Subhashini Edla, Actapharmica (2015) Vol.2 (1) 112-126 ISSN 2386-8937

LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY METHOD FOR THE SIMULTANEOUS ESTIMATION OF TELMISARTAN AND HYDROCHLOROTHIAZIDE IN PLASMA

SUBHASHINI EDLA*, B. SYAMA SUNDHAR
Dept. of Chemistry, Acharya Nagarjuna University, Nagarjuna Nagar, Guntur

Abstract:
A rapid, sensitive and selective analytical Liquid chromatography tandem mass spectrometry method was developed and validated for the determination Telmisartan and Hydrochlorothiazide in human plasma. Liquid-liquid extraction was used for sample preparation and analysis, followed by liquid chromatography tandem spectrometric analysis and an electrospray-ionization interface. Compounds were analyzed on a Aquasil-C18 (250×4.6mm×5µm) column with the mobile phase of pH 4.5 Acetate buffer solution, Methanol and Acetonitrile in the ratio of 60:20:20 (v/v) in isocratic condition at a flow rate of 0.5m L/min for 10min. a retention time of 4.39min and 5.73min were observed for Telmisartan and Hydrochlorothiazide respectively. The method was validated as per ICH guidelines as Linearity, precision, accuracy, recovery and different stability studies. All the results obtained were found to be within the acceptance limit. Hence the developed LC/MS/MS method was successfully applied for the determination of Telmisartan and Hydrochlorothiazide in human plasma.

Key Words: LCMS/MS Method, Telmisartan, Hydrochlorothiazide, Plasma

Introduction:

Telmisartan is an angiotensin II receptor antagonist (angiotensin receptor blocker, ARB) used in the management of hypertension. It is indicated for the treatment of essential hypertension [1,2] and also used to reduce the risk of heart attack, stroke, or death due to heart problems in certain patients. Sometimes it is also used to treat congestive heart failure (condition in which the heart is unable to pump enough blood to the rest of the body) and diabetic nephropathy (kidney disease in people with diabetes and high blood pressure). It works by relaxing blood vessels, which helps to lower blood pressure. 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) [3]. Telmisartan's activity at the PPAR-γ receptor has prompted speculation around its potential as a sport doping agent as an alternative to GW 501516 [4]. Telmisartan activates PPARδ receptors in several tissues [5-8]. Side effects with Telmisartan are similar to other angiotensin II receptor antagonists and include tachycardia and bradycardia (fast or slow heartbeat), hypotension (low blood pressure), edema (swelling of arms, legs, lips, tongue, or throat, the latter leading to breathing problems), and allergic reactions [9].

Hydrochlorothiazide is a diuretic drug of the thiazide class is used for the treatment of fluid retention (edema) in people with congestive heart failure, cirrhosis of the liver, or kidney disorders, or edema caused by taking steroids or estrogen. This medication is also used to treat high blood pressure (hypertension). It may also be used to treat patients with diabetes insipidus and certain electrolyte disturbances and to prevent kidney stones in patients with high levels of calcium in their blood, renal tubular acidosis [10, 11]. It is also sometimes used for treatment of hypoparathyroidism,[12] hypercalciuria, Dent's disease, osteoporosis and Menieres disease. The drug acts by inhibiting the kidneys' ability to retain water. This reduces the volume of the blood, decreasing blood return to the heart and thus cardiac output and, by other mechanisms, is believed to lower peripheral vascular resistance [10]. Side effects with the Hydrochlorothiazide includes Hypokalemia, an occasional side effect, can be usually prevented by potassium supplements or by combining

hydrochlorothiazide with a potassium-sparing diuretic, Hypomagnesemia, Hyponatremia, Hyperuricemia, High blood sugar, Hyperlipidemia, Hypercalcemia, Headache, Nausea/vomiting, Photosensitivity, Weight gain, Gout, Pancreatitis [13-16]. Literature review [17-42] reveals that various analytical methods have been reported for Telmisartan and Hydrochlorothiazide individually and combination. But very few bioanalytical methods have been reported for analysis of Telmisartan and Hydrochlorothiazide in combination by RP-HPLC. So present study aimed to develop a stable bioanalytical liquid chromatographic method and mass sepctrophotometric for Telmisartan and Hydrochlorothiazide.

Materials and Method:

Instrumentation:

An HPLC system (Shimadzu, Kyoto, Japan) consisting of an advance C18 column, a binary LC-20AD prominence pump, an auto-sampler (SIL-HTc) and a solvent degasser (DGU-20A3) was used for the study. Aliquots of the processed samples (20 mL) were injected into the column, which was kept at 30 1 C. The isocratic mobile phase was delivered into the electro-spray ionization chamber of the mass spectrometer. Quantitation was achieved with MS–MS detection in positive ion modefor both the analytes using a MDSSciex API-4000 mass spectrometer equipped with a Turboionspray TM interface at 500 1 C. The ion spray voltage was set at 5500 V. The source parameters, viz.the nebulizer gas, curtain gas, auxiliary gas and collision gas were set at 45, 20, 45 and 10 psi, respectively. Detection of the ions was carried out in the multiple-reaction monitoring mode (MRM)

Chemicals and reagents:

All chemicals and solvents were of analytical grade. HPLC grade Acetonitrile, Methanol and water were purchased from Merck (Mumbai, India). Ammonium acetate, glacial acetic acid, diethyl ether and dichloromethane were obtained in their highest grade from SD fine chemicals limited (Mumbai, India). The working standard drug Telmisartan having a purity of 99.36% and Hydrochlorothiazide with 98.91% pure were kindly provided by Medley Pharmaceuticals Ltd, Mumbai; Maharashtra, India.

Preparation of stock and standard solutions:

A stock solution of Telmisartan (mg/mL) was prepared by dissolving 25.16mg in the 25ml methanol. The standard stock solution was prepared as per the potency of Telmisartan. A standard concentration of 1001.59mcg/ml was obtained. The solution was filtered and was used as standard stock solution. Similarly 25.625mg of Hydrochlorothiazide was weighed accurately and was dissolved in 25ml methanol to get a standard stock concentration of 1008.91mcg/ml Hydrochlorothiazide solution. From the standard stock solutions, calibration curve dilutions were prepared by selected dilutions. 1:1 (v/v) of both the drug solutions were mixed to get a combined solution for the simultaneous analysis.

Extraction of drugs from plasma:

Prior to sample analysis, 100µL of each solution was extracted using 300µL of diethyl ether: dichloromethane (60:40% v/v) for protein precipitation. Further, each of the mixtures was vortex for a period of 5 min in a vortex mixer with subsequent centrifugation at 10000 rpm, for a period of 10 min at 4°C using a centrifuge. For each sample, an aliquot of a supernatant was isolated and subjected to dryness. The residue was reconstituted in 100µL of mobile phase and subsequently centrifuged at 10000 rpm for10 min at 4°C in a centrifuge. The supernatant was finally collected and directly injected for analysis. This procedure was followed for all samples of calibration curve plasma spiked dilutions and plasma spiked samples.

Chromatographic conditions:

The separation of the analytes was carried out on an Aquasil-C18 (250×4.6mm×5µm) column. Temperature was set to 20°C. The mobile phase composed of buffer solution, Methanol and Acetonitrile in the ratio of 60:20:20 (v/v) in isocratic condition at a flow rate of 0.5m L/min for 10min and the isocratic mobile phase comprised.

The flow rates of sheath gas and auxiliary gas were optimized and set to 30 psi and 5 psi, respectively. The needle spray voltage was set to 4.5 k V. Helium was used as collision gas tuned for each analyte to obtain good signal intensity in MS2experiment. The drugs were analyzed using multiple react ions monitoring (MRM) mode.

Mass parameters were tuned in both positive and negative ionization modes for the analytes. Good response was achieved in positive ionization mode. Data from the MRM mode were considered to obtain better selectivity. Protonated form of each analyte ion was the parent ion in the Q 1 spectrum and was used as the precursor ion to obtain Q 3 product ion spectra.

Method Validation:

To demonstrate the feasibility of the newly developed method, validation was performed in relation to specificity, linearity, LOQ, LOD, accuracy, precision, robustness, and solution stability. These parameters were validated in agreement with the ICH guidelines.

Linearity was tested for Telmisartan and Hydrochlorothiazide in the concentration range of 40.064ng/ml - 801.272ng/ml for Telmisartan and 20.178ng/ml - 908.019ng/ml for Hydrochlorothiazide in the method. For the determination of linearity, standard calibration curves containing at least 6 points (non-zero standards) were plotted and checked. In addition, blank plasma samples were also analyzed to confirm the absence of direct interferences, but these data were not used to construct the calibration curve.

System precision of the mass spectrometric response was established by injecting six individual preparations of the standard solution. The method precision was evaluated by spiking each analyte and determining the percent relative standard deviation (%RSD).

Recoveries of Telmisartan and Hydrochlorothiazide in spiked samples were studied at three different concentration levels. At each concentration level, three independent sample preparations were injected, and the percentage recoveries were determined by comparing the concentration of the spiked sample obtained with the concentration of the spiking standard. The robustness of the method was evaluated by changing mobile phase flow and column temperature, and the stability of the impurities in the sample solution was evaluated by analyzing spiked sample solution at different time intervals at room temperature.

Stock solution stability was determined by comparing the peak areas of freshly prepared solutions (LQC and HQC) with stability samples. Main stock solutions of Telmisartan and Hydrochlorothiazide were freshly prepared and aliquots of stocks were kept at room temperature for 8hours (stability sample). Areas of stability samples and freshly prepared samples were compared to determine mean % nominal concentration during stability period. The mean % change calculated. The areas of stability samples and freshly prepared samples were compared to determine mean % nominal concentration during stability period.

For the Long term stock solutions (LQC and HQC), the working solution of Telmisartan and Hydrochlorothiazide was prepared and stored in the refrigerator at 2-10°C for 11 days. Working solutions of

Telmisartan and Hydrochlorothiazide was compared against fresh stock solution prepared. The mean % concentration calculated. The mean % concentration was calculated by comparing freshly prepared and stability samples.

Samples were prepared at LQC and HQC levels, aliquot and frozen at -20±5°C. Six samples from each concentration were subjected to three freezes and thaw cycles (stability samples). These samples were processed and analysed along with freshly prepared calibration standards, LQC and HQC samples (comparison samples). Concentrations were calculated to determine mean % change after four cycles. The extracted plasma sample was incubated at 6H in bench top and 12H in auto injector for determining the bench top and auto injector stability.

Results and Discussion:

Sample preparation is an important part in the pharmaceutical analysis, because matrix effects in trace analysis were enlarged, causing loss of sensitivity, abnormal recovery, and analyte instability. Different diluents were evaluated with respect to chromatographic efficiency. Solubility of both Telmisartan and Hydrochlorothiazide were good in methanol. Good response and proper peak shapes were obtained for both drugs when Methanol and Acetonitrile at a ratio of 1:1 was used as the diluent. Good recoveries (95.0% to 104.0%) were also observed for both Telmisartan and Hydrochlorothiazide when this solution was used as a diluent. Therefore, Methanol and Acetonitrile in the ratio of 1:1 (v/v) was employed as the diluent throughout the analysis.

The present method was developed by testing different stationary phases to achieve good separation of the peaks. It is important to achieve proper separation among the two components, because of similar chemical. In order to obtain a short analysis time, various analytical columns like Kromasil C18 150 mm × 4.6 mm, 3.5 µm (Altmann Analytik, Munich, Germany), Hypersil BDS C8 150 mm × 4.6 mm, 3.5 µm and Aquasil-C18 (250×4.6mm×5µm) columns were evaluated. The tested columns were checked under the same conditions; with the Kromasil C18 and Zorbax Rx C8 columns, the peaks were overlapped. The resolution between components was poor with Hypersil BDS C8 column. On Aquasil-C18 (250×4.6mm×5µm) column, the separation and responses for both the compounds were found good. On this column, the analytes were well retained and separated from each other. This separation is achieved due to polar group technology that 'shields' the silica residual silanol surface from highly basic analytes; this reduced silanol activity for the symmetry column significantly improved the peak shape and resolution.

Different compositions of mobile phases using Acetate buffer with acetonitrile and methanol were tested; finally, good separation and response were observed at buffer solution, Methanol and Acetonitrile in the ratio of 60:20:20 (v/v). Both isocratic and gradient elution modes were evaluated. Isocratic elution was observed to be more efficient in achieving optimum separation of drugs. The column was thermostated at 20°C to avoid any shift in retention time. Retention times of Telmisartan and Hydrochlorothiazide were observed at 4.39min and 5.73min, respectively. Peaks were well separated from each other.

Selection of a detection method is also the most important part of pharmaceutical analysis. From the instrument simplicity and availability, first, we have evaluated with HPLC-UV and GC-FID. However, on these techniques sufficient sensitivity for the trace level analysis was not achieved. In view of this, a sensitive and specific mass LC-MS/MS technique in MRM mode was evaluated for the quantification of Telmisartan and Hydrochlorothiazide. Then, the possibility of using electrospray ionization (ESI) source under positive ion detection mode was evaluated during the early stage of method development. The signal intensity in positive mode was much higher than that in negative mode. Further, the method development was carried

out with ESI source operated in positive polarity mode. The ion source parameters were optimized to get proper response. The representative mass spectra of Telmisartan and Hydrochlorothiazide are shown in Figure 2 and 3 respectively and LC chromatogram of Telmisartan and Hydrochlorothiazide were given in figure 4-7.

The linear calibration curve of Telmisartan and Hydrochlorothiazide were given in figure 8 and 9 respectively. Accurate calibration curve was obtained for both the drugs in the study. Table 1 gives the results of the calibration curve for both the drugs. The precision of the method was evaluated at two levels, viz. repeatability and intermediate precision. The acceptance criteria included accuracy within ±15% deviation (SD) from the nominal values, except LLOQ QC, where it should be ±20% and a precision of ≤15% relative standard deviation (RSD), except for LLOQ QC, where it should be ±20%. Whereas batch acceptance criteria included 67% for overall quality control samples and 50% at each level respectively. The results confirmed that the method was found to be precise and accurate.

The results of the recovery conforms that the % recovery was found to be 3.27 for Telmisartan and 6.011 for Hydrochlorothiazide in three levels. The results of the recovery were given in table 2 and 3 for Telmisartan and Hydrochlorothiazide respectively.

Solution at 801.272ng/ml for Telmisartan and 908.019ng/ml Hydrochlorothiazide was used for stability study. Solution Stability studies like short term, long term studies were studied. % stability was found to be within the range of more than 98% for both the drugs in short term and long term stability studies. Stability of the drug in biological matrix was studies by bench top, freeze thaw and auto injector stabilities were studied. Accurate range of results was obtained for all the stability studies. Results were represented in table 4-7.

Conclusion:

The results of this study showed that the validated LC/MS/MS method proved to be a simple, rapid reliable, selective, and sensitive method sufficient for simultaneous monitoring of Telmisartan and Hydrochlorothiazide. A small plasma sample volume and LOQ were sufficiently sensitive to detect terminal phase concentrations of the drugs. The method utilized MRM mode for the quantitation, which provided the better sensitivity. The method was fully validated and presents good linearity, specificity, accuracy, precision, and robustness, and it is also found to be simple, sensitive, selective, cost effective, and stability indicating. The LOD and LOQ of the method were found very low for both Telmisartan and Hydrochlorothiazide. The method presented here could be very useful for the simultaneous estimation of Telmisartan and Hydrochlorothiazide in plasma and other biological samples.

Conflict of Interest statement:

We declared that we have no conflict of interest

References:

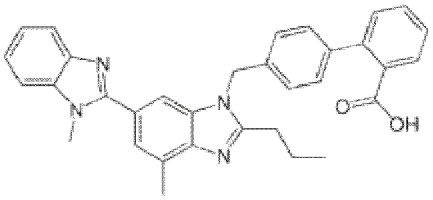
1. Pritor prescribing information
2. Drugs.com: Telmisartan
3. Benson, S. C.; Pershadsingh, H.; Ho, C.; Chittiboyina, A.; Desai, P.; Pravenec, M.; Qi, N.; Wang, J. et al,. "Identification of Telmisartan as a Unique Angiotensin II Receptor Antagonist with Selective PPAR - Modulating Activity". Hypertension; 2004, 43 (5): 993-95.
4. Sanchis- Gomar, F.; Lippi, G. "Telmisartan as metabolic modulator: A new perspective in sports doping?" Journal of Strength and Conditioning Research; 2011: 1.

5. Cytoplasmic and Nuclear Receptors: Advances in Research and Application: 2011 Edition. ScholarlyEditions. 2012, 21; 2.
6. Feng, X.; Luo, Z.; Ma, L.; Ma, S.; Yang, D.; Zhao, Z.; Yan, Z.; He, H. et al. . "Angiotensin II receptor blocker telmisartan enhances running endurance of skeletal muscle through activation of the PPAR-δ/AMPK pathway". Journal of Cellular and Molecular Medicine. 2011, 15 (7): 1572–1581.
7. He, H.; Yang, D.; Ma, L.; Luo, Z.; Ma, S.; Feng, X.; Cao, T.; Yan, Z. et al. "Telmisartan Prevents Weight Gain and Obesity Through Activation of Peroxisome Proliferator-Activated Receptor- -Dependent Pathways". Hypertension. 2010 55 (4): 869–879.
8. Li, L.; Luo, Z.; Yu, H.; Feng, X.; Wang, P.; Chen, J.; Pu, Y.; Zhao, Y. et al. "Telmisartan Improves Insulin Resistance of Skeletal Muscle Through Peroxisome Proliferator-Activated Receptor- Activation". Diabetes. 2012 62 (3): 762–774.
9. Drugs.com: Micardis.
10. Duarte JD, Cooper-DeHoff RM et al. "Mechanisms for blood pressure lowering and metabolic effects of thiazide and thiazide-like diuretics". Expert Rev CardiovascTher. June 2010 8 (6): 793–802.
11. "Hydrochlorothiazide". The American Society of Health-System Pharmacists. Retrieved 3 April 2011.
12. Mitchell, Deborah. "Long-Term Follow-Up of Patients with Hypoparathyroidism". J ClinEndocrinMetab. Endocrine Society. Retrieved 19 June 2013.
13. "Tour de France: AlexandrKolobnev positive for banned diuretic". Velonation. 2011-07-11. Archived from the original on 2011-07-12. Retrieved 2011-07-12.
14. "Kolobnev denies knowledge of doping product, says not fired by Katusha". Velonation. 2011-07-12.
15. "Press release: Adverse Analytical Finding for Kolobnev". Union CyclisteInternationale. 2011-07-11. Archived from the original on 2011-07-12.
16. "Kolobnev Tour de France's first doping case". Cycling News (Bath, UK: Future Publishing Limited). 2011-07-11.
17. Lakshmana Rao et al, Stability Indicating RP-HPLC Method for Simultaneous Determination of Telmisartan and Hydrochlorothiazide in Pharmaceutical Dosage Form, IJPCBS 2012, 2(3): 382-391.
18. Gangola R et al., Spectrophotometric Simultaneous Determination of Hydrochlorothiazide and Telmisartan in Combined Dosage Form by Dual Wavelength Method, Pharmacie Globale; 2011, 2 (04).
19. Sutirtho Mukhopadhyay et al, Simultaneous determination of related substances of telmisartan and hydrochlorothiazide in tablet dosage form by using reversed phase high performance liquid chromatographic method. J Pharm Bioallied Sci: 2011 3(3); 375–383.
20. Ismail Salama et al, Simultaneous HPLC–UV analysis of telmisartan and hydrochlorothiazide in human plasma. Bulletin of Faculty of Pharmacy: 2011 49 (1); 19-24.
21. Wankhede SB et al, RP-HPLC method for simultaneous estimation of telmisartan and hydrochlorothiazide in tablet dosage form. Indian journal of pharmaceutical science: 2007, 69 (2); 298-300.
22. Leena R. Bhat et al, Validated RP-HPLC Method for Simultaneous Determination of Telmisartan and Hydrochlorothiazide in Pharmaceutical Formulation. Journal of Liquid Chromatography and Related Technologies: 2007 30 (20).
23. J. Kavitha Et Al, Development and Validation of RP-HPLC Method for Simultaneous Estimation of Telmisartan and Hydrochlorothiazide in Tablets: It's Application to Routine Quality Control Analysis. International Journal of Pharmacy and Pharmaceutical Sciences: 2011 3(4); 1-6
24. Shravan Bankey et al, Simultaneous Determination of Ramipril, Hydrochlorothiazide and Telmisartan by RP-HPLC, International Journal of Pharmaceutical and Medical Sciences. 2012 1(2).

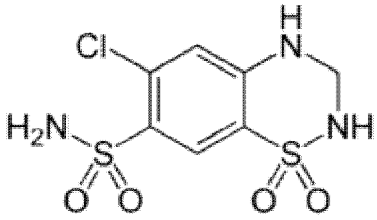
25. Shanmugasundaram P et al, Validated Simultaneous Estimation of Telmisartan and Hydrochlorothiazide in Tablet Dosage form by RP-HPLC. Trade science Inc: 2007 5; 1-6.
26. Ping MA et al, determination of Telmisartan and Hydrochlorothiazide in human plasma by HPLC-MS/MS. journal of Chinese journal of mass spectrometry society: 2011, 32(1); 43-49.
27. *Vasanth PM et al,* A Rapid Stability-Indicating RP-HPLC Method for the Simultaneous estimation of Enalapril Maleate and Hydrochlorothiazide in Solid Dosage Forms. International journal of chemical and life science: 2013 2 (1).
28. Rajesh nakum et al, Quantitative Estimation of Telmisartan and Hydrochlorothiazide by RP-HPLC Method, International Journal of Drug Discovery and Medical Research. 2012 1(2).
29. Qiao-Juan SHI et al, Determination of Telmisartan and Hydrochlorothiazide in Compound Telmisartan Capsules by HPLC, Chinese Journal of Pharmaceuticals.
30. Thirupathaiah sanikommu et al, Stability Indicating RP-HPLC Method for Simultaneous Determination of Telmisartan, Amlodipine and Hydrochlorothiazide from Their Combination Drug Product. International journal of advances in pharmaceutical research: 2013 4(3), 2013.
31. Mandlekar P et al, "Simultaneous RP-HPLC Determination of Ramipril, Hydrochlorothiazide and Telmisartan in the Bulk Drug and in Tablet. Inventi Rapid: Pharm Analysis & Quality Assurance: 2011.
32. Mhaske R. A. et al, Rp-Hplc Method for Simultataneous Determination of Amlodipine Besylate, Valsartan, Telmisartan, Hydrochlorothiazide and Chlorthalidone: Application to Commercially Available Drug Products. IJPSR: 2012 3(1); 141-149
33. Dhanalakshmi K. et al, Analytical Method Development and Validation of Telmisartan and Hydrochlorothiazide in Dissolution by RP-HPLC, International Journal of Biological & Pharmaceutical Research: 2013 4(3); 200-211.
34. Kalyan Kumar B. et al. Development and Validation of RP-HPLC Method for Simultaneous Estimation of Ramipril, Telmisartan and Hydrochlorothiazide in Pharmaceutical Dosage Forms, Journal of Pharmacy Research 2011, 4(10), 3306-3308.
35. Subhakar Nandipati et al, Development and Validation of RP-HPLC Method for Estimation of Telmisartan in Bulk and Tablet Dosage Form. Int. Res J Pharm. App Sci.: 2012 2(3); 39-43.
36. Jabir Aboobacker O et al, Method Development and Validation of Hydrochlorothiazide, Amlodipine Besylate and Telmisartan in Tablet Dosage Form by RP-HPLC Method. RJPBCS: 2012 3 (3); 509.
37. Susheel John Varghese et al, Simultaneous Determination of Ramipril, Hydrochlorothiazide and Telmisartan in tablet dosage form using High-Performance liquid chromatography method. Der Pharmacia Lettre: 2011 3(2); 83-90.
38. Prasad CVN et al, Simultaneous Determination of Telmisartan, Amlodipine Besylateand Hydrochlorothiazide in a Combined Poly Pill Dosage Form byStability-Indicating High Performance Liquid Chromatography. International Journal of Research in Pharmacy and Chemistry: 2011 1(3).
39. Santaji Nalwade et al, Rapid Simultaneous Determination of Telmisartan, Amlodipine Besylate and Hydrochlorothiazide in a Combined Poly Pill Dosage Form by Stability-Indicating Ultra Performance Liquid Chromatography. Sci Pharm: 2011 79; 69–84.
40. Delhiraj N et al, Validated chromatographical methods for the simultaneous estimation of antihypertensive drugs in multicomponent formulations. Der PharmaChemica: 2012 4(6); 2416-2421.
41. AjitPandey et al, UV-Spectrophotometric Method for estimation of Telmisartan in Bulk and Tablet Dosage Form. International Journal of ChemTech Research: 2011 3 (2); 657-660, April-June 2011.
42. Zhou Su-qin et al, Simultaneous Determination of Telmisartan and Hydrochlorothiazide in Human Plasma by RP-HPLC. Chinese Pharmaceutical Journal: 2007 19.

List of Figures:

Figure 1: structure of Telmisartan and Hydrochlorothiazide



Telmisartan



Hydrochlorothiazide

Figure 2: Mass spectrum of Telmisartan

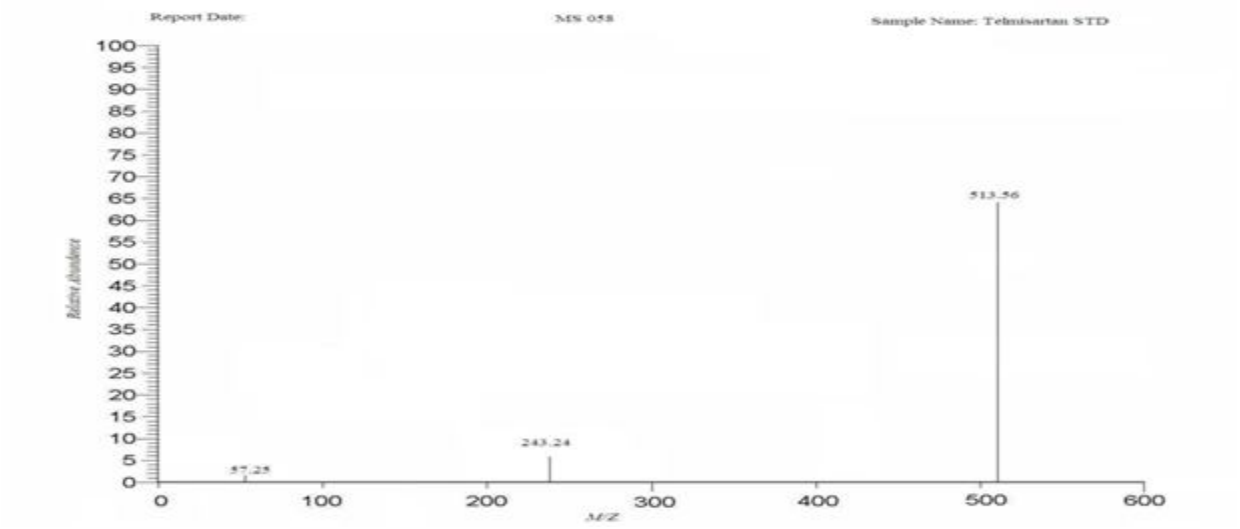


Figure 3: Mass spectrum of Hydrochlorothiazide

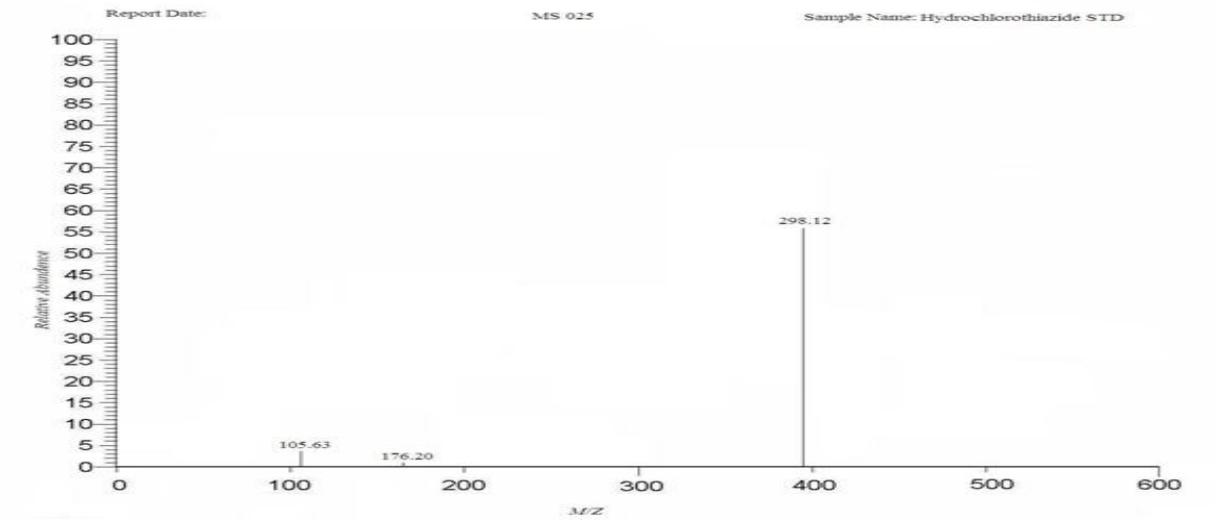


Figure 4: Standard LC chromatogram of Telmisartan and Hydrochlorothiazide

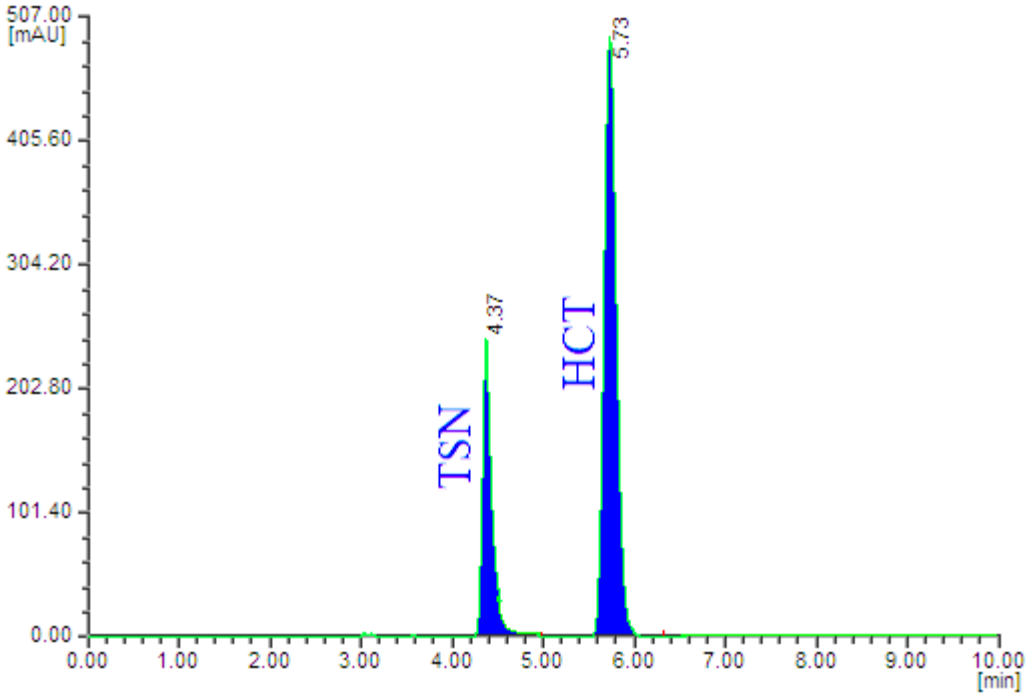


Figure 5: Blank chromatogram of Telmisartan and Hydrochlorothiazide

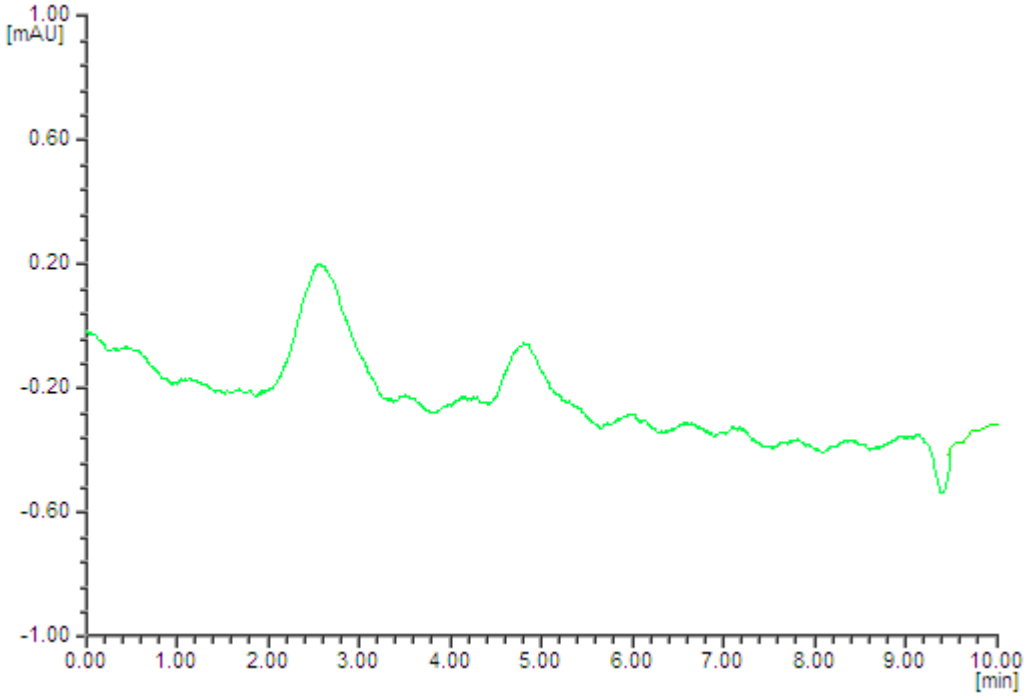


Figure 6: Standard LC chromatogram of Telmisartan

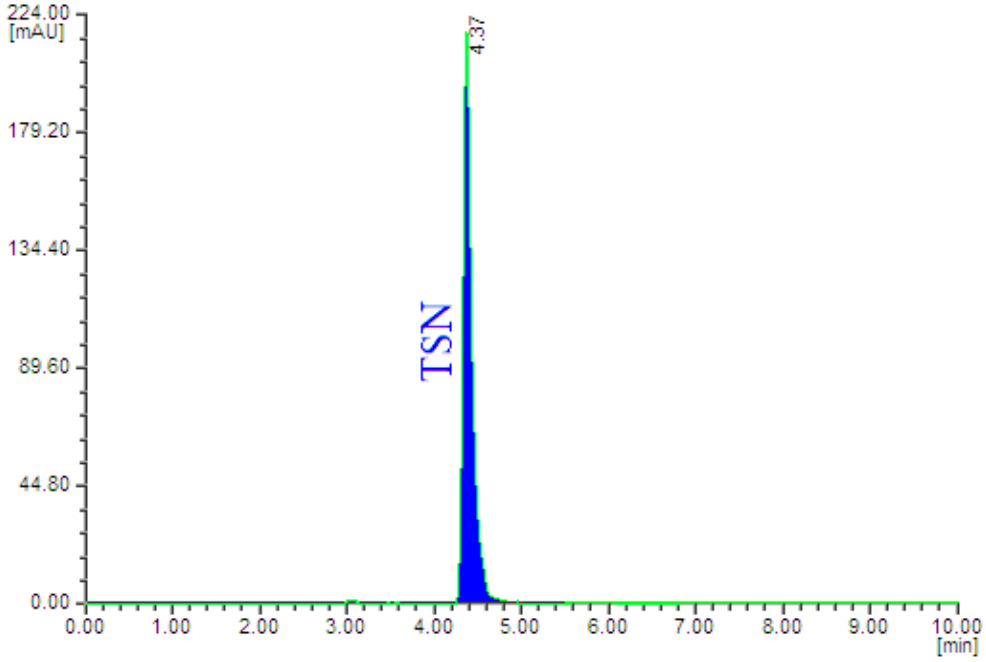


Figure 7: Standard LC chromatogram of Telmisartan

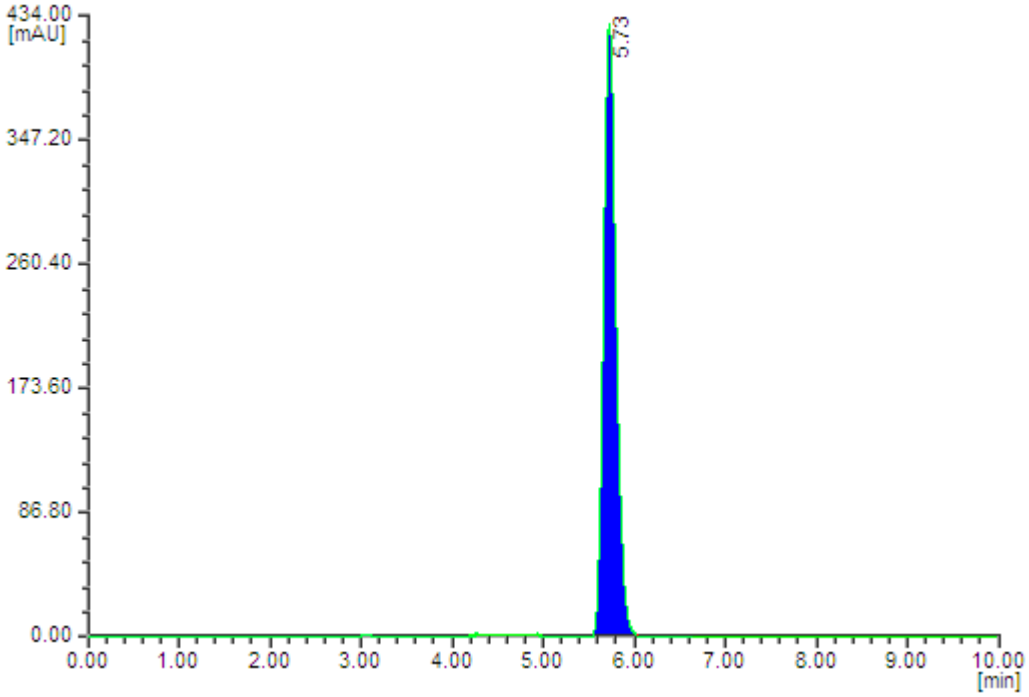


Figure 8: Linearity graph for Telmisartan

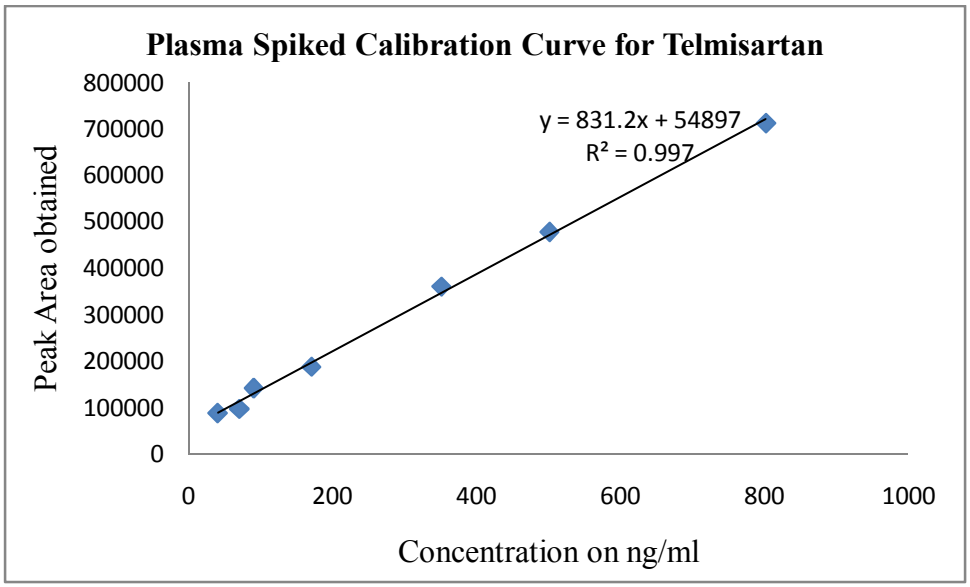
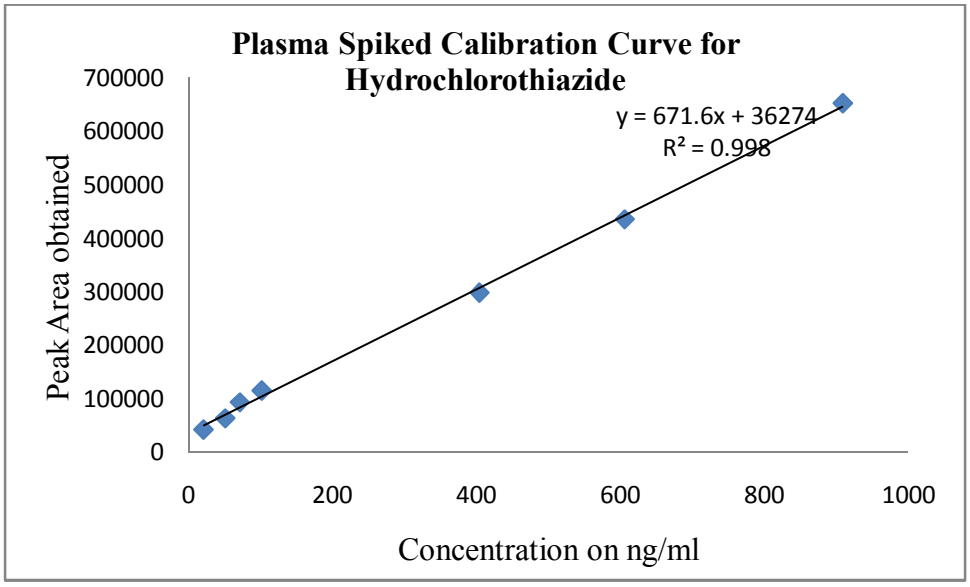


Figure 9: Linearity graph for Hydrochlorothiazide



List of Tables:

Table 1: Plasma spiked calibration curve

S.NO	Telmisartan		Hydrochlorothiazide		Sample vial code
	Concentration in ng/ml	Area at the retention	Concentration in ng/ml	Area at the retention time	
1	40.064	88251	20.178	42814	PSCC 001
2	70.111	97123	50.445	63817	PSCC 002
3	90.143	141935	70.624	93871	PSCC 003
4	170.27	187936	100.891	115827	PSCC 004
5	350.557	360369	403.564	298710	PSCC 005
6	500.795	478251	605.346	436107	PSCC 006
7	801.272	712258	908.019	652814	PSCC 007
	Slope Intercept r^2	831.27 54897 0.9976	Slope Intercept r^2	671.61 36274 0.9984	

Table 2: Recovery of Telmisartan

S. NO	Recovery at HQC level				Recovery at MQC level				Recovery at LQC level			
	Extracted	Aqueous	recovered	% recovery	Extracted	Aqueous	recovered	% recovery	Extracted	Aqueous	recovered	% recovery
1	736925	908211	650.154	81.1403	382863	441893	303.728	86.642	90176	105863	59.722	85.182
2	729963	909917	642.805	80.223	381190	459631	290.731	82.934	91589	106397	60.353	86.082
3	731580	905583	647.312	80.7855	380326	432816	308.043	87.872	93691	118580	55.395	79.011
4	739281	914792	647.541	80.8141	385601	445021	303.750	86.648	93902	107951	60.987	86.986
5	728284	909882	641.351	80.0416	376920	446179	296.141	84.477	93698	105821	62.079	88.544
6	731417	904861	647.684	80.832	382047	446377	300.036	85.588	90581	107486	59.084	84.272
SD	**4261**	**3591**	**3.3435**	**0.417**	**2878.38**	**8658**	**6.204**	**1.770**	**1698.42**	**4925**	**2.306**	**3.290**
Mean	**732908**	**908874**	**646.141**	**80.64**	**381491**	**445319**	**300.405**	**85.693**	**92272.8**	**108683**	**59.603**	**85.013**
CV	**0.581**	**0.395**	**0.517**	**0.517**	**0.75451**	**1.9443**	**2.065**	**2.065**	**1.841**	**4.533**	**3.870**	**3.869**
Standard Deviation					**2.74313**							
Average recovery of three levels					**83.782**							
% Recovery					**3.27413**							

Table 3: Recovery of Hydrochlorothiazide

S. NO	Recovery at HQC level				Recovery at MQC level				Recovery at LQC level			
	Extracted	Aqueous	recovered	% recovery	Extracted	Aqueous	recovered	% recovery	Extracted	Aqueous	recovered	% recovery
1	682239	849631	729.124	80.2983	275819	315896	352.365	87.3132	64281	85861	37.7663	74.8664
2	679228	845822	729.175	80.3039	286932	319671	362.233	89.7585	61820	82661	37.7265	74.7874
3	671852	841028	725.368	79.8846	265102	328476	325.703	80.7067	60528	86394	35.342	70.0604
4	642811	842836	692.525	76.2676	295017	339178	351.02	86.98	65936	84105	39.5475	78.3972
5	637492	849928	681.063	75.0054	259670	329541	317.998	78.7975	62820	80281	39.4733	78.2501
6	637028	842014	686.964	75.6553	279954	330208	342.146	84.7811	64559	84028	38.7571	76.8303
SD	**21540.5**	**3886**	**22.81**	**2.512**	**13230.5**	**8295**	**16.970**	**4.205**	**1979.76**	**2225.1**	**1.567**	**3.105**
Mean	**658442**	**845210**	**707.37**	**77.902**	**277082**	**327162**	**341.911**	**84.723**	**63324**	**83888**	**38.102**	**75.532**
CV	**3.271**	**0.460**	**3.224**	**3.224**	**4.77493**	**2.536**	**4.963**	**4.963**	**3.126**	**2.652**	**4.111**	**4.111**
Standard Deviation					**4.771**							
Average recovery of three levels					**79.386**							
% Recovery					**6.011**							

Table 4: Short term stability results for Telmisartan and Hydrochlorothiazide

S. NO	Telmisartan			Hydrochlorothiazide		
	Fresh Stock	Room Temperature stock	% Stability	Fresh Stock	Room Temperature stock	% Stability
1	912581	901526	98.789	846628	841028	99.339
2	911286	904789	99.287	851573	840856	98.741
3	913952	902851	98.785	846692	843942	99.675
4	910179	896925	98.544	859015	844103	98.264
5	913631	897530	98.238	848715	836925	98.611
6	909425	904744	99.485	845593	838128	99.117
SD	**1850.15**	**3458.07**	**0.463**	**5031.9**	**2932.12**	**0.516**
Mean	**911842**	**901394.17**	**98.855**	**849703**	**840830**	**98.96**
CV	**0.2029**	**0.384**	**0.468**	**0.592**	**0.349**	**0.522**
% Stability	**98.855**			**98.96**		
% Change	**1.145**			**1.04**		

Table 5: Long term stability results for Telmisartan and Hydrochlorothiazide

S. NO	Telmisartan			Hydrochlorothiazide		
	Fresh Stock	Room Temperature stock	% Stability	Fresh Stock	Room Temperature stock	% Stability
1	942891	929671	98.598	866917	856921	98.847
2	951273	931157	97.885	861801	844281	97.967
3	955107	930179	97.390	865528	859362	99.288
4	946931	936901	98.941	869018	851029	97.930
5	952037	945143	99.276	860581	849361	98.696
6	953975	941128	98.653	869286	845536	97.268
SD	**4621.51**	**6442.746**	**0.697**	**3649.52**	**6043.65**	**0.740**
Mean	**950369**	**935696.5**	**98.457**	**865522**	**851082**	**98.333**
CV	**0.486**	**0.689**	**0.7078**	**0.422**	**0.710**	**0.753**
% Stability	**98.457**			**98.333**		
% Change	**1.543**			**1.667**		

Table 6: Freeze Thaw Stability results for Telmisartan and Hydrochlorothiazide

S.NO	Telmisartan				Hydrochlorothiazide			
	Area at HQC		Area at LQC		Area at HQC		Area at LQC	
	Fresh	stability	Fresh	stability	Fresh	stability	Fresh	stability
1	802.141	802.128	40.154	41.221	908.123	908.589	20.361	19.152
2	801.361	803.631	40.914	41.014	909.632	910.281	20.498	19.365
3	800.943	802.063	41.225	39.651	907.581	911.476	19.936	18.637
4	799.75	804.586	39.879	39.481	906.636	911.693	19.881	18.992
5	801.22	804.125	39.425	40.691	908.036	913.571	20.781	19.705
6	802.58	805.636	39.661	40.361	908.921	914.286	19.632	19.553
N	**6**	**6**	**6**	**6**	**6**	**6**	**6**	**6**
SD	**0.988**	**1.405**	**0.715**	**0.713**	**1.041**	**2.093**	**0.435**	**0.3905**
Mean	**801.333**	**803.695**	**40.209**	**40.403**	**908.155**	**911.64**	**20.1815**	**19.234**
% CV	**0.123**	**0.175**	**1.778**	**1.765**	**0.115**	**0.230**	**2.153**	**2.030**
Accuracy	**100.008**	**100.302**	**100.564**	**100.846**	**100.015**	**100.40**	**100.017**	**95.322**
Stability	**100.293**		**100.482**		**100.380**		**95.305**	

Table 7: Bench-top stability results for Telmisartan and Hydrochlorothiazide

S. NO	Telmisartan				Hydrochlorothiazide			
	Area at HQC		Area at LQC		Area at HQC		Area at LQC	
	Fresh	stability	Fresh	stability	Fresh	stability	Fresh	stability
1	795.653	795.514	39.636	38.157	905.693	916.635	21.119	18.874
2	799.154	794.825	40.185	39.636	907.759	911.125	19.898	19.896
3	798.582	796.621	41.128	38.571	905.596	910.141	21.556	19.254
4	799.125	797.103	42.225	38.253	906.674	914.471	20.693	18.631
5	796.251	796.634	42.174	37.556	907.581	911.392	21.175	19.204
6	799.936	794.415	39.996	38.142	906.058	917.856	20.225	18.874
N	**6**	**6**	**6**	**6**	**6**	**6**	**6**	**6**
SD	**1.74182**	**1.09561**	**1.1275**	**0.69502**	**0.94085**	**3.19527**	**0.62715**	**0.44437**
Mean	**798.117**	**795.852**	**40.891**	**38.386**	**906.56**	**913.603**	**20.7777**	**19.1222**
% CV	**0.21824**	**0.13767**	**2.75736**	**1.81063**	**0.10378**	**0.34974**	**3.0184**	**2.32384**
Accuracy	**99.6062**	**99.3236**	**102.063**	**95.8113**	**99.8393**	**100.615**	**102.972**	**94.7674**