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Der Pharmacia Lettre, 2013, 5 (3):81-89
(http://scholarsresearchlibrary.com/archive.html)

ISSN 0975-5071
USA CODEN: DPLEB4

A rapid stability-indicating simultaneous determination of hydrochlorothiazide, ramipril and telmisartan, in combined pharmaceutical dosage form by ultra performance liquid chromatography

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ABSTRACT

A simple, isocratic rapid stability-indicating ultra-performance liquid chromatography (UPLC) method was developed with 5 minutes run time and validated for the simultaneous quantitative determination of hydrochlorothiazide, ramipril and telmisartan present in tablets. Chromatographic separation achieved isocratically on Waters Acquity BEH Shield RP18 column (100 mm x 2.1, 1.7µm) column. The separation was achieved on simple Isocratic method. The mobile phase consisted of a mixture of 0.1% triethylamine at pH 3.5 with methanol and acetonitrile (3:2:5 v/v) and the flow rate was 0.15 mL/min. The column temperature was maintained at ambient and the detection was carried out at 215 nm. The retention times of Hydrochlorothiazide, Ramipril and Telmisartan are 1.5, 1.9 and 2.9 minutes respectively. The method was validated in terms of system suitability, linearity, precision, limit of detection, limit of quantitation and accuracy. The developed method was linear for hydrochlorothiazide, ramipril, telmisartan in the range of 6.2-18.7 µg/mL, 1.2-3.7 µg/mL and 20-60 µg/mL respectively. The accuracy of the method was evaluated in the range of 80% to 120% in triplicate and the mean recoveries obtained for hydrochlorothiazide, ramipril, telmisartan were 99.7%, 99.6% and 100.3% respectively. Validation parameters such as specificity and robustness were also determined. The method was found to be rapid and stability-indicating, which can be applied for simultaneous quantitative determination of hydrochlorothiazide, ramipril and telmisartan present in combination tablets.

Keywords: Hydrochlorothiazide, Ramipril and Telmisartan; UPLC; Validation

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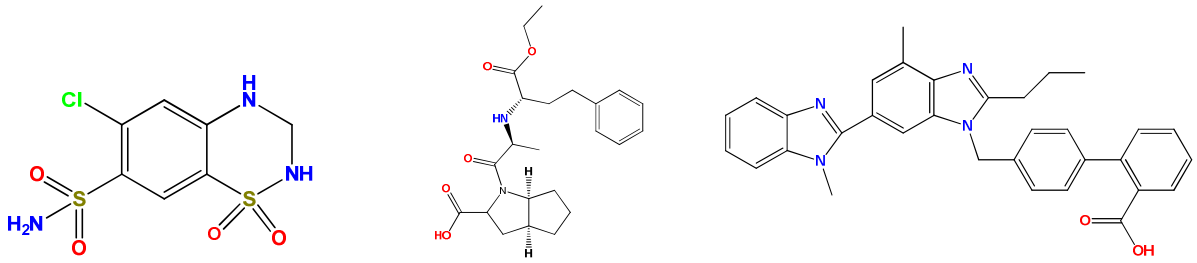
INTRODUCTION

A combination drug or fixed-dose combination (FDC) is a formulation of two or more active ingredients combined in a single dosage form, available in certain fixed doses. FDC therapies are hypothesized to enhance compliance by decreasing the number of required pills. UPLC system involves significant technological advances in particle size performance, system optimization, data processing, detector design and control. When all brought together, the specific achievements in each area have created a step-function progress in chromatographic performance. This new technique of analytical separation science uses the principles and practicality of High Performance Liquid Chromatography (HPLC) with increasing the attributes of speed, sensitivity and resolution. Now a day's pharmaceutical industries are in search of new ways to reduce cost and time for analysis of drugs. Analytical laboratories are not exception in this trend. UPLC with better resolution, assay sensitivity and high sample throughput allows a greater number of analysis to be performed in a shorter period of time and it also imparts cost

effective advantage over HPLC [1-5] analysis, so that conventional assay was transferred and optimized for UPLC system [6].

Hydrochlorothiazide is chemically 6-Chloro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide [7]. A thiazide diuretic often considered the prototypical member of this class. It reduces the re absorption of electrolytes from the renal tubules. This results in increased excretion of water and electrolytes, including sodium, potassium, chloride, and magnesium. It has been used in the treatment of several disorders including edema, hypertension, diabetes insipidus, and hypoparathyroidism.

Figure 1: Structures of A) Hydrochlorothiazide B) Ramipril and C) Telmisartan.



(A) **(B)** **(C)**

Ramipril is chemically (2S,3aS,6aS)-1-[(2S)-2-{[(2S)-1-ethoxy-1-oxo-4-phenylbutan-2-yl]amino}propanoyl]-octahydrocyclopenta [b] pyrrole-2-carboxylic acid [7]. Ramipril is a prodrug belonging to the angiotensin-converting enzyme (ACE) inhibitor class of medications. It is metabolized to ramiprilat in the liver and, to a lesser extent, kidneys. Ramiprilat is a potent, competitive inhibitor of ACE, the enzyme responsible for the conversion of angiotensin I (ATI) to angiotensin II (ATII). ATII regulates blood pressure and is a key component of the rennin angiotensin-aldosterone system (RAAS). Ramipril may be used in the treatment of hypertension, congestive heart failure, nephropathy, and to reduce the rate of death, myocardial infarction and stroke in individuals at high risk of cardiovascular events.

Telmisartan is chemically 2-(4-{[4-methyl-6-(1-methyl-1H-1,3-benzodiazol-2-yl)-2-propyl-1H-1,3-benzodiazol-1-yl]methyl}phenyl)benzoic acid [7]. It belongs to the class of drugs called angiotensin II receptor blockers. It is used to treat high blood pressure and to reduce the risk of heart attacks, strokes, and related deaths in certain people. Telmisartan decreases the effectiveness of a chemical known as angiotensin II, which normally causes blood vessels to narrow (constrict). By blocking the effects of angiotensin II, telmisartan causes blood vessels to relax, which can lower blood pressure. Based on clinical studies, telmisartan has been shown to significantly lower systolic and diastolic blood pressure. Angiotensin converting enzyme inhibitors (ACE inhibitors) are very useful group of medications often used to reduce cardiovascular risks in people at high risk for such problems. The structures of telmisartan, ramipril and hydrochlorothiazide were given in Figure 1.

The ever-increasing need for speed and efficient use of time in the pharmaceutical and other fields, there is a demand for the development of fast and high throughput analytical procedures. The rapid quantitative determination of combination drugs with big difference in label claims (12.5 mg for hydrochlorothiazide, 2.5 mg for ramipril and 40mg of telmisartan) with shorter run time is a challenge. For UPLC based assays, the processes of reducing analysis time while adequately resolving analytes from degradation products is often accomplished with column with small particles. The theoretical advantages for small particles are to get well resolved peaks with high theoretical plates over small concentration. Present drug stability test guidance Q1A (R2) issued by international conference on harmonization (ICH) [8] suggest that stress studies should be carried out on a drug product to establish its inherent stability characteristics, leading to identification of degradation products and hence supporting the suitability of the proposed analytical procedures. It also requires that analytical test procedures for stability samples should be stability indicating and they should be fully validated. Accordingly, the aim of the present study was to establish inherent stability of hydrochlorothiazide, ramipril and telmisartan through stress studies under a variety of ICH recommended test conditions [8] and to develop a rapid stability-indicating reverse phase assay method. Literature survey reveals that a variety of spectrophotometric and chromatographic methods including UV, colorimetric determination, and a stability indicating HPLC methods have been reported for determination

hydrochlorothiazide, ramipril and telmisartan either single or in combination with other drugs [9-11]. It is important to develop a rapid stability indicating method [12], which can quantify hydrochlorothiazide, ramipril and telmisartan simultaneously [13-16] in the combined dosage form.

Hence UPLC method with 5 minutes run time was developed for simultaneous quantitative determination of hydrochlorothiazide, ramipril and telmisartan in pharmaceutical dosage forms in the presence of degradation products.

MATERIALS AND METHODS

2.1. Instrumentation:
Acquity UPLC system (Waters) was used for developing the method and validation consisting of a binary solvent manager, a sample manager and a UV detector. The output signal was monitored and processed using Empower software. The water bath equipped with MV controller was used for hydrolysis studies. Thermal stability studies were performed in a dry air oven.

2.2. Chromatographic Conditions:
The chromatographic column used was Waters Acquity BEH Shield RP18 (100mm x 2.1, 1.7µm) column. The mobile phase contains a mixture of 0.1% TEA at pH 3.5, methanol and acetonitrile in the ratio 3:2:5 v/v respectively. The flow rate was 0.15 mL/min and column temperature was maintained at ambient. The run time is 5 minutes. The detection wavelength is 215nm. The mobile phase is used as diluent.

2.3. Preparation of Standard Solutions:
Standard stock solutions of hydrochlorothiazide, ramipril and telmisartan (0.1 mg/mL of hydrochlorothiazide, 0.01 mg/mL of ramipril and 1.0 mg/mL of telmisartan) were prepared by dissolving appropriate amounts of the drug substances of hydrochlorothiazide, ramipril and telmisartan in diluent. Working solutions of hydrochlorothiazide, ramipril and telmisartan 0.0125 mg/mL, 0.0025 mg/mL and 0.04 mg/mL respectively were prepared from above stock solutions in diluent for assay determination. However, the precision and accuracy results show that method is precise, linear and accurate without internal standard.

2.4. Preparation of Sample Solution
Accurately weighed and made the Tablets powder with Mortar and Pestle. 276.5 mg of Tablets Powder was weighed into a 100ml clean dry volumetric flask, 70ml of diluent was added and sonicated to dissolve it completely and made up to the volume with diluent. Further pipetted 1ml of the above stock solution into a 10ml volumetric flask and diluted up to the mark with diluent to get a final hydrochlorothiazide, ramipril and telmisartan concentration of 0.0125 mg/mL, 0.0025 mg/mL and 0.04 mg/mL respectively. The solution was then filtered through 0.45 µ (Nylon 66-membrane) filter to analyze.

2.4.1. Preparation of Sample for Degradation studies:
The standard stock solutions of hydrochlorothiazide, ramipril and telmisartan were exposed to stress conditions [11] like acid, base, peroxide and thermal. The acid and base samples were neutralized before analysis. The samples were exposed to 0.1N hydrochloric acid and 0.1N sodium hydroxide at room temperature for 90 minutes under acid and base degradation respectively. The oxidative degradation conditions applied were 3% hydrogen peroxide at ambient conditions. The sample was refluxed at 80°C for 60 minutes under thermal degradation condition.

Table 2

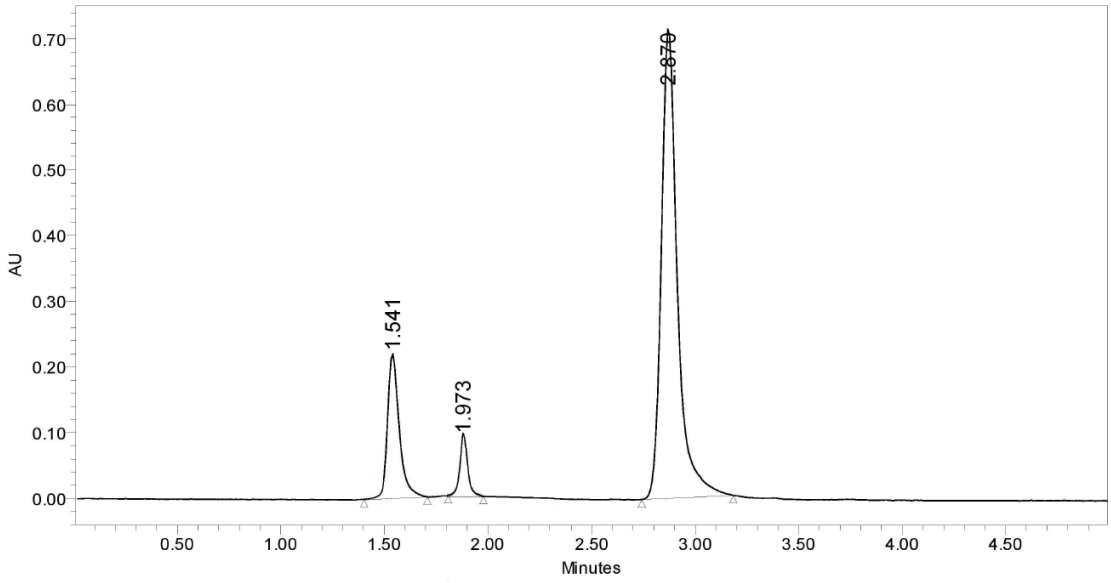
S. No	Linearity Level (%)	Hydrochlorothiazide		Ramipril		Telmisartan	
		Concentration µg/mL	Response	Concentration µg/mL	Response	Concentration µg/mL	Response
1	80	6.2	1288769	1.2	680457	20	2934769
2	90	9.3	1510609	1.8	793400	30	3431278
3	100	12.5	1730105	2.5	908614	40	3926868
4	110	15.6	1910018	3.1	1031325	50	4435442
5	120	18.7	2104268	3.7	1117367	60	4868744
Correlation Coefficient		0.999		0.999		0.999	

RESULTS AND DISCUSSION

3.1. Chromatographic Conditions

A Reverse phase UPLC method was proposed as a suitable method for quantitative determination of hydrochlorothiazide, ramipril and telmisartan tablets (Teram H Pharmaceuticals). The mobile phase conditions were optimized so that the three drugs would be separated in short run time of 5 min in the presence of their degradation products. The final choice of the stationary phase that gave a satisfactory resolution and run time was the reverse phase column Waters Acquity BEH Shield RP18 (100 mm x 2.1, 1.7µm). Triethyl amine solution with different pH values and concentrations in combination with different volume fractions of acetonitrile as modifier were also tested. The best results were obtained by the use of a mixture of 0.1% triethylamine in UPLC water with pH 3.5, methanol and acetonitrile in the ratio of 3:2:5 v/v/v respectively. All the experiments were carried out at an ambient column temperature. Under the optimum chromatographic conditions, the retention times obtained for hydrochlorothiazide, ramipril and telmisartan peaks were 1.54, 1.97 and 2.87 minutes respectively (Figure 2). The result of resolutions (Rs), tailing factors and theoretical plate numbers were reported in Table 1. Validation was done after establishing the optimal conditions for the separation. Specificity, linearity, precision, accuracy, limit of detection and limit of quantification were determined under the validation for the combination tablets.

Figure 2



3.2. Linearity

The linearity was determined for three drugs hydrochlorothiazide, ramipril and telmisartan separately in the range of 50% to 150% of each drug with respect to test concentration of 12.5 µg/mL for hydrochlorothiazide, 2.5 µg/mL for ramipril and 40 µg/mL for telmisartan, prepared from standard stock solution in diluent. The correlation co-efficient values calculated for the concentration and the response for hydrochlorothiazide, ramipril and telmisartan, are found to be linear in the range of 6.2 - 8.7µg/ml, 1.2 - 3.7 µg/ml and 20 - 60 µg/ml respectively and the values are given in Table 2.

3.3. Accuracy:

To check the accuracy of the developed method, analytical recovery study experiments were carried out by the standard addition method. From that total amount of the drug found, the percentage recovery was calculated. The recovery values for the three compounds are in the range of 99.7% – 100.3%. The accuracy data are reported in Table 3.

Table 3

% Concentration at Specification level	% Recovery		
	Hydrochlorothiazide	Ramipril	Telmisatan
80	99.6	99.5	100.5
100	99.9	99.8	100.1
120	99.8	99.6	100.3
Mean	99.7	99.6	100.3

3.4. Precision:

The System precision of the proposed method was determined by injecting standard solution for five times and measured the response for them into UPLC. The Method Precision of the proposed method was determined by injecting six sample solutions into UPLC prepared individually. The % RSD for the areas of system precision was given in the Table 4. The % assay calculated from the method precision data and the % RSD calculated for % assay values were given in Table 4.

Table 4

Precision	Hydrochlorothiazide	Ramipril	Telmisartan
System Precision (% RSD for the response)	0.4	0.3	0.4
Method Precision (% Assay)	99.0	99.8	100.4
% RSD (Assay)	0.2	0.2	0.4

3.5. Intermediate Precision / Ruggedness:

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different days by using different instruments. The Intermediate Precision of the proposed method was determined by injecting six sample solutions into UPLC prepared individually. The % RSD for the areas of system precision was given in the Table 5. The % assay calculated from the intermediate precision data and the % RSD calculated for % assay values were given in Table 5.

Table 5

Intermediate Precision	Hydrochlorothiazide	Ramipril	Telmisartan
System Precision (%RSD for the response)	0.2	0.3	0.3
Method Precision (%assay)	100.0	99.8	99.9
Assay %RSD	0.2	0.4	0.2

3.6. Limits of Detection and Quantitation

For determining the limit of detection (LOD) and limit of quantitation (LOQ), the standard deviation and slope method was adopted and studies were carried out to evaluate the detection and quantization limits of the method by using equations, LOD = 3.3 σ/S and LOQ = 10 σ/S, where σ is the standard deviation and S is the slope of the curve. The LOD for Hydrochlorothiazide, Ramipril and telmisartan was 0.005µg/mL, 0.002µg/mL and 0.005µg/mL respectively and LOQ was 0.02µg/mL, 0.007 µg/mL and 0.02µg/mL respectively.

3.7. Robustness

As part of the Robustness, deliberate change in the flow rate, mobile phase composition was made to evaluate the impact on the method. The results reveal that the method is robust for the flow rate and mobile phase composition changes. The results are summarized in Table 6A and 6B.

Table 6A

S. No	Change in Flow Rate (ml/min)	System Suitability Results					
		Hydrochlorothiazide		Ramipril		Telmisartan	
		USP Plate Count	USP Tailing	USP Plate Count	USP Tailing	USP Plate Count	USP Tailing
1	0.1	8512	1.39	8197	1.13	8009	1.31
2	0.15	8132	1.39	8167	1.10	7914	1.35
3	0.2	7713	1.30	7516	1.14	7534	1.29

Table 6B

S. No	Change in Organic Composition in the Mobile phase	System Suitability Results					
		Hydrochlorothiazide		Ramipril		Telmisartan	
		USP Plate Count	USP Tailing	USP Plate Count	USP Tailing	USP Plate Count	USP Tailing
1	10% Less	8439	1.33	8303	1.24	8221	1.25
2	Actual	8132	1.47	8167	1.13	7914	1.35
3	10% More	8116	1.34	7922	1.10	7627	1.33

3.8. Forced Degradation Studies:

The International Conference on Harmonization (ICH) guidline entitled stability testing of new drug substances and products requires that stress testing be carried out to elucidate the inherent stability characterstics of the active substance. The aim of this work was to perform the stress degradation studies [11] on the hydrochlorothiazide, ramipril and telmisartan using proposed method.

The degradation products were well separated from hydrochlorothiazide, ramipril and telmisartan peaks in all the degradation conditions. The peak purity is checked for Hydrochlorothiazide, Ramipril and Telmisartan and the results are summarized in Figures 3-6 and Table 7.

Figure 3. Acid Degradation of hydrochlorothiazide, ramipril and telmisartan standard solution

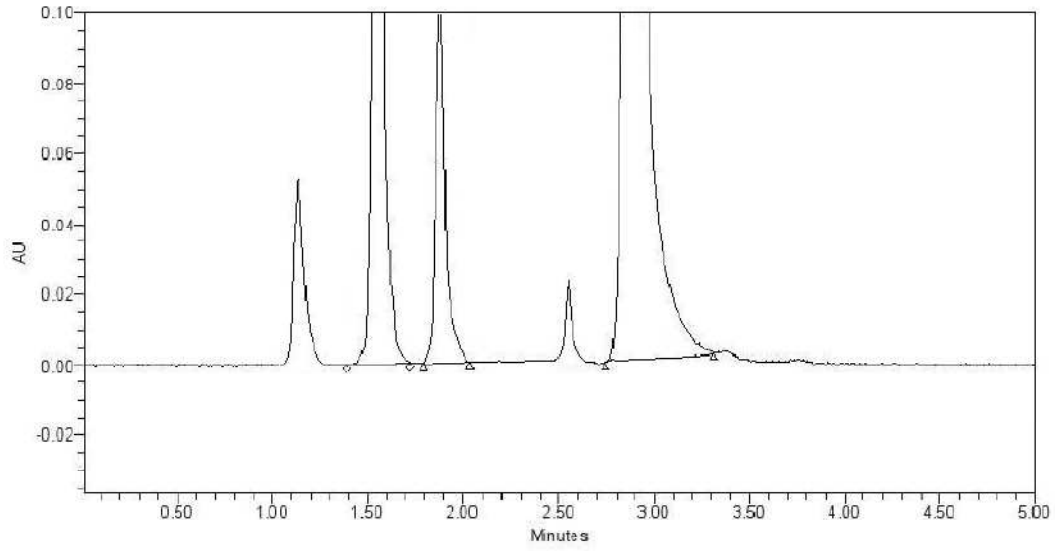


Table 7

Degradation Condition	Hydrochlorothiazide		Ramipril		Telmisartan	
	Purity angle	Purity Threshold	Purity angle	Purity Threshold	Purity angle	Purity Threshold
Acid	0.5	0.6	0.4	0.5	0.5	0.6
Base	0.5	0.6	0.5	0.6	0.5	0.6
Oxidative	0.4	0.5	0.5	0.6	0.5	0.6
Thermal	0.4	0.5	0.5	0.6	0.5	0.6

Figure 4. Base Degradation of hydrochlorothiazide, ramipril and telmisartan standard solution

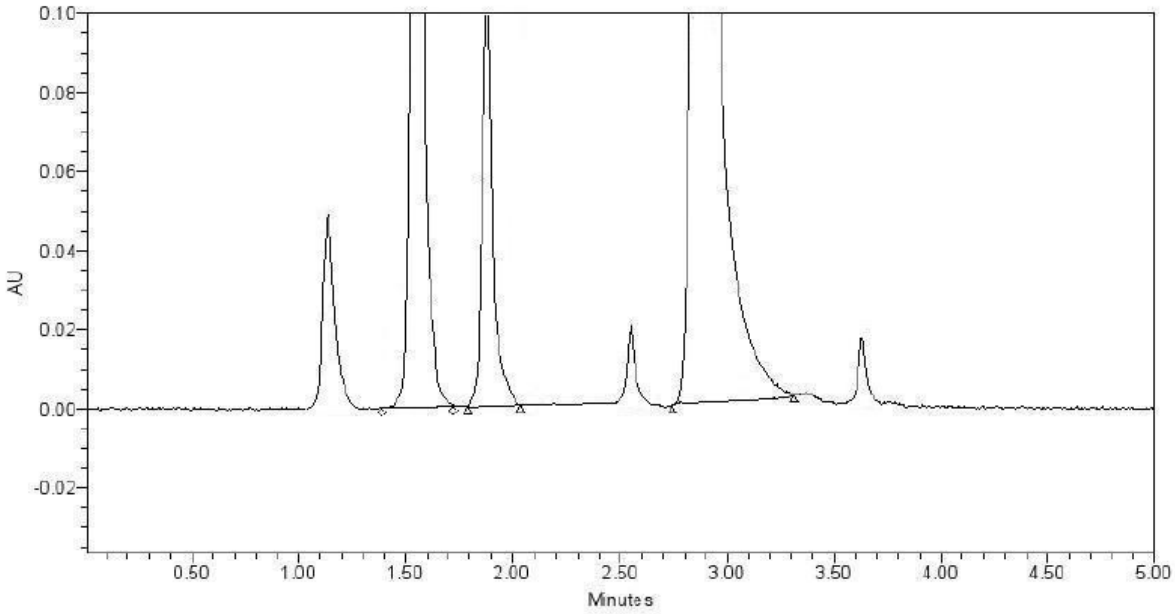


Figure 5. Peroxide Degradation of hydrochlorothiazide, ramipril and telmisartan standard solution

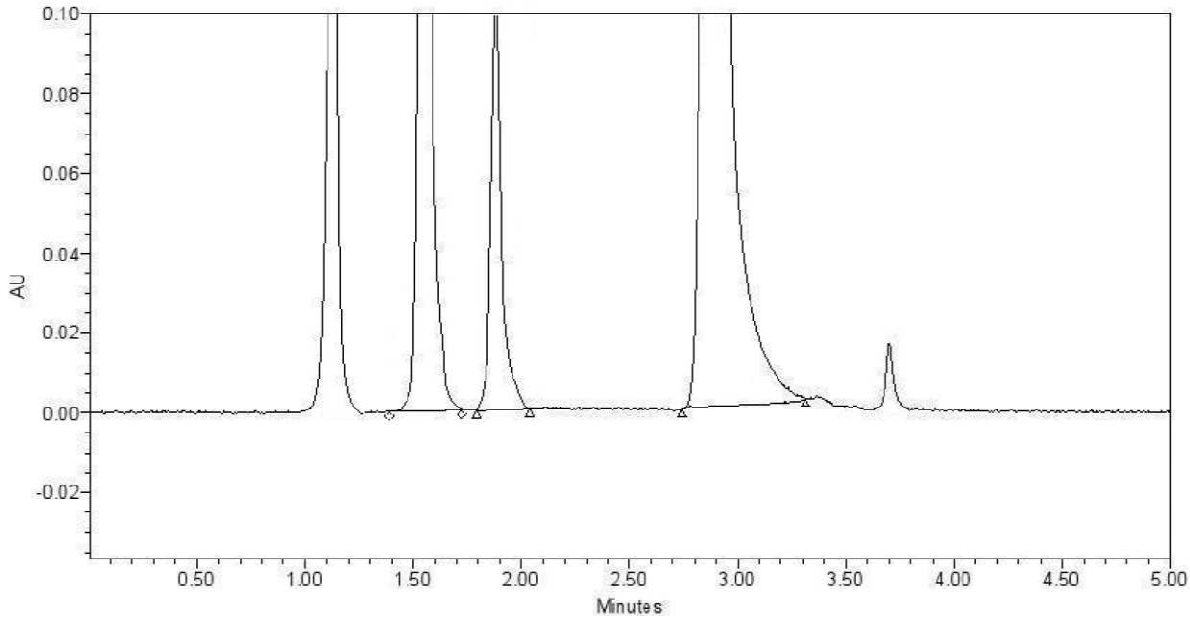
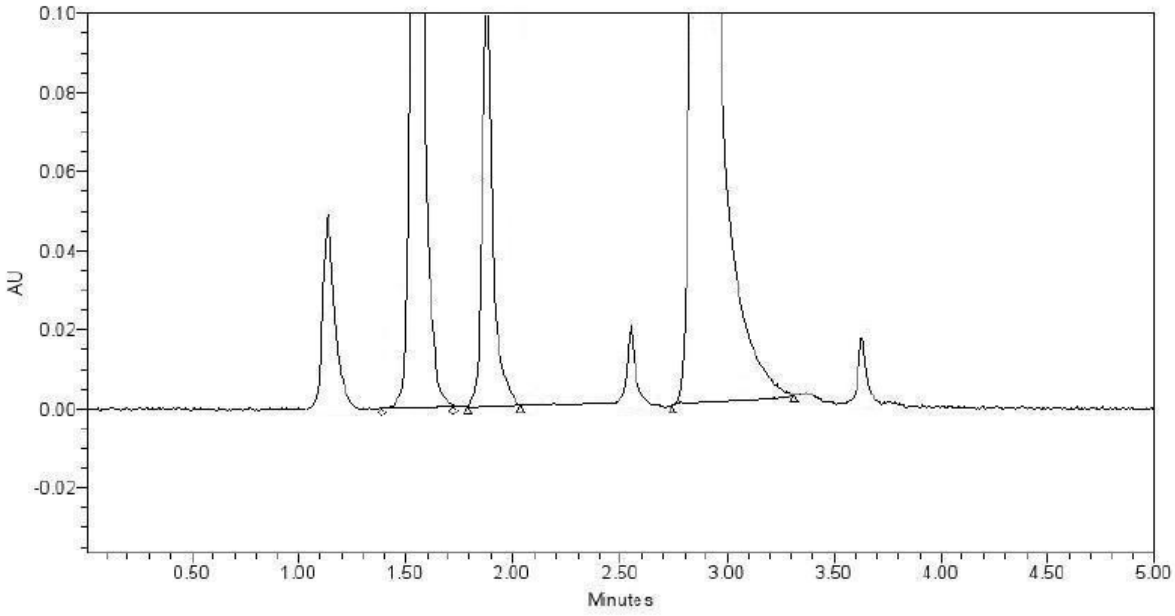


Figure 6. Thermal Degradation of hydrochlorothiazide, ramipril and telmisartan standard solution



CONCLUSION

The established UPLC method proves to be simple, linear, precise and accurate. The total runtime was 5.0 minutes within which three drugs and their degradation products were well separated. The method was validated and shows satisfactory data for all the method validation parameters tested. The Developed method is stability indicating and can be used for simultaneous quantitative determination of the drugs hydrochlorothiazide, ramipril and telmisartan in presence of degradation products in stability by the industry. The adopted UPLC method also can be used separately for assay estimation of Telmisartan tablets, simultaneous estimation of Hydrochlorothiazide and Telmisartan and simultaneous estimation of Ramipril and Hydrochlorothiazide in tablets [17].

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