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Simulataneous Estimation and Validation of Telmisartan, Amlodipine Hydrochlorthiazide and Losartan in Bulk and Pharmaceutical Dosage Form by RP-HPLC Method

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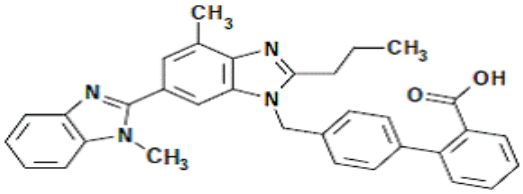
ABSTRACT
A Simple, accurate reverse phase high-performance liquid chromatographic method was developed and validated for simultaneous estimation of telmisartan,amlodipine,losartan and hydrochlorothizide in bulk and pharmaceutical dosage forms. Chromatography was carried out by using Qualisil C-18, column. A mixture of 0.1m triethylamine: acetonitile: methanol 50:40:10 (V/V/V) and pH 2.5 was adjusted by using orthophosphoric acid as mobile phase. Detection was at 231nm. The retention times were found to be 4.1, 6.1, 7.6, 8.6 for telmisartan, amlodipine, losartan and hydrochlorothizide respectively .

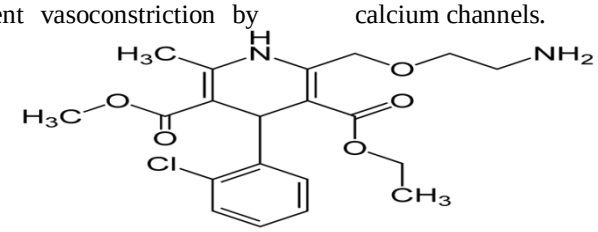
Key Words: Telmisartan, amlodipine, losartan, and hydrochlorothizide Method Development and validation, RP- HPLC.

I. INTRODUCTION

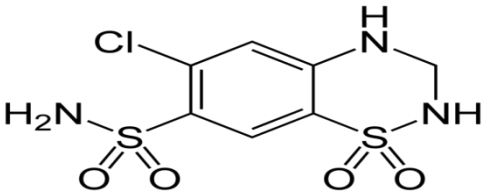
Telmisartan interferes with the binding of angiotensin II to the angiotensin II AT_1-receptor by binding reversibly and selectively to the receptors in vascular smooth muscle and the adrenal gland. As angiotensin II is a vasoconstrictor, which also stimulates the synthesis and release of aldosterone, blockage of its effects results in decreases in systemic vascular resistance.



Amlodipine decreases arterial smooth muscle contractility and subsequent vasoconstriction by inhibiting the influx of calcium ions through L-type calcium channels.



Hydrochlorothiazide, a thiazide diuretic, inhibits water reabsorption in the nephron by inhibiting the sodium-chloride symporter in the distal convoluted tubule, which is responsible for 5% of total sodium reabsorption.



Losartan competitively inhibits the binding of angiotensin II to AT1 in many tissues including vascular smooth muscle and the adrenal glands. Losartan is metabolized to its active

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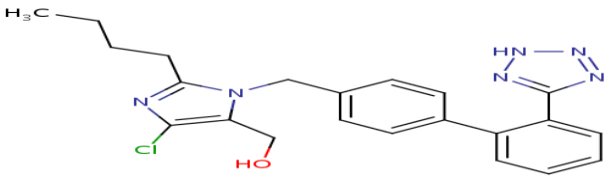
metabolite, E-3174, which is 10 to 40 times more potent than losartan and acts as a non-competitive AT1 antagonist. Inhibition of angiotensin II binding to AT1 inhibits its AT1-mediated vasoconstrictive



To ensure the effectiveness of these drugs, quality and efficacy assessments and maintenance of proper dosage schedule are of great importance. Manufacturers have to evaluate their products during and after manufacturing processes and at various time intervals during the shelf life of every product. Therefore, it is needed to study and determine the potency and efficacy of Telmisartan, amlodipine, losartan and hydrochlorthiazide. Several methods have been previously reported in the literature for determination of amlodipine and losartan in the pharmaceutical formulations but there is no report on the simultaneous determination of these drugs in pharmaceutical dosage forms.

Therefore, a rapid and sensitive reversed phase high performance liquid chromatographic method was developed and validated according to the guidelines of FDA, ICH, and USP with respect to accuracy, precision, specificity and linearity. The developed method was found to be simpler, accurate, reproducible, efficient and less time consuming, and was applied successfully for the study of Telmisartan, amlodipine, losartan and hydrochlorthiazide formulations.

Experimental work:

Working standards of were kind gifts from NATCO pvt ltd, Telangana. HPLC grade aceonitrile, mehanol and water were purchased from MERCK pvt ltd,Mumbai.

Equipment: agilent technology, Column: qualisil C18, Flow rate: 1ml / min, Wave length: 231nm, Injection volume: 20 µl. Run time:10minutes

Preparation of phosphate buffer(20 mM):

Weighed 6.2 gms of KH2PO4 in to 1000 ml beaker dissolved and diluted to 1000 ml with HPLC water. Adjusted the pH to 2.5 with orthophosphoric acid.

Peparation of mobile phase:

Mixed a mixture of above buffer 600 ml and 400 ml of acetonitrile HPLC and degassed in ultrasonic water bath for 30 mins. Filtered through 0.45 µ filter under vacum filtration.

Diluent preparation:

Used the mobile phase as diluent.

Preparation of standard and sample solutions:

Standard solution preparation:

Accurately weighed and transferred 10 mg of each telmisartan, losartan. amlodipine, hydrochlorthazide, working standard in to a four 10 ml clean dry volumetric flasks. About 10 ml of diluent was added and sonicated to disslove it complettely and made volume up to the mark with the same solvent (working standard).

Further 0.4 ml of losartan, 0.32 ml of telmisartan, 0.04 ml of amlodipine and 0.10 ml of hydrochlorthiazide pippetted out from the above stock solutions in to a 10 ml volumetric flask and diluted up to the mark with diluent.

Sample solution preparation:

Accurately weighed and transferred 10 mg of working standard in to a 10 ml clean dry volumetric flask. About ml of diluent was added and sonicated to disslove it complettely and made volume up to the mark with the same solvent (working standard).

Further 0.4 ml of losartan, 0.32 ml of telmisartan, 0.04 ml of amlodipine and 0.10 ml of hydrochlorthiazide pippetted out from the above stock solution in to a 10 ml volumetric flask and diluted up to 10 ml volumetric flask and diluted up to the mark with diluent.

Procedure:

20 µl of standard and sample solutions was injected in the chromatographic system and measured areas of peaks.

Validation summary:

Linearity and Calibration

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A minimum of five concentrations were recommended for linearity studies. Varying quantities of the mixed standard stock solution was diluted with the mobile phase to give concentrations of 20, 24, 28, 32, 36, 40 µg / ml for telmisartan, 6.25, 7.5, 8.75, 10, 11.25, 12.5µg / ml for hydrochlorthiazide, 2.5, 3, 3.5, 4, 4.5, 5 and 20, 24, 28, 32, 36, 40 µg / ml for losartan,. The injections were made at in interval of 10 minutes and the peak areas were determined.

A calibration curve was determined for each of the drug independently by plotting the peak areas obtained against concentrations.. From the data obtained, correlation coefficient. Y-intercept and slope were calculated to provide mathematical estimates of the degree of linearity.

Presicion:

Preparation of stock solution:

Accurately weighed and transferred 10 mg of telmisartan, amlodipine, losartan and hydrochlorthiazide in to a 10 ml clean dry volumetric flask. About 10ml of diluent was added and sonicated to dissolve it completely and made volume up to the mark with the same solvent (working standard).

Further 0.4 ml of losartan, 0.32 ml of telmisartan, 0.04 ml of amlodipine and 0.10 ml of hydrochlorthiazide pippetted out from the above stock solution in to a 10 ml volumetric flask and diluted up to 10 ml volumetric flask and diluted up to the mark with diluent.

Procedure:

The standard solution was injected 5 times and measured the area for al five injections in HPLC. The %RSD for the area of the replicate injections was found to be with in the specified limits.

The results are summraized

		Hctz		LH	
S No	Name	RT	Area	RT	Area
1	Injection-1	4.123	9279095	8.143	40148582
2	Injection-2	4.13	9076221	8.17	40139725
3	Injection-3	4.13	9446204	8.167	38569464
4	Injection-4	4.13	9268974	8.18	40170213
5	Injection-5	4.143	9210246	8.317	39543439
6	Injection-6	4.132	9246091	8.162	40129439
Avg		4.131	9254472	8.190	39783477
Std Dev		0.007	119480.0	0.063	642014.7
% RSD		0.157	1.29	0.775	1.61

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		TEL		AML	
S No	Name	RT	Area	RT	Area
1	Solution-1	5.677	51140734	7.433	2696457
2	Solution-2	5.717	53100886	7.523	2468461
3	Solution-3	5.72	53281573	7.537	2771447
4	Solution-4	5.747	53004378	7.57	2798935
5	Solution-5	5.773	52557831	7.603	2690216
6	Solution-6	5.712	53401578	7.542	2568936
Avg		5.724	52747830	7.535	2665742
Std Dev		0.033	839236.6	0.057	125497.0
% RSD		0.571	1.59	0.761	4.708

		TEL		HCTZ	
S No	Name	RT	Area	RT	Area
1	Solution-1	5.67	63496293	4.13	9650622
2	Solution-2	5.7	66154044	4.13	9988456
3	Solution-3	5.7	66325107	4.143	9981655
4	Solution-4	5.707	66515564	4.147	9928193
5	Solution-5	5.847	65302263	4.15	9752722
6	Solution-6	5.712	66495163	4.142	9950815
Avg		5.723	65714739	4.140	9875411
Std Dev		0.063	1175377.3	0.009	140086.7
% RSD		1.095	1.79	0.205	1.419

Acceptence criteria:
The %RSD for the area of fove standard injections results should not be more than 2%

ACCURACY:
Preparation of standard solution:

Accurately weighed and transferred mg of losartan and hydrochlorthiazide in to a 10 ml clean dry volumetric flask. About ml of diluent was added and sonicated to dissolve it completely and made volume up to the mark with the same solvent (working standard).

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Further ml of pip petted out from the above stock solution in to a 10 ml volumetric flask and diluted up to 10 ml volumetric flask and diluted up to the mark with diluent.

Limit of detection and Limit of quantification

The limit of detection and limit of quantification were calculated based on the standard deviation of the response and the slope.

The limit of detection (LOD) may be expressed as

$$LOD = \frac{3.3\sigma}{S}$$

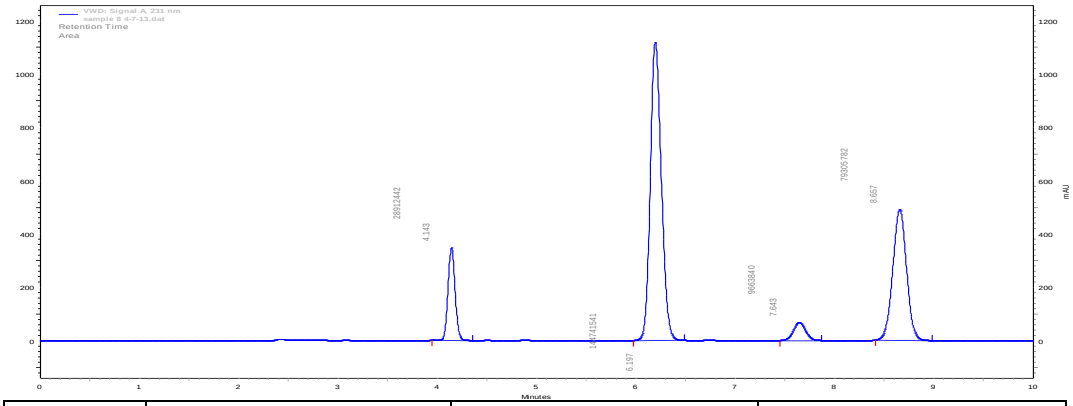
The limit of quantification (LOQ) may be expressed as

$$LOQ = \frac{10\sigma}{S}$$

Where σ=the standard deviation (SD) of peak areas of six injections of each drug.
S =the slope of the calibration curve

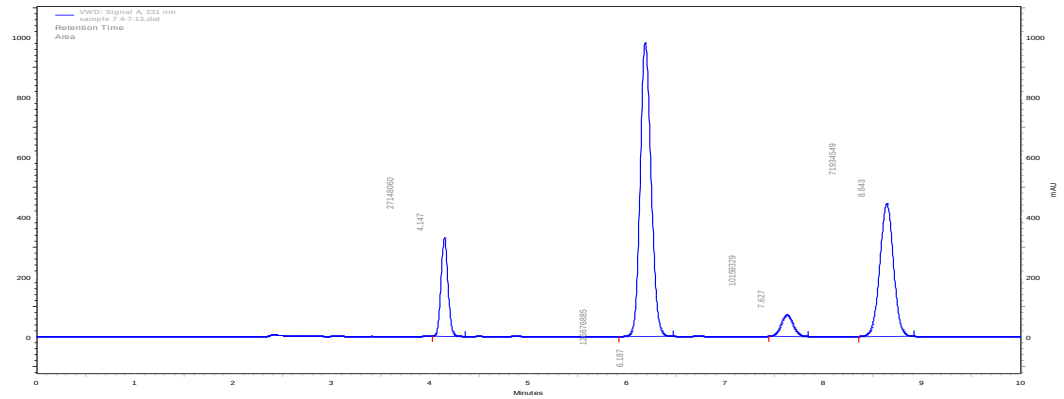
Assay:

Standard:



S.no	Name of the drug	Retenton time(rt)	Area
1.	Hydrochlorthiazide	4.147	27148060
2.	Telmisartan	6.187	126676885
3.	Amlodipine	7.627	10159329
4.	losartan	8.643	71934549

Sample:



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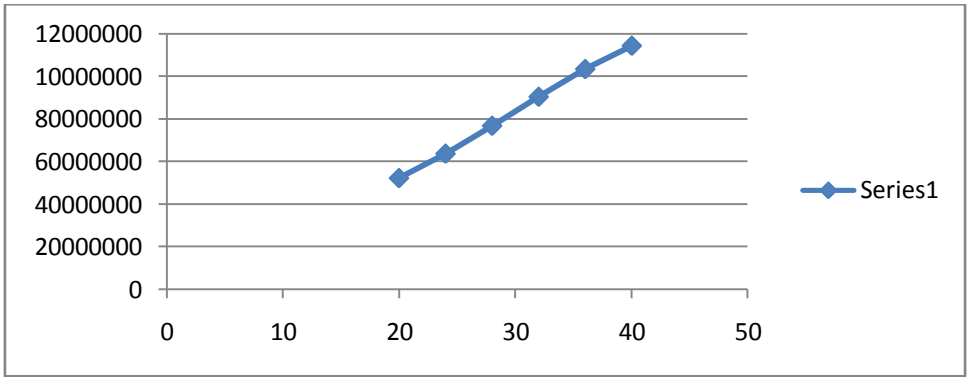
S.no	Name of the drug	Retenton time(rt)	Area
1.	Hydrochlorthiazide	4.137	25987523
2.	Telmisartan	6.190	113101528
3.	Amlodipine	7.617	9450807
4.	losartan	8.633	64363742

Linearity:

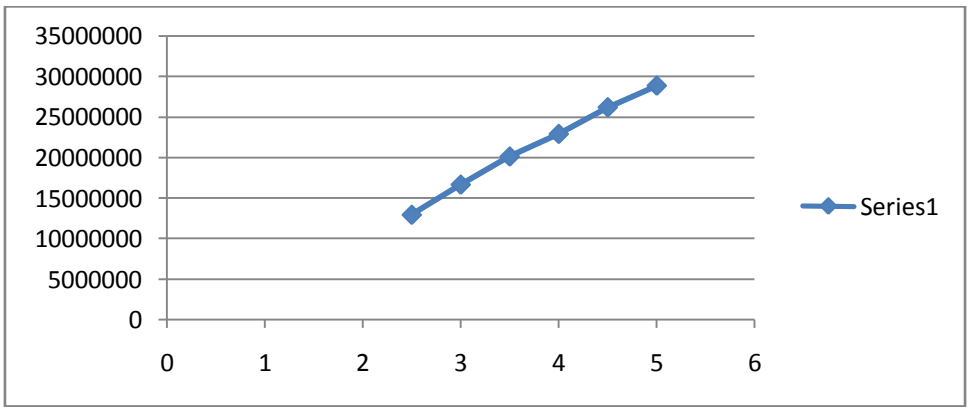
Telmisartan

Conc.	Area
20	52292887
24	63707065
28	76758179
32	90338543
36	103307821
40	114235629



Amlodipine:

Conc.	Area
2.5	12950320
3	16670932
3.5	20133272
4	22914765
4.5	26201280
5	28862733



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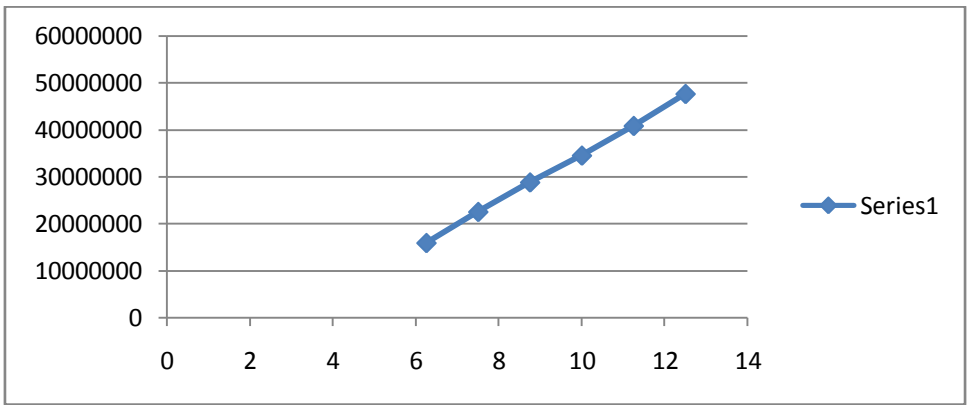
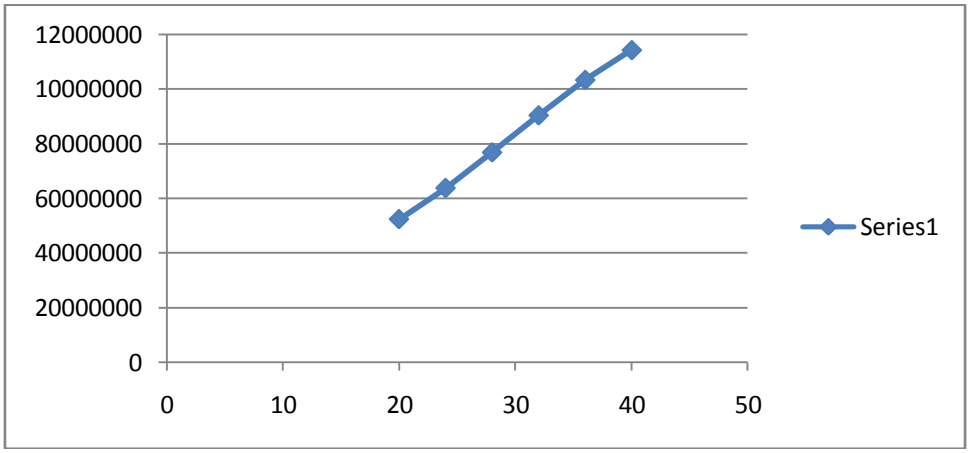
Hydrochlorthiazide:

Conc.	Area
6.25	15950320
7.5	22565125
8.75	28849908
10	34561296
11.25	40874434
12.5	47685753



Losartan

Conc.	Area
20	52292867
24	70707065
28	79758179
32	89033854
36	104407821
40	124235629



Linearity

Hydrochlorthiazide 5 µgm, telmisartan 20 µgm, amlodipine 2 µgm, losartan 20 µgm:

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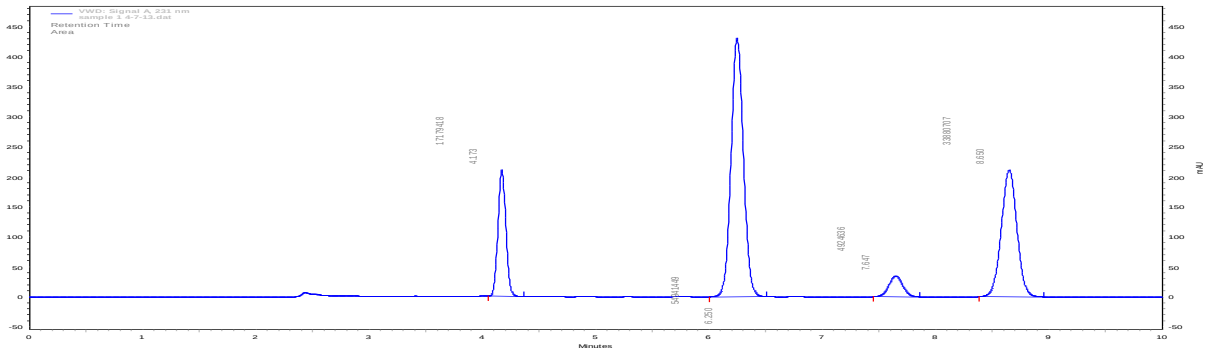

S.no	Name of the drug	Retenton time(rt)	Area
1.	Hydrochlorthiazide	4.173	52292867
2.	Telmisartan	6.250	54941449
3.	Amlodipine	7.647	4924363
4.	losartan	8.650	33880707

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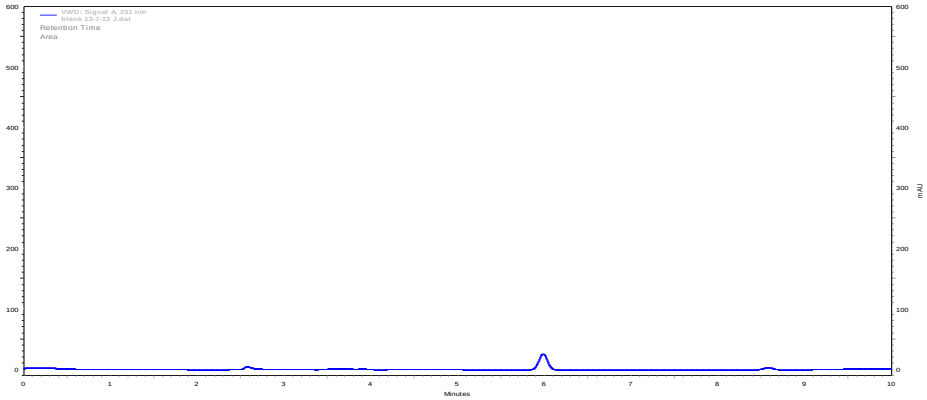

II. RESULTS AND DISCUSSION:

Hctz is a thiazide and ibresartan is an angiotensin 2 receptor antagonist and their combination is used in the treatment of high blood pressure, the 3 drugs are simultaneously estimated by RP – HPLC .

From the results shown in the system suitability the %RSD for the retention times, peak areas and no. of theoritical plates and tailing factor were found to be with in the limits i,e., % RSD for the retention times not more than 2.0% peak areas are not more than 2.0 % and the number of theoritical plates not less than 2000 and tailing factor not more than 2.0. so the method passed the system suitability.

From the results shown in the precision table found that % RSD is not more than 2.0% wich indicates that the proposed method was good reproducibility.

In case of accuracy 50%, 100%%, 150%, of solutions with respective target assay concentrations the % recovery for each levels are between 98.0% to 102%. It indicates the method was accurate and also reveals that the commonly used excepients and additives present in the pharmaceutical formulation were not interfering with the proposed method.

From the results shown in linearity table it was found that the method was linear and the correlation coefficeient is not less than 0.999.

Incase of the LOD and LOQ the signal to noise ratios are within the limits for the hydrochlorthiazide losartan amlodipine and telmisartan.

III. SUMMARY AND CONCLUSION:

A RP-HPLC method is deveoped and validated as per ICH guidelines for simultanious estimation of

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hydrochlorthiazide, telmisrtan , losartan and amlodipine.

In present study an attempt has been made to modify experimental condition, in orderto estimate simultaniously the drugs in combination. The mobile phase was selected after trying various combinations of polar solvents. The preparation of solvents and variation of buffers were found to be quite critical as slight varation in it adversly affected the resolution of the peaks. Considering all fact the following parameter were fixed for this method.

Equipment: high performance liquid chromatography
Coloumn :
Mobile phase :
Mode : isocratic
Flow rate : 1ml/min
Wavelenght :231nm
Injection volume: 20 µl.
Run time: 10 mins.

The praposed methos was found to be rapid accurate, precise, specific, robust and ecnomical. The mobile phase is simple to prepare and economical. The sample recoveries in all formulations were in good agrement with their respective lable claims and they suggested non interference of formulation excepients in the estimation.