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 C₁₈ 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)
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_{2}O_{4}.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_{4}O_{2}.It is useful in high blood pressure, kidney disorders in diabetes, also in heart failure. (12-14)Cilnidipine is Hyperkalemia, antiarrhythmic drug; it is a calcium channel inhibitor, Hypotensive drug. Chemical formula is C_{27}H_{30}N_{4}O_{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 | ![Image](image1) | Diuretic<br>Anti Hypertensive<br>Thiazide drug |
| Telmisartan    | ![Image](image2) | Angiotensin two receptor,<br>Antihypertensive |
| Cilnidipine    | ![Image](image3) | Hyperkalemia,<br>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:

Chlorthalidone, Telmisartan, Cilnidipine 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 C₁₈(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

![Chromatogram of selected Drugs.](image)

| Name | Retention Time | Area    | % Height | USP Height | USP Resolution | USP Tailing | USP Plate Count |
|------|---------------|---------|----------|------------|----------------|-------------|----------------|
| CHL  | 1.782         | 1623300 | 44.24    | 1.48       | 1.42           | 1.28        | 8669           |
| TEL  | 2.266         | 2029927 | 49.10    | 3.19       | 3.12           | 1.28        | 7325           |
| CIL  | 2.828         | 339137  | 6.65     | 3.12       | 1.28           | 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 drugs and 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 concentration in 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 Chlorothalidone 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
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 Chlorthalidone, 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 resolved 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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