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A New Stability Indicating RP-HPLC Method for Determination of Chlorthalidone, Telmisartan and Cilnidipine in Bulk and Tablet Dosage Form

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Abstract : In present study, accurate, precise, rapid and sensitive stability indicting HPLC-UV method has been established for quantification of Telmisartan, Cilnidipine and Chlorthalidone simultaneously in Tablet and bulk. Telmisartan, Cilnidipine and Chlorthalidone were resoluted on Sunsil C_{18} column (4.6mmx250mm; 5µm) using mobile phase containing Acetonitrile and Potassium dihydrogen phosphate in 50:50(v/v) ratio with flow rate of 1ml/min at 238 nm. Concentrations were linear over the range of 40-120 µg/ml for Telmisartan, 10-30 µg/ml for Cilnidipine and 6.25-18.75 µg/ml for Chlorthalidone. The percentage recovery was found to be 99.70-100.51% for Telmisartan, 98.41-100.49% for Cilnidipine and 99.34-100.48% for Chlorthalidone. % RSD for peak area was 0.069% for Telmisartan, 0.058% for Cilnidipine and 0.057% for Chlorthalidone shows that the proposed method is precise. Force-degradation studies have not shown any observable change in the results and hence the proposed method is stability indicating and hence the method is suitable for routine analysis of Telmisartan, Cilnidipine and Chlorthalidone in bulk and tablet dosage form.
Keywords : HPLC, Telmisartan, Cilnidipine, Chlorthalidone, Acetonitrile, Potassium dihydrogen phosphate.

Introduction:

Hypertension frequently referred to as a high blood pressure which is the consequence of higher pressure levels of blood. Blood pressure measurement flowing via blood vessels and blood resistance amount as blood is pumped by heart. (1-3)

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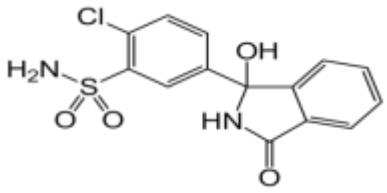
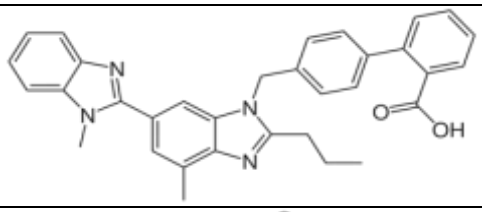
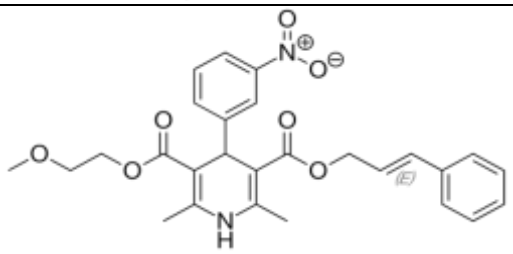
Although blood pressure levels are alarmingly large, there may be no signs. Some individuals of hypertension have narrowness of breath, nosebleeds, headaches and chest pain with blood in urine. These symptoms are not very particular and will not be disclosed unless a health-threatening blood pressure rate is met. (4, 5)

Chlorthalidone/ Telmisartan/ Cilnidipine Combination Formulation:

This three combination drugs is used in elevated blood pressure chemotherapy [6-8]. Chlorthalidone is a diuretic, anti hypertensive, Thiazide drug. Chemical formula is $C_{14}H_{11}ClN_2O_4S$.It is useful for treating high blood pressure, oedema, and hypertrophy of ventricles and prevention of calculi from kidneys. (9-11).Telmisartan is an antagonist of angiotensin two receptor, antihypertensive, It is a derivative of benzene, cardiovascular drug.

Chemical formula is $C_{33}H_{30}N_4O_2$.It is useful in high blood pressure, kidney disorders in diabetes, also in heart failure. (12-14)Cilinidipine is Hyperkalemia, antiarrhythmic drug; it is a calcium channel inhibitor, Hypotensive drug. Chemical formula is $C_{27}H_{28}N_2O_7$.It is useful in Hypertension, diabetes with albumunaria; It is also used in kidney diseases of chronic nature.(15-17)

Table No.1: Tabulated form of selected Drugs

Drug	Structure	Properties
Chlorthalidone		Diuretic Anti Hypertensive Thiazide drug
Telmisartan		Angiotensin two receptor, Antihypertensive
Cilnidipine		Hyperkalemia, Ant arrhythmic drug

Review of Literature:

Literature review reveals that few methods have been reported for determination of chlorthalidone, telmisartan and cilinidipine by UV-spectroscopic method (18) and High Performance Liquid Chromatography Methods. (19-21)

In the proposed analytical work, we have made an attempt to develop a new, simple accurate, precise and sensitive method and to validate the method according to ICH [Q2-R1] guidelines (22).

Experimental Section:

Instrument employed:

The HPLC system consisted of waters 2695 solvent delivery model and waters 2669 PDA detector with reverse phase ODS Sunsil C_{18}(4.6x250mm:5µm).Data acquisition was performed by empower 2 software.

Materials:

Chlortahlidone, Telmisartan, Cilinidipine were obtained as gifts from Rainbow laboratories, Hyderabad. Acetonitrile, Methanol and Millipore water system are of HPLC grade and Potassium dihydrogen phosphate were procured from Yarrow chem. products Mumbai. All reagents used in the present study were of analytical grade.

Preparation of stock solution:

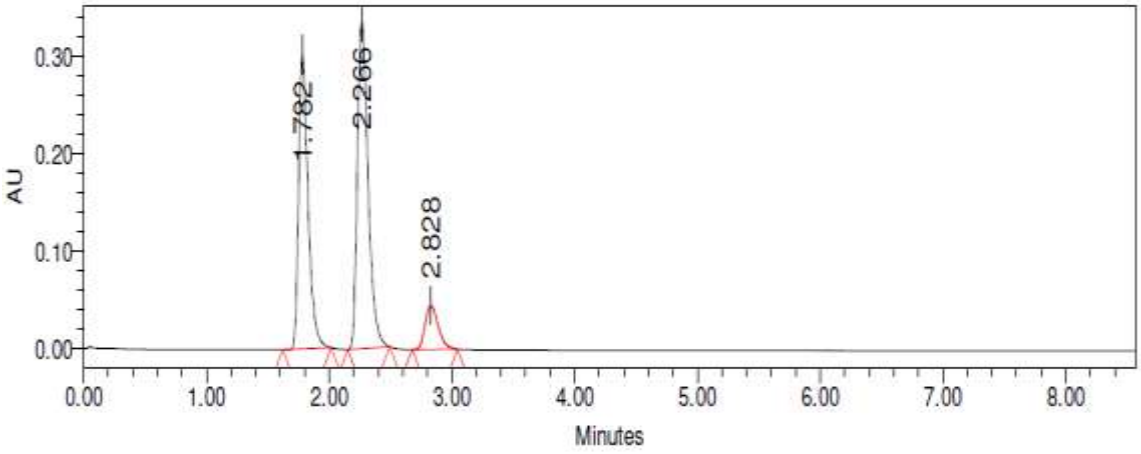
Stock solution of Telmisartan (400 µg/ml), Cilnidipine (100 µg/ml) and Chlorthalidone (65 µg/ml) was prepared by the direct weighing 40 mg, 10 mg and 6.5 mg Telmisartan, Cilnidipine and Chlorthalidone, respectively with succeeding dissolution in diluent in a volumetric flask (capacity 100 ml).

Preparation of working standard:

Solutionis developed from stock solution with concentration level of 80 µg/ml telmisartan, 20 µg/ml cilnidipine and 12.50µg/ml chlorthalidone concentration.

Method Development:

A simple RP-HPLC method was developed on Sunsil –ODS C_{18}(4.6x250mm:5µm)column using Acetonitrile: Potassium dihydrogen phosphate(50:50)as mobile phase with flow rate of 1.0ml/min at 238nm detection with runtime 8minutes.The retention times for Chlorthalidone, Telmisartan and Cilnidipine were 1.782,2.266,2.828mins respectively. RP-HPLC Chromatogram of 3selected drugs it is represented in Figure-1



Name	Retention Time	Area	% Height	USP Resolution	USP Tailing	USP Plate Count
CHL	1.782	1623300	44.24		1.48	8669
TEL	2.266	2029927	49.10	3.19	1.42	7325
CIL	2.828	339137	6.65	3.12	1.28	9349

Fig No.1: Chromatogram of selected Drugs.

Method Validation:

Selectivity:

The method selectively eluted for Chlorthalidone, Telmisartan, and Cilnidipine. There was no interference of placebo and mobile phase at retention time of drugsand were represented in Figure-2 and Figure -3.

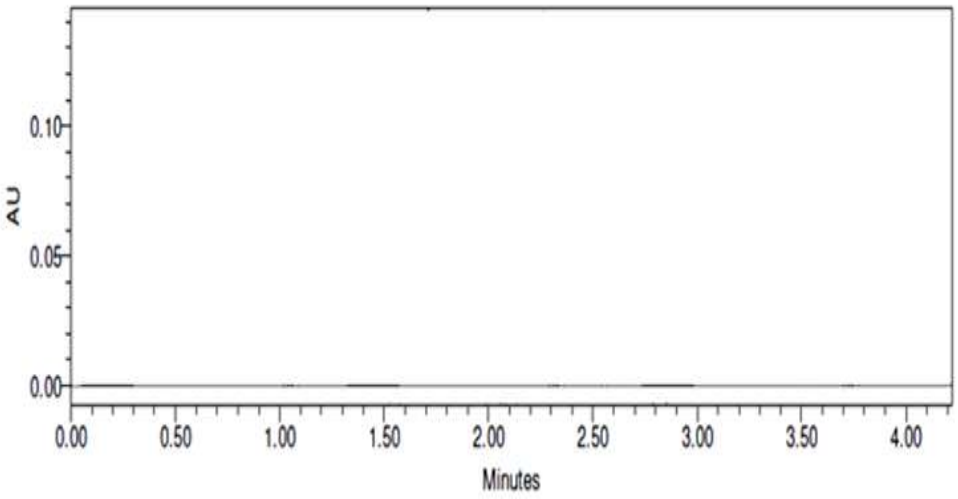


Fig No.2: Chromatogram of Mobile phase

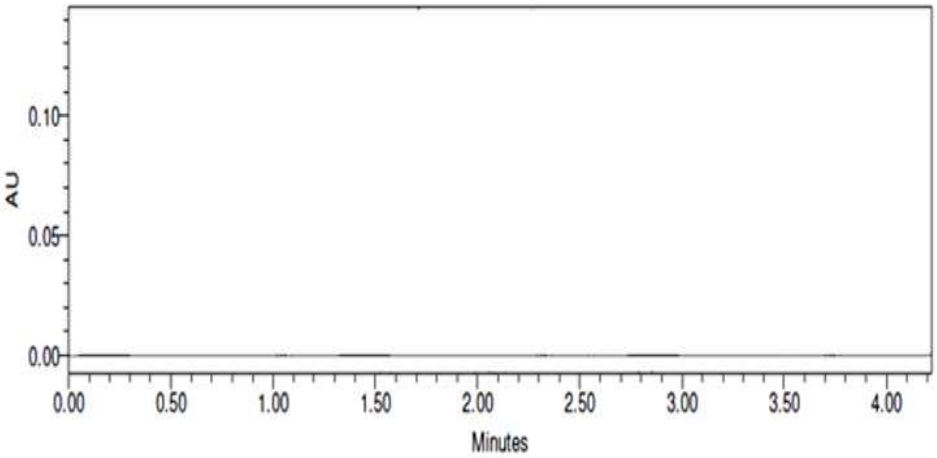


Fig No.3: -Chromatogram of Placebo

Sensitivity:

The studies were performed by injecting lowest concentrationin the calibration curve for six times and assay was determined and it was between 99-102%.The results are tabulated in Table-2.

Table No.2:%Assay of Selected Drugs

S.NO	**CHLORTHALIDONE N=6.25**	**TELMISARTAN N=40**	**CILNIDIPINE N=10**
1	6.21	39.8	9.9
2	6.19	41	10.2
3	6.20	41.2	10.1
4	6.23	40.9	10
5	6.21	40.5	9.8
6	6.24	41.1	9.9
Mean Concentration	**6.21**	**40.75**	**9.98**
%Assay	**99.36%**	**101.87%**	**99.8%**

Linearity:

A series of solutions were prepared using Chlorthalidone 6.25µg/ml to 18.75 µg/ml, Telmisartan 40 µg/ml to 120 µg/ml and Cilnidipine 10 µg/ml to 30 µg/ml of target concentrations. Data was illustrated in Table-3 and in Figure 4, 5, 6.

Table No.3-Data achieved with Linearity Test

Area of chlorthalidone	µg/ml of chlorthalidone	Area of Telmisartan	µg/ml of telmisartan	Area of cilnidipine	µg/ml of cilnidipine
1811831	6.25	2254147	40	368028	10
2726578	9.38	3385450	60	574575	15
3632954	12.50	4519670	80	766332	20
4549363	15.625	5649318	100	957883	25
5451538	18.75	6776558	120	1147103	30

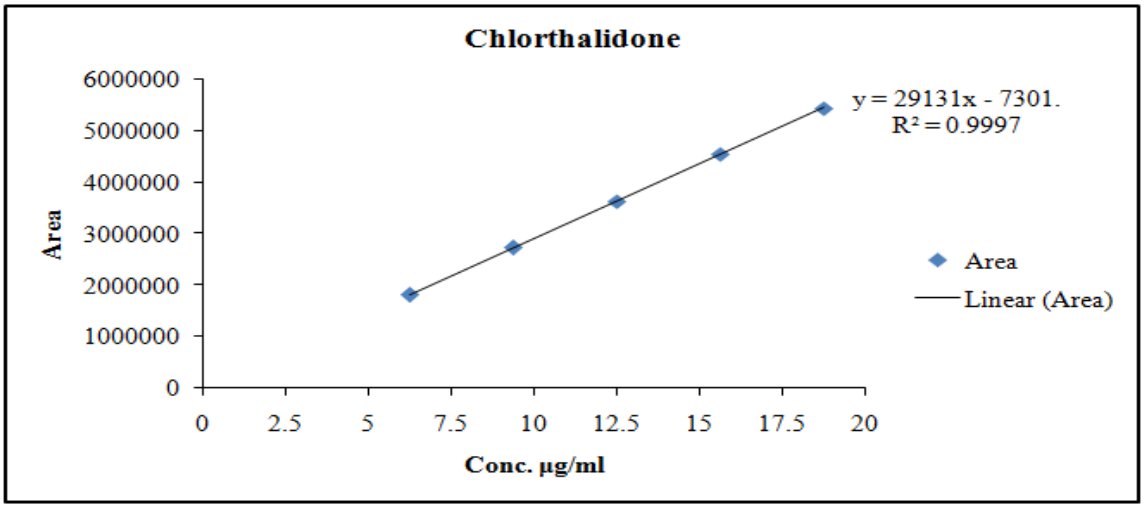


Fig No.4: Linearity of Chlorthalidone

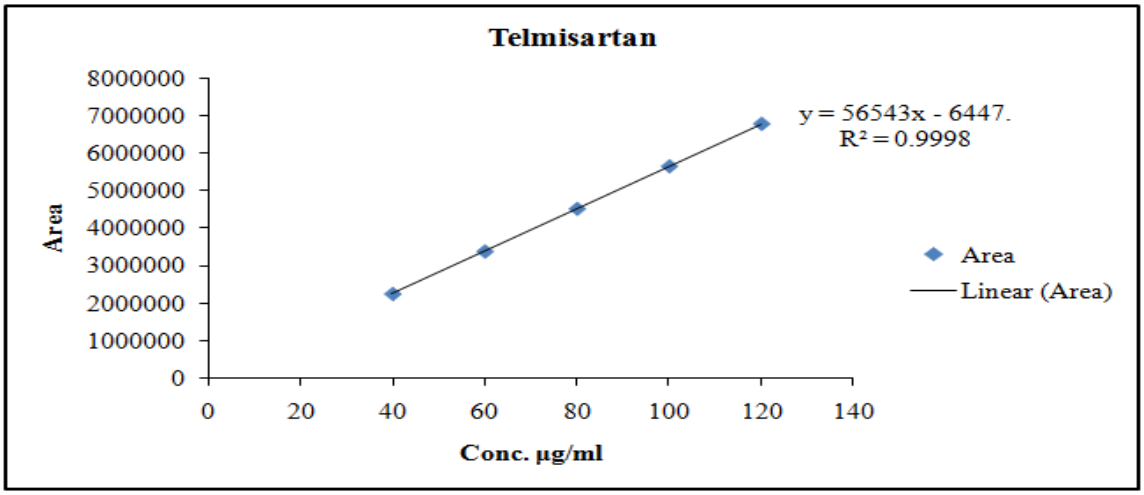


Fig No.5: Linearity of Telmisartan

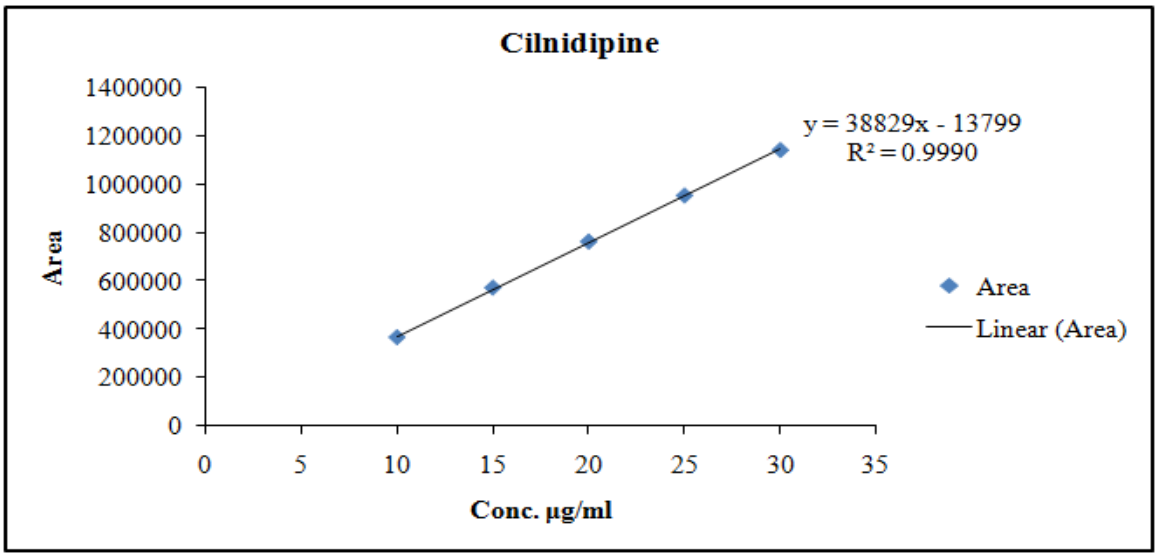


Fig No.6: Linearity of Cilnidipine

Accuracy:

A study of accuracy was conducted. Drug Assay was performed in triplicate as per test method with equivalent amount of Chlorthalidone, Telmisartan and Cilnidipine in to each volumetric flask for each spike level to get the concentration of Chlorthalidone, Telmisartan and Cilnidipine solutions equivalent to 50%, 100%, and 150% of the labelled amount as per test method. The average %recovery of Chlorthalidone, Telmisartan and Cilnidipine. The result for Accuracy data is shown in Table-4.

Table No.4:Accuracy Data for selected drugs

Spiked conc. level	**50%**	**100%**	**150%**
% of Chlorthalidone recovery	99.79	100.48	100.36
	99.34	100.10	100.44
	100.02	100.17	100.17
% RSD of Chlorthalidone	0.34%	0.22%	0.137%
% of Telmisartan recovery	99.88	100.35	100.43
	100.17	100.34	100.51
	99.70	100.42	100.49
% RSD of Telmisartan	0.23%	0.0039%	0.041%
% of Cilnidipine recovery	100.30	100.38	99.67
	100.49	100.37	98.41
	100.49	100.45	99.04
% RSD of Cilnidipine	0.1%	0.043%	0.63%

Precision:

Working Standard solutions were injected six times in to HPLC column and %RSD for peak areas was determined and it was shown that %RSD for selected drugs was less than 2%.The result for precision data is shown in Table -5.

Table No.5: Precision Data for Selected Drugs

Sample inj. No.	Area of chlorthalidone	Area of telmisartan	Area of cilnidipine
1	3626458	4503984	765901
2	3623339	4504322	765184
3	3625508	4509615	765051
4	3623412	4500829	765989
5	3628428	4505725	765180
6	3627294	4501970	765000
Avg.	**3625740**	**4504408**	**765384.2**
SD	**2068.526**	**3091.524**	**441.1972**
RSD	**0.00057**	**0.0069**	**0.0058**
%RSD	**0.057%**	**0.069%**	**0.058%**

Degradation studies:

Force degradation study was performed for selected drugs in different circumstances i.e.,Acid, Base, Peroxide, Heat Dry and Sunlight.% Degradation for selected drugs was within the limits as per ICH guidelines. The result of degradation studies is shown in Table-6

Table No.6: Results of Degradation studies

Degraded with	Area of Chlorthalidone	% Chlorthalidone Assay	% Chlorthalidone Degraded
Acid	3245488	89.02	10.98
Base	3516601	96.46	3.54
Peroxide	3544535	97.23	2.77
Heat dry	3496994	95.92	4.08
Sunlight	3461731	94.95	5.05
Degraded with	**Area of Telmisartan**	**% Telmisartan assay**	**% Telmisartan Degraded**
Acid	4082968	90.05	9.95
Base	4302757	94.90	5.1
Peroxide	4358184	96.12	3.88
Heat dry	4180839	92.21	7.79
Sunlight	4299203	94.82	5.18
Degraded with	**Area of Cilnidipine**	**% Cilnidipine assay**	**% Cilnidipine Degraded**
Acid	703872	91.80	8.2
Base	718601	93.72	6.28
Peroxide	742354	96.81	3.19
Heat dry	709813	92.57	7.43
Sunlight	728486	95.01	4.99

Conclusion:

A simple, sensitive and accurate HPLC-UV Method has been developed for determination of Chlortahlidone, Telmisartan, and Cilnidipine in bulk and tablet dosage form.Both Placebo and mobile phase did not interfere at retention times of drugs which shows that the method selectively resoluted the drugs. The proposed method resulted better %Recovery compare to existing methods. %RSD less than 1% for peak areas shows that the method is precised. The %Assay for lowest concentration in calibration curve for selected drugs was between 99-101% which shows that the developed method was sensitive. % degradation for selected drugs

was less than 10%which shows that the proposed method was stability indicating. Hence; the developed method can be used for determination of selected drugs in bulk and tablet dosage form.

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