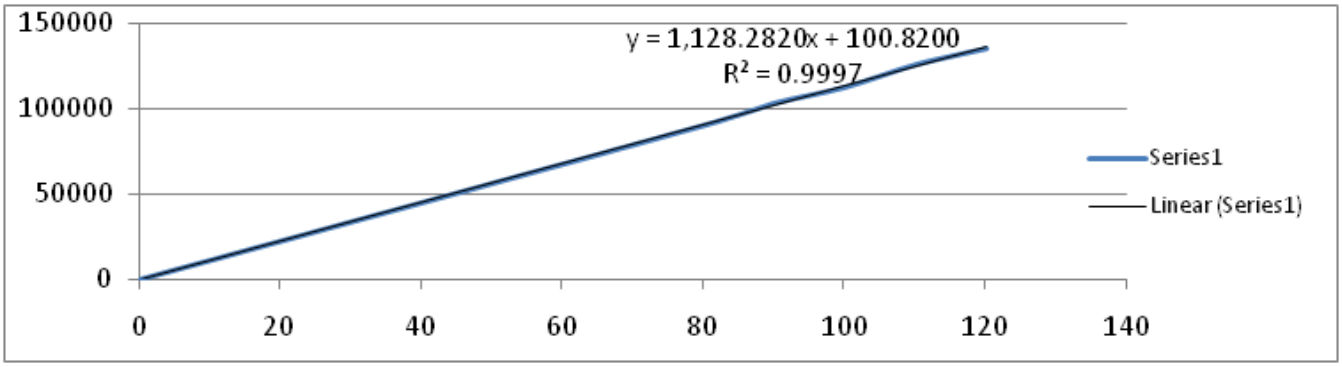


Figure 7. Calibration curve for Hydrochlorothiazide

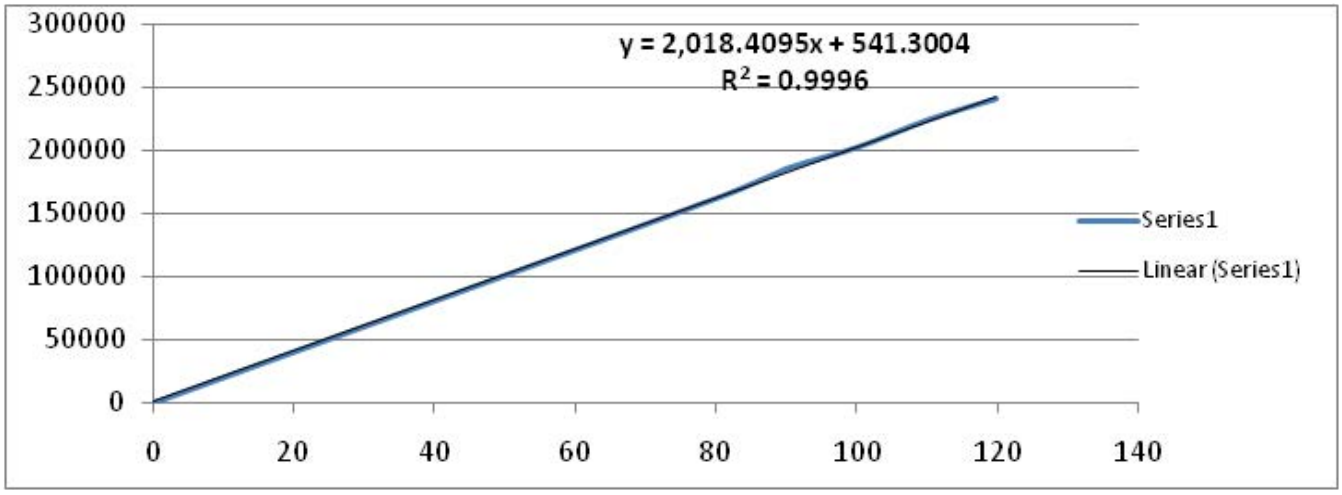


Figure 8. Calibration curve for Losartan

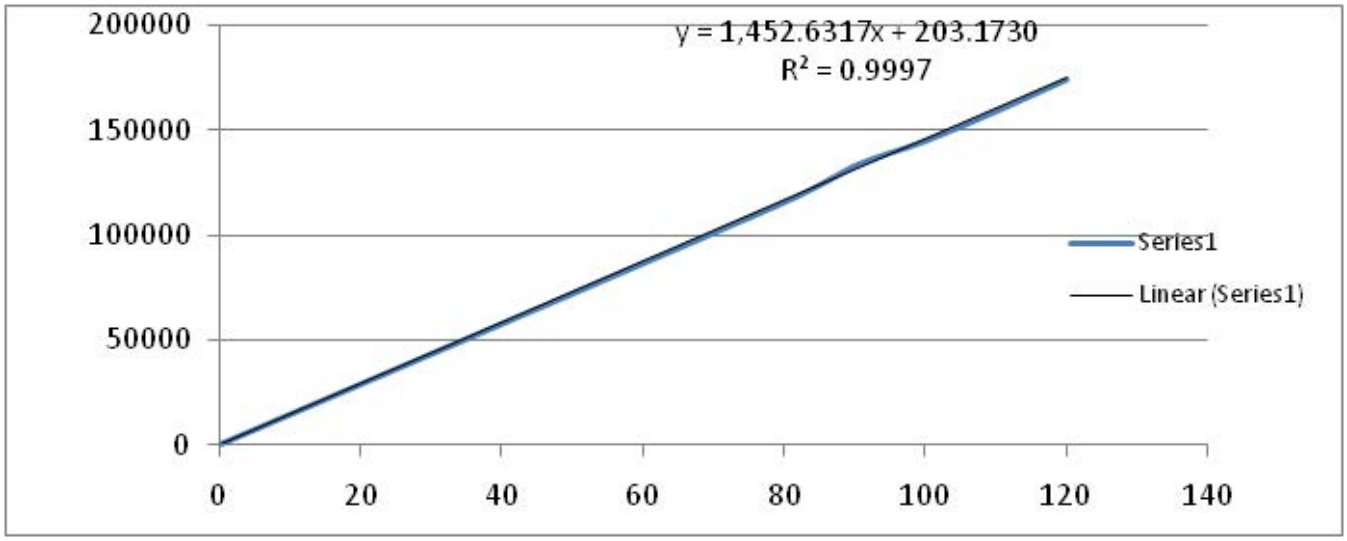


Figure 9. Calibration curve for Amlodipine

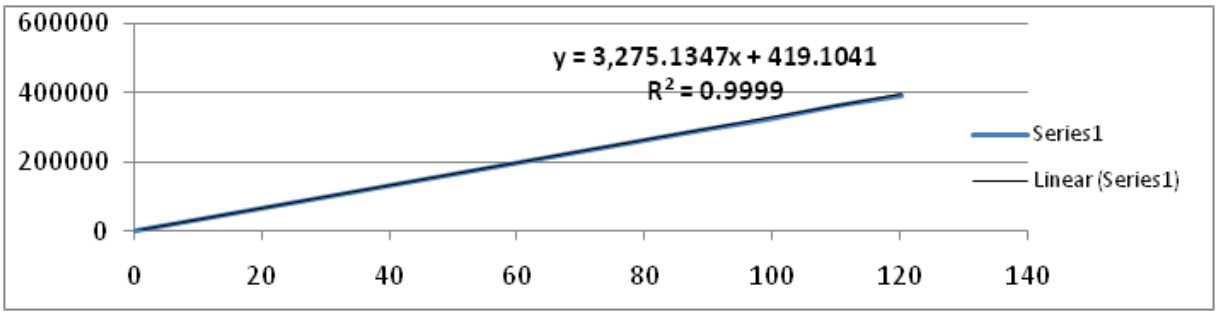


Figure 10. Calibration curve for Metoprolol

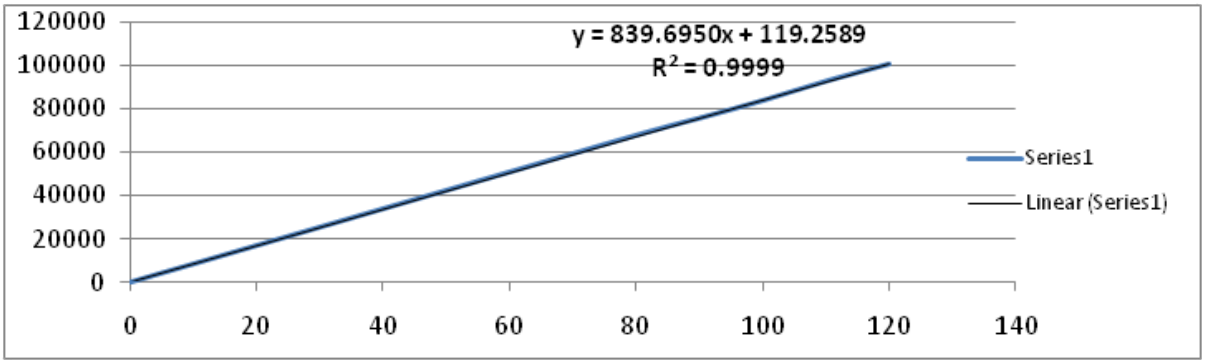


Figure 11. Calibration curve for Ramiprill

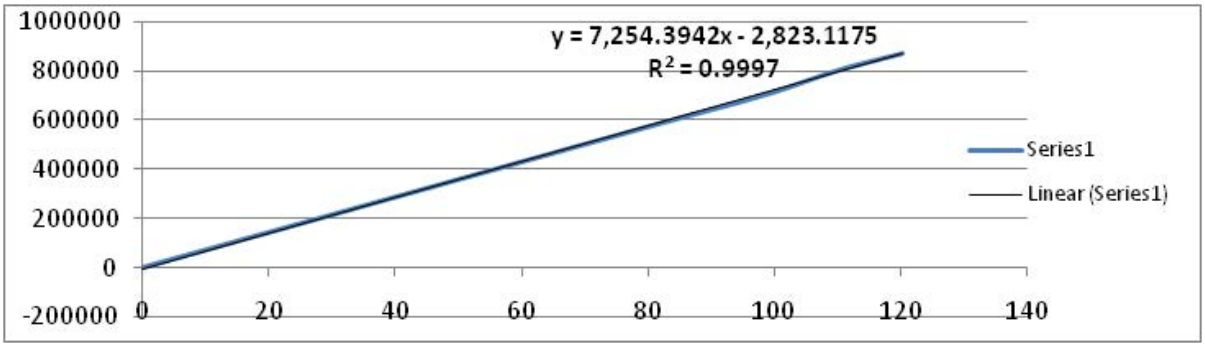


Figure 12. Calibration curve for Telmisartan

Table 4. Results of marketed formulation analysis

Combination	Labeled claim (mg)	Mean estimated Amount (mg)	% of Assay
Hydrochlorothiazide Losartan	12.5mg	12.3	98.4
	50mg	49.5	99.1
Hydrochlorothiazide Amlodipine	12.5mg	12.7	101.6
	5mg	4.9	99.8
Hydrochlorothiazide Metoprolol	12.5mg	12.4	99.2
	50mg	50.2	100.4
Hydrochlorothiazide Ramipril	12.5mg	12.7	101.6
	2.5mg	2.4	99.7
Hydrochlorothiazide Telmisartan	12.5mg	12.6	100.8
	40mg	40.1	100.2

quantity of methanol. These were sonicated for 5mins and volume was made up to the mark with mobile phases A (flask-1) mobile phases -B (flask-2, 3) mobile phases -C (flask-4, 5). From all the flasks five different test solutions were prepared using mobile phases –A, B, C. Each test solution 12.5 µg/ ml Hydrochlorothiazide concentrations, injected 6 times in to the column. Each time peak area and retention time was recorded for five combinations. Using above calculated regression equations content of Hydrochlorothiazide was calculated in each combination. The results were obtained as shown in table no: 4

2.5 Method validations

The analytical method was validated for various parameters as per ICH guidelines

2.6 Accuracy, as Recovery

Accuracy is expressed as the closeness of the results from standard samples to that of the actual known amounts to determine the accuracy of the proposed Method, recovery studies were carried out in different recovery levels (80%, 100% and 120%) by adding placebo to the pre-analyzed

Table 5. Accuracy results

S. No	Name of the drug	% of recovery								
		80			100			120		
		Amount added	Amount found	recovery	Amount added	Amount found	recovery	Amount added	Amount found	recovery
1	Hydrochlorothiazide	6.4	6.34	99.06	8	8.1	101.2	9.6	9.5	99.2
2	Losartan	20.8	20.9	100.4	26	25.9	99.61	31.2	31.3	100.3
3	Amlodipine	19.2	19.5	101.5	24	24.1	100.4	28.8	28.6	99.30
4	Metoprolol	20.8	20.5	99.7	26	25.8	99.23	31.2	31	99.35
5	Ramipril	0.96	0.93	99.6	1.2	1.19	99.16	1.44	1.4	100
6	Telmisartan	19.2	19.1	99.47	24	24.2	100.8	28.8	28.7	99.72

Table 6. Precision results

S. No	Method	Combination	Intraday		Interday	
			mean±SD	RSD	mean±SD	RSD
1	A	Hydrochlorothiazide	0.429±0.004	0.97	0.681±0.003	0.67
		Losartan	0.585±0.003	0.61	0.579±0.004	0.86
2	B	Hydrochlorothiazide	0.782±0.005	0.57	0.585±0.003	0.61
		Amlodipine	0.681±0.003	0.67	0.681±0.003	0.67
3	C	Hydrochlorothiazide	0.449±0.004	0.92	0.776±0.005	0.78
		Metoprolol	0.782±0.005	0.58	0.392±0.005	1.28
4	D	Hydrochlorothiazide	0.412±0.005	1.31	0.782±0.005	0.58
		Ramipril	0.579±0.004	0.86	0.392±0.005	1.22
5	E	Hydrochlorothiazide	0.776±0.005	0.78	0.429±0.004	0.97
		Telmisarstan	0.392±0.005	1.22	0.412±0.005	1.31

formulation .the solutions were suitably diluted in the range and then each of the dilution was observed 6 times. The% recovery of the drugs were shown in the table no.5

Table 7. LOD and LOQ results

S.No	Method	Drug Name	LOD	LOQ
1	A	Hydrochlorothiazide	0.06	0.20
		Losartan	0.131	0.401
2	B	Hydrochlorothiazide	0.03	0.1
		Amlodipine	0.105	0.325
3	C	Hydrochlorothiazide	0.032	0.1
		Metoprolol	0.14	0.45
4	D	Hydrochlorothiazide	0.03	0.1
		Ramipril	0.105	0.325
5	E	Hydrochlorothiazide	0.03	0.1
		Telmisartan	0.105	0.325

Precision: Precision is the level of repeatability of results as reported between samples analyzed on the same day (intra-day) and samples run on 3 different days (inter-day).to check the intra-day and inter-day variation of the method. The precision of proposed method i.e. the intra and inter-day variations in the peak area of the drug solutions were calculated in terms of % RSD and the results were presented in the table.6, statically revolution revealed that relative standard deviation of drugs at different concentration levels for 6 times was less than 2.0 . The results are shown in table no: 6

LOD and LOQ: Limit of detection and quantification were established based on signal to noise ratio (S/N) 3:1 and 10:1 respectively. The results were shown in table no: 7

2.6 System suitability parameters

For assessing system suitability, six replicates of working standards samples of Hydrochlorothiazide and its combinations were injected and studied the parameters like plate number (N), tailing factor(K),resolution, relative retention time and peak asymmetry of samples. The results were tabulated in table 8.

3. RESULTS

Optimized chromatographic conditions: The optimized chromatographic conditions Table no: 3). Different mobile phases were used for setting of optimized, better resolution /good peak shape were obtained by using mobile phases.

Accuracy: The percentage recovery of Hydrochlorothiazide in different combined dosage forms were obtained in a range from 98% to 103%, respectively. The results were as shown in table no: 5

Precision: The precision of the method was determined by repeatability (Intraday precision) and intermediate precision (Interday precision) of Hydrochlorothiazide in different combined dosage forms were obtained. The obtained results of repeatability (Intraday precision) and intermediate precision (Interday precision) were less than 2.0 as shown in table no: 6

Linearity: The calibration curve for Hydrochlorothiazide (6-10µg/ml) Losartan (26-39 µg/ml), Amlodipine (21-32µg/ml), Metoprolol (26-39µg/ml), Ramipril (1.2-2.4µg/ml), Telmisartan (21-32 µg/ml).The data for the peak area versus concentration were treated by linear regression analysis, and the correlation coefficient (R^2) was obtained (0.999). The regression equation for the calibration curve was calculated. The result were shown fig no 7-12.

LOD and LOQ: The results of LOD and LOQ data were within the acceptance criteria; the signal-to-noise ratio for the LOD and LOQ were well within the acceptance criteria. The results were shown in table no: 7

Robustness: The robustness of the assay method was established by introducing small changes in the chro-

Table 8. System suitability Parameters

Parameters	METHOD-A		METHOD-B		METHOD-C		METHOD-D		METHOD-E	
	HCT	L	HCT	A	HCT	M	HCT	R	HCT	T
Plate count	238	445	2268	2607	2276	2521	2230	2581	2268	2607
Tailing Factor	1.25	1.26	1.25	1.26	1.25	1.26	**0.00**	**3.25**	1.25	1.26
R_t (min)	0.59	0.99	0.600	1.067	0.588	1.002	0.61	1.25	0.600	1.067
Resolution	0	2.39	0	2.92	0	2.58	0	3.25	0	2.92
Asymmetry	1.61	1.76	1.73	1.88	1.72	1.79	1.74	1.71	1.73	1.88

Combination	λmax (nm)	Linearity Range(µg/mL	Regression Equation (y=mx+c)	Correlation Coefficient (r2)	Accuracy indicated by % recovery	Precision indicated by % RSD	LOD (µg)	LOQ (µg)
Hydrochlorothiazide		6-10µg/ml	y = 1,128.2820x + 100.8200	0.999	100.10	0.67	0.06	0.20
		26-39 µg/ml	y = 2,018.4095x + 541.3004	0.999	100.3	0.86	0.131	0.401
Losartan	254nm							
Hydrochlorothiazide		6-10µg/ml	y = 3,011.8777x + 993.8273	0.999	99.79	0.61	0.03	0.1
Amlodipine	238nm	21-32µg/ml	y = 1,452.6317x + 203.1730	0.999	99.30	0.67	0.105	0.325
Hydrochlorothiazide		6-10µg/ml	y = 878.8702x - 55.5391	0.999	101.33	0.78	0.032	0.1
Metoprolol	275nm	26-39µg/ml	y = 3,275.1347x + 419.1041	0.999	99.35	1.28	0.14	0.45
Hydrochlorothiazide		6-10µg/ml	y = 2,393.2881x + 656.1689	0.999	100.27	0.58	0.03	0.1
Ramipril	224nm	1.2-2.4µg/ml	y = 839.6950x + 119.2589	0.999	100	1.22	0.105	0.325
Hydrochlorothiazide		6-10µg/ml	y = 1,236.7819x - 675.2482	0.999	100.10	0.97	0.03	0.1
Telmisartan	276nm	21-32 µg/ml	y = 7,254.3942x - 2,823.1175	0.999	99.72	1.31	0.105	0.325

matographic condition which included flow rate and column oven temperature (28 C and 32 C).

4. DISCUSSION

4.1 Optimized chromatographic conditions:

Most of all reported UPLC methods till date use C-8 or C-18 columns. Most of this use complex mobile phase compositions. Hence, attempts were directed towards development of a Simple and better method on commonly used C18 column with good resolution. Different logical Modifications were tried to get good separation among the drugs .These changes included change in different mobile phase composition in gradient modes on different C18 columns.

Accuracy: Percentage of RSD value of replicated sets was less than 2.0 which indicates that this method is highly accurate.

Precision: Percentage of RSD value of replicated sets was less than 2.0 which indicates that this method is highly precise.

Linearity: The statically analysis revealed that the proposed method was linear

LOD and LOQ: All the results of LOD and LOQ data were within the acceptance criteria; the signal-to-noise

ratio for the LOD and LOQ were well within the acceptance criteria.

Robustness: The developed method was unaffected by the small deliberated changes, it indicated the proposed method was robustness.

5. CONCLUSION

To developed simple, rapid, accurate and precise UPLC quantitative analysis of hydrochlorothiazide in its different combinations. The experimental design describes the scouting of the key UPLC method components including column, pH, and mobile phase. Their interrelationships are studied and the preliminary optimized conditions are obtained for each combination of drugs.

All the validated parameters were found within acceptance criteria. The validated method is specific, linear, precise, accurate, robust and rugged for determination based on the knowledge of method obtained through the method development and the results of risk assessment along with robustness and ruggedness studies, detailed analytical method performance control strategy can be defined to manage the risk. This approach has been successfully used in the laboratory to develop UPLC method hydrochlorothiazide in its different combinations.

6. ACKNOWLEDGEMENT

The authors are grateful to Piramal Health Care & Orchid Chemicals and Pharmaceuticals, Chennai, Ltd, India for providing drugs as a gift samples.

7. REFERENCES

1. www.wikipedia.org/wiki/Losartan Potassium
2. www.wikipedia.org/wiki/Hydrochlorothiazide
3. Ramana Rao G, Murthy SSN, Khadgapathi P. High performance liquid chromatography and its role in pharmaceutical analysis. *Eastern Pharmacist*, 29(346), 1986, 53.
4. Snyder LR, Kirkland JJ, Glajch JL. Practical HPLC method development. 2nd ed. New York: Wiley InterScience; 1997, 1-57.
5. Byyny RL. Losartan potassium lowers blood pressure measured by ambulatory blood pressure monitoring. *J. Hypertension*, 13, 1995, S29-S33.
6. Lastra OC, Lemus IG, Sanchez HJ, Perez RF. Development and validation of an UV derivative spectrophotometric determination of Losartan potassium in tablets. *J Pharm Biomed Anal., 33,* 2003, 175-80.
7. Zarapkar SS and Kanyawar NS. Simultaneous estimation of Amlodipine and Losartan potassium in pharmaceutical dosage by RP-HPLC. *Indian drugs*, 39(6), 2002, 338-341.
8. Deanne L Hertzog, et al. Developed and validated stability-indicating HPLC method for the simultaneous determination of losartan potassium, hydrochlorothiazide, and their degradation products. *Pharmacopoeial Forum*, 31(5), 2005, 1453-1463.
9. Wang ZY, Tain, Tain XL, Tain T. Simultaneous determination of Valsartan and Hydrochlorothiazide in tablets by RP-HPLC. Indian journal of pharmaceutical sciences, 70(3), 2008, 372-374.
10. Shah NJ, Suhagia BN, Shah RR, Shah PB. Development and validation of a HPTLC method for the simultaneous estimation of telmisartan and hydrochlorothiazide in tablet. *Indian Journal of Pharmaceutical Sciences,* 69(2), 2007, 202-205.
11. Garg G, Saraf S. Spectrophotometric and column high-performance liquid chromatographic methods for simultaneous estimation of metoprolol tartrate and hydrochlorothiazide in tablets. JAOAC Int. 2008;91:1045–50.
12. Gupta KR, Tajne MR, Wadodkar SG. New Spectrophotometric method for simultaneous determination of metoprolol tartrate and hydrochlorothiazide in tablets. Indian J Pharm Sci. 2008;70:511–3.
13. Bilal YILMAZ, Determination of Ramipril in pharmaceutical preparations by High-performance liquid chromatography. International Journal of Pharmaceutical Sciences Review and Research, Volume 1, Issue 1, March – April 2010; Article 008, pp 39-42.
14. Rao KV Kanna, Rao ME, Nagoji KE, Rao SS. Determination of Metoprolol Tartrate by reverse phase HPLC. Indian J Pharm Sci. 2003;65:204–6.
15. Badulescu M, Balala UD, Cacovean I, Ilie M, Baconi DL. UVVisible spectrophotometeric assay of metoprolol. Note-2. Method validation. Farmacia. 2008;LVI(4):363–70.
16. Sunil Jawla, K Jeyalakshmi, T Krishnamurthy, Y. Kumar Development and Validation of Simultaneous HPLC method for Estimation of Telmisartan and Ramipril in Pharmaceutical Formulations. Int.J. PharmTech Res.2010, Vol.2, No.2, pp 1625- 1633.
17. M. M. Baing, V. V.Vaidya , R. T. Sane, S. N. Menon, K. Dalvi; Simultaneous RP-LC Determination of Losartan Potassium, Ramipril, and Hydrochlorothiazide in Pharmaceutical Preparations, Chromatographia 2006, 64, pp 293–296 September (No. 5/6)
18. Tambe, Vijaya Vichare, Ujjawala Kandekar And Shashikant Dhole, Novel UV Spectrophotometric Methods For Estimation of Ramipril and Hydrochlorothiazide by

Simultaneous Equation And Area Under Curve Method, Int J Appl Pharm , Vol 2, Issue 4, 20-22.

19. Bankey, G. G Tapadiya, S. S Saboo, S. Bindaiya, Deepti Jain, S. S. Khadbadi; Simultaneous Determination of Ramipril, Hydrochlorothizide and Telmisartan by Spectrophotometry. Int.J. ChemTech Res.2009, Vol.1, No.2, pp 183-188.
20. Budawari S. The Merck Index. Whitehouse Station, NJ: Merck and Co. Inc.; 2006. p. 927, 1573.
21. Satana E, Altinay S, Goger NG, Ozkan SA, Senturk Z. Simultaneous determination of Valsartan and Hydrochlorothiazide in tablets by first order UV spectrophotometry and Liquid chromatography. J Pharm Biomed Anal 2001;25:100913
22. Lande NR, Shektar BM, Kadam SS, Dhaneshwar SR. Simultaneous spectrophotometric estimation of Losartan potassium and Hydrochlorothiazide in tablet dasage form. Indian drugs 2000;37:57781.
23. Dinc E, Ustundag O. A new application of chemometric technique to HPLC data for the simultaneous analysis of two compound mixture. J Liq Chrmatogr Relat Technol 2005;28:217994.
24. Ulu ST, Saglik S. Comparison of UV and Second derivative spectrophotometric and HPLC methods for the determination of Losartan in tablets. Turk J Pharm Sci 2004;1:16575.
25. Dinc E, Ustundag O. Application of multivariate calibration techniques to HPLC data for quantitative analysis of binary mixture of Hydrochlorothiazide and Losartan in tablet. Chromatographia 2005;61:237 44.
26. DL, Mc Cafferty JF, Fang XG, Tyrell RJ, Reed RA. Development and validation of stability indicating HPLC method for simultaneous determination of Losartan Potassium, Hydrochlorothiazide and their degradation product. J Pharm Biomed Anal 2002;30:74760.