Ultra performance liquid chromatographic method for simultaneous quantitation of degradation products of telmisartan and hydrochlorothiazide in combination dosage form

Narasimha Reddy G P¹, Sreenivasulu Reddy T¹, Sidda Reddy K¹, Shashi Kumar K N²

¹Department of Chemistry, Sri Krishnadevaraya University Anantapur, Anantapuram, Andhra Pradesh, India
²Department of H&S, Annamacharya Institute of Technology & Sciences, Kadapa, 516003, Andhra Pradesh, India

ABSTRACT
This work is intended to thrive a stability indicating Ultra performance liquid chromatographic method for the estimation of Telmisartan (TLM) and Hydrochlorothiazide (HCTZ) and degradation products pharmaceutical dosage forms. Separation was carried out on Zorbax Eclipse XDB C-18(50 x 2.1 mm, 1.7 μm) column using a gradient method. Mobile phase A is 10mM KH₂PO₄ having 1% (v/v) of trimethylamine and mobile phase B is acetonitrile used in this work. 0.5 mL/minute is the flow rate and at 271nm noticed wave length is monitored. Method development trails were carried out on six different columns. For specificity, limit of quantification, limit of detection, linearity, accuracy, method precision, robustness and stability this method is validated. Correlation coefficient of the impurities is more than 0.99. Stability indicating method confirmed that there were no interference of all impurities of TLM and HCTZ. Hence, developed LC method was stability indicating and well applied for drug product stability study as well as to quality monitoring.

INTRODUCTION
Telmisartan is an angiotensin II receptor antagonist. Telmisartan keeps blood vessels from narrowing, which lowers blood pressure and improves blood flow. Telmisartan (TLM) is an angiotensin receptor blocker (ARB) has an extensive period of working, and has the lengthy half-life of any ARB (Benson et al., 2004). Hydrochlorothiazide is the 3, 4-dihydro derivative of chlorothiazide. Its chemical name is 6-Chloro-3, 4-dihydro-2H-1, 2, 4-benzothiadiazine-7-sulfonamide 1, 1-dioxide. In recent studies, it was found that the drug has uniquely potent activity which could prevent stroke. Hydrochlorothiazide (HCTZ) belongs to the thiazide class of diuretics, working to reduce re-absorption of sodium (Na) in the distal convoluted tubule at kidneys. It improves the osmolarity in the channel, results fewer water to be mopped up from the gathering tubule. Which cause to improve the urination output.

From the literature it was observed that no UPLC method was published for the simultaneous quantitation of degradation products related to both TLM and HCTZ. Few test methods were published for simultaneous estimation of TLM and HCTZ. Validated RP-HPLC method concurrent for the quantification of TLM and HCTZ in its dosage forms (Bhat et al., 2007). Four respective methods for determination of TLM and HCTZ in homogenize dosage
forms and spectrofluorimetric technique used for analysis TLM in human plasma (Bebawy et al., 2005). Diffuse Reflectance spectroscopy is used to determination of HCTZ in formulations (Gotardo et al., 2005). Telmisartan and Congo red ion complex determination by spectrometric methods (Qin et al., 2009). Determination of telmisartan by LC/APCI-MS/MS in human blood plasma (Li et al., 2005; Hempen et al., 2006). Low level Impurities of TLM and HCTZ, however run time of this method was 60 minutes with HPLC technique (Reddy et al., 2012). Different HPLC and spectroscopic methods have been reported for the evaluation additionally TLM or HCTZ in its dosage forms (Martín et al., 1995; Farthing et al., 1998). Development and stability-Indicating LC Method to Quantify Hydrochlorothiazide in Oral Suspension (Tagliari et al., 2008). Solid-phase microextraction coupled to HPLC used to determination of TEL in rat tissues (Nie et al., 2006).

The goal of the present research work was to develop a short, specific, stability indicating fast LC (UPLC) method for simultaneous estimation of TLM, HCTZ and its degradation products in TLM and HCTZ oral dosage forms. Chemical structures of TLM, HCTZ and impurities presented in Figure 1. To develop a short run time UPLC method different development trails were taken using different UPLC columns, different gradient programs with different mobile phase pH’s. Finally, UPLC method was developed with run time of 5.5 minutes in which all degradation products related to HCTZ and TLM were separated. After successful method development, the method was validated according to ICH Q2A validation guidelines to use for intended purpose. Specificity study was conducted using different stress conditions to prove the stability indicating capability of the method. Specificity was conducted using acid, base, peroxide, humidity and thermal conditions.

EXPERIMENTAL

Reagents and Standards

TLM, HCTZ related impurities, standards and tablet samples of were obtain from Biosys Medisciences Laboratories (Hyderabad, Telangana, India). HPLC grade acetonitrile (ACN) and methanol (MeOH) and Ortho-phosphoric acid was collect from Qualigens Fine chemicals (Mumbai, India) and potassium dihydrogen orthophosphate is market from Merck Fine chemicals (Mumbai, India) and potassium di-

Ortho-phosphoric acid was collect from Qualigens Laboratories (Hyderabad, Telangana, India). HPLC samples of were obtain from Biosys Medisciences Reagents and Standards conditions.

using acid, base, peroxide, humidity and thermal capability of the method. Speciϐicity was conducted ent stress conditions to prove the stability indicating pose. Speciϐicity study was conducted using differ-

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UPLC columns, different gradient programs with dif-

etent development trails were taken using different

Chromatographic conditions for UPLC

The chromatographic separation was achieved on Zorbax Eclipse XDB C-18, 50 x 2.1 mm, 1.7 μm column using mobile phase-A consist of 10 mM KH₂PO₄ having 1% (v/v) of trimethylamine and the pH maintained to 2.7 with Orthophosphoric acid and mobile phase-B was ACN. Flow rate was set to 0.5 mL/min with column temperature of 45 °C and the detection wavelength was carried out at 271 nm. Sample solution contains a concentration of 250 μg mL⁻¹ of HCTZ and 800 μg mL⁻¹ of TLM and the injection volume is 5 μL. The sample solvent consist of Methanol: water in the ration of 50:50 which was used for test and reference solutions preparation.

Impurity stock solutions and Standard prepara-

10 tablets of each of the above strength were transferred into a mortar pestle and crushed in to a fine powder. About 25 mg of sample HCTZ powder is accurately weighed and transferred into a 100 mL volumetric flask. About 70 mL of solvent was added and sonicated for 30 min. with intermittent shaking. Further diluted up to the mark with solvent. Again 20 mL of water was added and further sonicated for 15 minutes. The solution is cooled to attain room temperature. The volume was made up to 100 ml mark with MeOH and mixed. The solution was filtered through 0.2 μm nylon membrane filter.

RESULTS AND DISCUSSION

Method development

The main aim of the current project work was to have a shorter run time and robust fast LC method to simultaneous estimation of TLM and HCTZ degra-

dation products in a single method. TLM and HCTZ were having pKa of 3.5, 4.1, 6.0 and 7.9, 9.2 acid and basic functional groups. The pH of selected buffer should have the pH ± 1.5 units from the pKₐ values of the analytes (Heinisch and Rocca, 2004).

Method development trials were carried out on six different UPLC columns (50 x 2.1 mm, 1.7 μm and 100 x 2.1 mm, 1.7 μm) and the respective chromatograms were shown in Figure 2. Relative retention time is used to understand the retention time for all impurities against the product peaks of
Figure 1: Chemical structures of TLM, HCTZ and impurities
### Table 1: RRT values

| Impurity Name     | RRT's w.r.t Telmisartan |
|-------------------|-------------------------|
| Chlorothiazide    | 0.27                    |
| Hydrochlorothiazide| 0.32                    |
| Telmisartan       | 1.00                    |
| Telmisartan imp-A | 0.25                    |
| Telmisartan imp-B | 0.96                    |
| Telmisartan imp-C | 1.11                    |
| Telmisartan imp-D | 1.32                    |
| Telmisartan imp-E | 0.92                    |
| Telmisartan imp-F | 0.83                    |
| Telmisartan imp-G | 1.07                    |
| Telmisartan imp-H | 1.51                    |

### Table 2: Force Degradation

| Type of stress | Stress condition |
|----------------|------------------|
| Thermal        | 50°C for 5days-It was observed that at 105°C the tablet become sticky to the petry dish. So selected at 50°C for thermal degradation. |
| Acid           | 5ml 1N HCl 50°C 1Hr |
| Base           | 5ml 1N NaOH 50°C 1Hr |
| Oxidation      | 5ml 10% H₂O₂ 50°C 1Hr |
| Photolytic     | 4000lux hours-5days |

### Table 3: Degradation results

| RRT w.r.t to Telmisartan | % Degradation | Name (known/unknown) | Remarks |
|--------------------------|---------------|----------------------|---------|
|                          | Thermal       | Acid                 | Base    | Oxidation | light | Chlorothiazide | Minor |
| 0.27                     | 3.06          | 1.04                 | 0.19    | 0.90      | 3.19  | Major          |
| 1.07                     | 0.01          | 0.04                 | 0.06    | 0.06      | 0.01  | Imp-G          | Minor |
| 1.11                     | –             | 1.32                 | –       | –         | –     | Imp-C          | Minor |
| 1.32                     | 0.06          | 0.04                 | 0.06    | 0.09      | 0.06  | Imp-D          | Minor |
| 0.92                     | –             | 0.04                 | –       | –         | –     | Imp-E          | Minor |
| 0.27                     | –             | –                    | –       | 0.11      | –     | Chlorothiazide | Minor |
| 0.96                     | –             | –                    | –       | 0.07      | –     | Imp-B          | Minor |

### Table 4: Linearity results

| Parameter | % Imp-A | % Imp-B | % Imp-C | % Imp-D | % Imp-E | % Imp-F | % Imp-G | % Imp-H |
|-----------|---------|---------|---------|---------|---------|---------|---------|---------|
| Slope     | 394615  | 406269  | 344374  | 386706  | 364374  | 396706  | 344345  | 386433  |
| Intercept | 1813.05 | 24.90   | 5152.79 | 6164.21 | 5652.79 | 1764.21 | 5132.77 | 1453.31 |
| Correlation | 0.9955 | 0.9968  | 0.9953  | 0.9924  | 0.9993  | 0.9944  | 0.9973  | 0.9955  |
Telmisartan and Hydrochlorothiazide. Telmisartan, Hydrochlorothiazide and known impurities were injected into the system and established the relative retention time (RRT) values and represented in Table 1. Inertsil ODS 3V 150 x 4.6 mm, 5 μm column provided the separation between the TLM and its positional isomer and the separation between HCTZ and its related impurities. To increase the resolution the column length was increased from 100 to 150 mm. In these columns, all the known impurities of TLM and HCTZ were well separated and also injected the stress degradation samples to verify any unknown impurities co-eluting with the known impurities. Analysis of a huge amount of samples which were exposed to different storage conditions with these chromatographic conditions clearly indicates that the final conditions of this method will adequately separate all the impurities of TLM and
HCTZ. Few chromatograms related to UPLC method development are shown in Figure 3, Figure 4, Figure 5, Figure 6, Figure 7, Figure 8, Figure 9.

**Chromatographic conditions**

**Selection of Wave length**

Telmisartan, HCTZ and its impurities have UV absorption sites. So UV /PDA detector was selected for degradation products method of Telmisartan+HCTZ tablets. Based on the wavelength maxima of the impurities of both Telmisartan and Hydrochlorothiazide wavelength selected at 271nm in which all impurities having optimal absorption. To compensate the response, response factor for all the impurities calculated at selected wavelength and correction factor applied.

**Buffer & pH selection**

In both assay and degradation products method development of Telmisartan+HCTZ, all the impurities and degradation products were well separated in buffer pH 2.7 hence pH 2.7 was selected for the purpose.
Figure 8: TLM, HCTZ and impurities chromatograms on X-bridge UPLC column

Figure 9: TLM, HCTZ and impurities chromatograms on Eclipse UPLC column

Figure 10: Method development trial-1 chromatogram
Buffer

1 litre of 0.01M KH$_2$PO$_4$ in water containing 1ml TEA was adjusted to pH 2.7 with OPA (ortho phosphoric acid).

Column Selection

Based on the different method development trails using different columns, the best suitable column was identified as Zorbax Eclipse XBD C-18, 50X2.1, 1.7 $\mu$m column. Hence this column was selected in which all the Telmisartan and HCTZ impurities were well separated. (For column selection ref: method development trials).

Column

Zorbax Eclipse XBD C-18, 50X2.1, 1.7 $\mu$m

Diluent Selection

During method development trails with different solvents like methanol, acetonitrile were used along with water as extraction solvent. From the method
Figure 14: Method Development trail-5 chromatogram

Figure 15: Specificity chromatogram

Table 5: Precision results

| Analyte   | Method precision (% RSD) | Intermediate precision (% RSD) |
|-----------|--------------------------|-------------------------------|
| HCTZ      | 1.12                     | 0.96                          |
| TLM       | 1.35                     | 0.78                          |
| %Chloro   | 1.52                     | 0.94                          |
| % Imp-A   | 1.25                     | 0.68                          |
| % Imp-B   | 1.21                     | 0.91                          |
| % Imp-C   | 1.11                     | 0.94                          |
| % Imp-D   | 1.32                     | 0.98                          |
| % Imp-E   | 1.25                     | 0.79                          |
| % Imp-F   | 1.51                     | 0.93                          |
| % Imp-G   | 1.38                     | 0.88                          |
| % Imp-H   | 1.34                     | 0.85                          |

development trials methanol: water in the ratio 80:20 was finally selected as solvent.

DEVELOPMENT TRIALS

Trial-1

Method development trials were initiated based on the understandings from the literature survey and presence of impurities nature. The chromatogram shown in Figure 10 shows that the peak shapes of HCTZ (plate count 875) and TLM is not good. Hence to separate HCTZ impurity and to enhance the peak concentration & injection volume

The test solution was selected to contain 250 ppm of HCTZ and 800 ppm of Telmisartan. The injection volume was selected at 5μl.
**Table 6: Accuracy results**

| Amoun added | %Chloro | % Imp-A | % Imp-B | % Imp-C | % Imp-D | % Imp-E | % Imp-F | % Imp-G | % Imp-H |
|-------------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| LOQ         | 98.3–   | 97.8–   | 98.1–   | 97.3–   | 97.8–   | 99.1–   | 97.3–   | 99.3–   | 99.3–   |
|             | 100.2   | 99.4    | 100.1   | 99.2    | 100.3   | 100.8   | 100.2   | 100.8   |          |
| 50%         | 97.3–   | 98.2–   | 97.3–   | 98.2–   | 99.3–   | 98.2–   | 99.2–   | 101.8   |          |
|             | 99.7    | 99.5    | 99.9    | 99.7    | 100.1   | 100.8   | 100.2   | 101.8   |          |
| 100%        | 98.6–   | 98.7–   | 98.7–   | 98.7–   | 98.6–   | 98.7–   | 98.7–   | 98.7–   |          |
|             | 99.4    | 99.4    | 100.1   | 99.1    | 100.5   | 99.4    | 100.2   | 100.2   |          |
| 150%        | 99.1–   | 99.2–   | 97.6–   | 99.2–   | 98.1–   | 99.1–   | 99.2–   | 98.7–   |          |
|             | 99.6    | 99.8    | 100.2   | 100.1   | 100.2   | 100.9   | 99.6    | 99.4    |          |

**Table 7: System suitability results**

| S. No | Name | Retention Time | Area   | USP Tailing | USP Plate Count |
|-------|------|----------------|--------|-------------|-----------------|
|       |      |                |        |             |                 |
| **Control condition** | | | | | |
| 1     | HCTZ | 0.913          | 100763 | 1.06        | 9628            |
| 2     | TLM  | 2.968          | 139963 | 1.26        | 179452          |
| **Flow minus** | | | | | |
| 1     | HCTZ | 1.003          | 111708 | 1.07        | 10118           |
| 2     | TLM  | 3.081          | 155945 | 1.28        | 175128          |
| **Flow plus** | | | | | |
| 1     | HCTZ | 0.831          | 92122  | 1.07        | 9684            |
| 2     | TLM  | 2.892          | 128092 | 1.25        | 185244          |
| **Minor component minus** | | | | | |
| 1     | HCTZ | 1.005          | 101151 | 1.05        | 9685            |
| 2     | TLM  | 3.080          | 140397 | 1.27        | 195118          |
| **Minor component plus** | | | | | |
| 1     | HCTZ | 0.834          | 101542 | 1.11        | 10055           |
| 2     | TLM  | 2.873          | 140220 | 1.26        | 168604          |
| **Temperature minus** | | | | | |
| 1     | HCTZ | 0.945          | 100994 | 1.07        | 9891            |
| 2     | TLM  | 2.956          | 140670 | 1.30        | 180057          |
| **Temperature plus** | | | | | |
| 1     | HCTZ | 0.874          | 100820 | 1.10        | 10034           |
| 2     | TLM  | 3.000          | 140563 | 1.24        | 182700          |
| **pH plus** | | | | | |
| 1     | HCTZ | 0.916          | 100443 | 1.06        | 9121            |
| 2     | TLM  | 3.222          | 137864 | 1.13        | 166156          |
| **pH minus** | | | | | |
| 1     | HCTZ | 0.948          | 97128  | 1.19        | 11783           |
| 2     | TLM  | 2.849          | 129311 | 1.36        | 162729          |
shapes, different UPLC column was used by adopting the same gradient program.

**Trial-2**

Acquity BEH C18 column was used for this development trail. The trail-2 chromatogram is shown in Figure 11. The chromatogram shown in Figure 11 shows that the peak shapes of HCTZ (plate count 904) is not good. Hence the method needs to be optimized further to improve the peak shape. Refer trail 3 for further development trails.

**Trial-3**

Development Trail-2 Chromatographic conditions were used for this development trail and modified gradient program was used. The chromatogram shown in Figure 12 shows that resolution between imp-B of Telmisartan and Telmisartan main peak is very less and the peak purity of Telmisartan is not good. Hence, further trials were made to improvise the resolution between TLM impurities.

**Trial-4**

Trial 4 objective was to resolve the above mentioned peaks and verify the peak purity of TLM. The chromatographic condition for trail-4 and the chromatogram are shown in Figure 13 respectively. In this trial different column chemistry was used and column temperature was also decreased to study the impact of column temperature of peak separations. The chromatogram shown in Figure 13 shows that resolution between imp-B of Telmisartan and Telmisartan main peak is very less and hence the method need to modified for better resolution between impurity B and Telmisartan.

**Trial-5:**

Trial-5 was carried out for evaluation of different gradient programs. Method chromatographic conditions were applied as per method development trail-4. development trail chromatogram was shown in Figure 14 and spiked chromatogram was shown in Figure 14.

**Method validation**

After successful method validation, According to ICH guidelines (Q2(R1), 2005), same equipment in same lab or different laboratories by different operators it is proven that elaborated test method is capable of giving reproducible and reliable results, and through stress testing active substances inherent stability characteristics, stability indicating capability is proved.

According to the ICH guidelines, parameters like robustness, precision, specificity, linearity, accuracy, LOD and LOQ of determination proved that TLM and HCTZ and its related substances are proved validated and quantify by developed UPLC method.

**Specificity**

Specificity of the developed method was proved by injecting the stressed degradation samples and impurities spiked solution. In the degradation study the chromatograms of tablet powder containing the TLM and HCTZ are recorded and shown in Figure 15. The diluent and the placebo chromatograms shows that no interference was observed at the RT’S of TLM, HCTZ and their related substances. Except in acid degradation, TLM establish to be stable in all degradation conditions, whereas HCTZ face degradation in all the circumstances and form major degradants of chlorothiazide. Spectral purity of TLM, HCTZ and their impurities of unknown and known was tested whether the peaks found to be pure or not. Purity angle and purity threshold was measured and found that purity angle for all the peaks was less than its purity threshold, it indicates that all peaks found to be pure. Spectra of well-known impurities in degradation samples were much same to the spectra be given for the single impurities indicates that there was no co-elution peak at the retention time of particular well-known impurities.

All the degradation samples are subjected to LC-MS analysis to confirm the known degradants using the experimental conditions mentioned in the experimental section. This specificity study also helpful for knowing the way of degradation and products that could form during storage, and helps to facilitate formulation development and packaging.

Degradation results of TLM and HCTZ under various stress conditions are shown in Table 4, which shows that TLM undergoes degradation in acid and formed TLM imp-C and is stable in other stress conditions.

The specificity of the method was established by injecting the placebo solution in which TLM and HCTZ were absent, to prove the absence of interference of the excipients which takes part in the pharmaceutical preparation. According to ICH guidelines, degradation study were also conducted by apply the tablet powder to accelerated degradation at different conditions to evaluate the interference of degradation impurities. The force degradation conditions and degradation results are summarized in Table 2 and Table 3 respectively.

**Limit of detection and quantification**

The LOD and LOQ for impurities TLM, HCTZ and its impurities were determined by injecting a series of solutions with known concentration. Calculated the
S/N ratio for these solutions and selected the concentration at which level S/N is about 2 to 3 was LOD and the S/N ratio was about 10 was LOQ.

**Linearity**

The linearity of the method in multiple reaction monitoring mode was satisfactorily investigated by injecting dissimilar concentrations. The TLM, HCTZ and their connected impurities linearity were determined at the range from LOQ to 120%. The least-squares method was used to calculate the correlation co-efficient and calibration equation. The relative response factor for all the impurities was determined against their respective standard. Data evaluation was done by Linear regression method.

Table 4 contains the values of correlation coefficient. Response factor and relative response factor for all impurities were obtained from linearity experiment and results were shown in Table 4.

**Precision**

The present method was verify through method precision and intermediate precision. Method precision was checked by impurities spiked sample by injecting six individual preparations. Table 5 given Precision results and between two analysts the results for all the compounds were listed and short variation between the two analysts be revealed that this method has good intermediate precision.

**Accuracy**

Accuracy of the present method was carried out by injecting the impurities spiked solution at three different concentration levels of 50%, 100% and 150% to their specification limit, in triplicate. The % recovery and the RSD were then calculated for each impurity. The mean of percentage recoveries and their RSDs were designed.

Table 6 reports three different concentration levels recovery percentage results of TLM+HCTZ impurity samples between 86-105%.

**Robustness**

Robustness of the method indicates the reliability of an analysis with respect to deliberate variations in method parameters. The values of RT, RRT and resolution were compared against the normal condition to assess the sensitivity of each impurity at different chromatographic conditions. Also the resolution between the remaining impurities from analytes was not significantly affected and the elution pattern of the impurities remained unchanged and the results were shown in Table 7.

**Solution stability**

The solutions of controlled sample TLM and HCTZ, placebo solution containing TLM, placebo solution containing HCTZ and impurities spiked solution in duplicate are prepared. The freshly prepared solutions and the solutions stored at room temperature and freezer up to 7 days injected at different time intervals. The % change of impurity come by at initial against was compared the % impurity come by at different time intervals.

Under these conditions, the % difference of the impurity is well within the acceptance limits indicating that the test solutions are stable up to 7 days when the solutions are stored at freezer conditions. However % change for HCTZ impurities were found out of the specification limit when the test solutions stored at room temperature hence it was concluded that test solutions are not stable at room temperature.

**CONCLUSION**

TLM+HCTZ tablet samples stored at accelerated (temperature: 40±2 °C, relative humidity 75±5%) and long term (temperature: 30±2 °C, relative humidity 65±5%) storage conditions. Stability samples were analyzed by using the developed LC method for period of 6 months at different intervals, i.e. initial, 1, 2, 3 and 6 months. The effect of storage conditions results were proved that the method is having stability indicating capability. Stability result clearly indicates that the dosage form was stable under long term and accelerated conditions. Stability indicating method confirmed that there were no interference of impurities. Hence, developed LC method was stability indicating and well applied for drug product stability study as well as to quality monitoring. This is the short run time method and from literature it was evident that no UPLC method was developed for simultaneous estimation of TLM, HCTZ and its impurities. The current ULC method is very short run time method and it is very helpful for routine analysis of TLM and HCTZ.

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