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An Improved Validated UPLC Method for Separation of Losartan Potassium, Amlodipine and Hydrochlorothiazide impurities in Losartan Potassium, Amlodipine and Hydrochlorothiazide Tablets

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ABSTRACTA rapid, specific, sensitive Ultra-performance liquid chromatographic method has been developed for determination of Losartan Potassium, Amlodipine and Hydrochlorothiazide impurities and its degradation products in pharmaceuticals preparation. 16 impurities including degradation as well as process related impurities have been well separated. UPLC was performed on a C18 column with "mobile phase A" consisting of 90:10:0.1 v/v/v of water, Acetonitrile and TFA; while "mobile phase B" consisted of 10:90:0.1v/v/v of water, Acetonitrile and TFA. The mobile phase was pumped in a gradient manner at the flow-rate of 0.35 mL min^{-1}. Ultraviolet detection was performed at 238 nm. Losartan Potassium, Amlodipine and Hydrochlorothiazide and its degradation products along with process impurities were chromatographed with a total run time of 20 minutes. Calibration showed that response of impurities was a linear function of concentration over the range LOQ to 150% of the target concentration ($r^2 \geq 0.999$) and the method was validated over this range for precision, accuracy, linearity and specificity. For precision study, percentage relative standard deviation of each impurity was <15% (n=6). The method was found to be precise, accurate, linear and specific. The method was successfully employed for estimation of Losartan Potassium, Amlodipine and Hydrochlorothiazide impurities and its degradation products in finished product Tablets formulation.

Keywords: UPLC–Losartan Potassium, Amlodipine and Hydrochlorothiazide, Impurities and Method validation

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INTRODUCTION

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Hypertension is the "silent killer" of humans because this disease is usually asymptomatic until the damaging effects of hypertension such as coronary heart disease and stroke. Amlodipine besylate is chemically described as (3-Ethyl 5-methyl (4RS)-2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-6-methyl-1, 4-dihydropyridine-3, 5-dicarboxylate benzene sulphonate). Amlodipine besylate is a calcium-channel blocking agent; a dihydropyridine derivative with an intrinsically long duration of action. Amlodipine besylate is an anti-hypertensive and an antianginal agent in the form of the besylate salt [1-3]. Losartan Potassium is chemically described as (1H-Imidazole-5-methanol, 2-butyl-4-chloro-1-[[2'-(lHtetrazol- 5-yl)[1,1'-biphenyl]-4-yl]methyl] monopotassiumsalt). Losartan Potassium is an angiotensin II receptor (type AT1) antagonist. It is indicated for the treatment of hypertension. It is indicated to reduce the risk of stroke in patients with hypertension and left ventricular hypertrophy [1-3]. Hydrochlorothiazide is chemically described as (6-chloro-1,1-dioxo-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide). Hydrochlorothiazide is a diuretic drug of the thiazide class that acts by inhibiting the kidney's ability to retain water. This reduces the volume of the blood, decreasing blood return to the heart and thus cardiac output and, is believed to exert hypotensive efficacy through a combined vasodilator and diuretic effect. Losartan potassium (LOS), chemically, is 2-butyl-4-chloro-1-[p-(o-1H-tetrazol-5-ylphenyl)benzyl]imidazole-5-methanol monopotassium salt. It is an angiotensin II receptor blocker and chemically is used as an antihypertensive agent. The preparation of new combinations of drugs in pharmaceuticals for pharmacological activity development, as well as the requirements of modern industrial-scale pharmaceutical analysis, encourages researchers to develop new and efficient methods for multi quantification with separation procedures. Ultra performance liquid chromatography is a dominant separation technique, especially in pharmaceutical analysis. So, it is necessary to develop a validated analytical method for estimation of impurities of these drugs in combination with each other in its pharmaceutical preparations. Our scope is development of a validated analytical method for estimation of degradation impurities as well as process related impurities of these drugs in combination with each other in its pharmaceutical preparations and it should be characterized by a simplicity, accuracy, preciseness and sensitivity. Losartan Potassium and Hydrochlorothiazide, Losartan Potassium and Amlodipine drugs are official in the British Pharmacopoeia, Indian Pharmacopoeia and United States Pharmacopoeia but combination of these three drugs is not official. Based on the literature survey, no official method has yet been developed for their separation and its impurities[6-8]. Several methods have been reported using HPLC with UV and fluorescent detection for the determination of Losartan Potassium, Amlodipine and Hydrochlorothiazide individually in pharmaceutical

dosage forms as well as in biological fluids[9-17]. Thus, application of an UPLC method with high sensitivity and selectivity will find use for the determination of Losartan Potassium , Amlodipine , and hydrochlorothiazide impurities and its degradation products in pharmaceutical formulations.

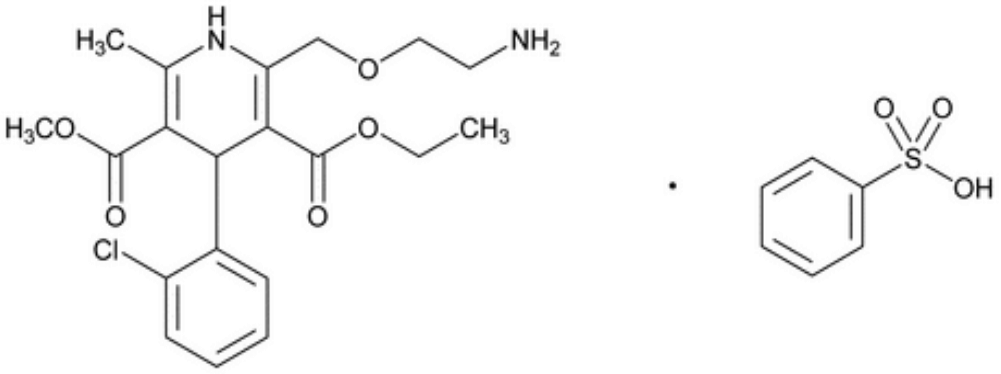

Figure 1A: Chemical structure of Amlodipine besylate.

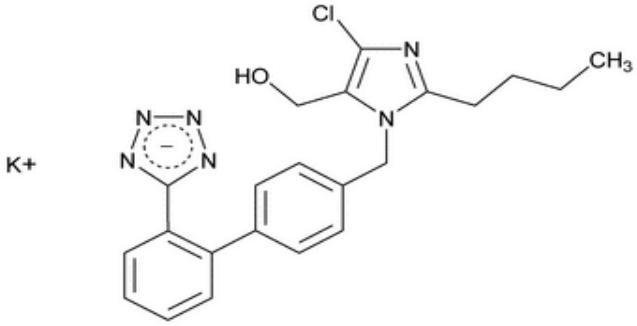

Figure 1B: Chemical structure of Losartan Potassium

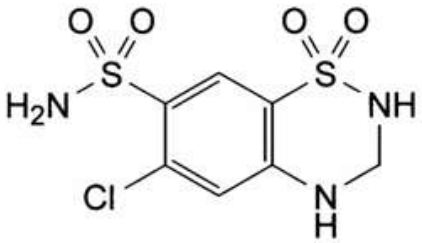

Figure 1C: Chemical structure of Hydrochlorothiazide

MATERIAL AND METHOD

Chemicals and Reagents

Losartan Potassium and it impurities, Losartan Impurity J, Losartan Impurity-D, Losartan AG 6, Losartan AG 12 and Losartan -PH3COH, Losartan – 1-H Dimer , Losartan – 2-H Dimer , Losartan – MBCT and Hydrochlothiazide and its impurities, Chlorothizide, Benzothiazide and 5-Chlorothizide impurities from USP and Amlodipine and it impurities, Amlodipine -Methyl ester, Amlodipine -Ethyl ester, Amlodipine Impurity A, Amlodipine Impurity B and Amlodipine Impurity D. Acetonitrile (HPLC-grade from J.T. Baker, USA), and Trifluoroacetic acid were from

Merck (Darmstadt, Germany. Water was purified by a Millipore (Bedford, MA, USA) Milli-Q water-purification system and passed through a 0.22 µm membrane filter (Durapore; Millipore, Dublin, Ireland) before use.

Equipment

UPLC analysis was performed with a Waters Alliance H class system equipped with a quaternary solvent manager, sample manager, column-heating compartment, and Photodiode array detector. This system was controlled by Waters Empower software. The specificity study was conducted by using heating oven, stability chamber and heating mantel (Thermo Lab, India).

Standard and Sample Preparation

The standard stock solution of Losartan Potassium, Amlodipine and Hydrochlorothiazide was prepared by dissolving an accurately weighed amount of working standards in diluent (Mix 700 volumes of Water with 300 volumes of Acetonitrile), resulting in a concentration of 1.2mg/mL, 0.6mg/mL and 1.5mg/mL respectively. Above solution further diluted in diluent to get a concentration of 4.8 µg mL^{-1}, 0.48 µg mL^{-1} and 1.2 µg mL^{-1} respectively. The impurity stock solutions for Losartan Potassium, Amlodipine and Hydrochlorothiazide impurities was prepared by dissolving an accurately weighed amount in diluent, resulting in a concentration of 4.8 µg $mL^{-1}$of each impurity of Losartan Potassium impurities, 0.48 µg $mL^{-1}$of each impurity of Amlodipine impurities and 1.2 µg mL^{-1} for Hydrochlorothiazide impurities. The test solution was prepared by dissolving an accurately weighed portion of the powder, equivalent to 50 mg of Losartan Potassium (5mg of Amlodipine and 12.5 mg of Hydrochlorothiazide) in 35mL diluent. After sonicating for around 30minutes, volume made up to 50mL.Above solution was filtered through 0.22µ PVDF filter to eliminate insoluble excipients. The clear liquid used for chromatographic analysis.

Chromatography

The analytes were separated on an Waters HPLC with BEH C18 column (100 mm x 2.6 mm, 1.7µ) at column oven temperature of 25^{o}C with a gradient run program at a flow-rate of 0.35 mL min^{-1} (Table 1). Before use, the mobile phase was filtered through a 0.22 µm Millipore filter. UV detection was performed at 238 nm. The sample injection volume was 3.3 µL in partial-loop mode.

Table 1: Gradient program for elution of Losartan Potassium, Amlodipine and Hydrochlorothiazide and impurities

Time (minutes)	*Solution A* (%)	*Solution B* (%)	Elution
0–3.57	90 →85	10 →15	linear gradient
3.57-7.15	85 →75	15 →25	linear gradient
7.15-11.91	75 →50	25 →50	linear gradient
11.91-16.67	50 →10	50 →90	linear gradient
16.67-17.39	10 →90	90 →10	Initial gradient
17.39-20.0	90	10	re-equilibration

Method Validation

The method was validated for specificity, precision, accuracy, sensitivity and linear range as per the International Conference on Harmonization (ICH) guidelines[19].

Specificity

A study was conducted to demonstrate the interference from placebo. Sample solutions were prepared by taking the placebo equivalent to the amount present in the sample solution and analyzed as per test method. Chromatograms of placebo preparations are not showing any interference at the retention time of known impurities as well as analyte peaks. A study was conducted to demonstrate the known impurities interference by spiking the sample solution with all the known impurities at 0.5% spike level and analyzed as per test method. It is found that all the known impurities are separated from each other and also from analyte peaks. The known impurities of Losartan Potassium, Amlodipine, and hydrochlorothiazide were injected individually to confirm the retention time. A study was conducted to demonstrate the effective separation of degradants from Losartan Potassium, Amlodipine, and hydrochlorothiazide peaks. The drug product was subjected to hydrolysis by refluxing the test solution in 1 N Sodium hydroxide solution at 55ºC for 30 minutes. Similarly the acidic hydrolysis was performed by refluxing test solution in 1N Hydrochloric acid solution at 55ºC for 30 minutes. The neutral hydrolysis was done in water at refluxing temperature of 60ºC for 30 minutes. Oxidation studies were performed in 3 % Hydrogen Peroxide solution at Bench top for 30 minutes. On photo stability study drug product was sufficiently spread on petri plates (1 mm thick layer) and exposed to sunlight and UV light at ambient conditions for 7 days. Humidity study was performed separately by exposing drug product to humidity at 25°C, 90% RH for 5 days. Thermal degradation study was performed by heating drug product at 85° C for 30 minutes. Similarly placebo samples were prepared as like as drug product by exposing formulation

matrices without drug substance. Stressed samples were injected into the UPLC system with photo diode array detector by following test method conditions.

Precision

The precision of test method was evaluated by using six samples spiked with known Impurities at 0.5% level and analyzed as per test method.

Accuracy

To confirm the accuracy of the method, recovery studies were carried out by standard addition technique. Samples were prepared in triplicate by spiking all known impurities in test preparation at the level of LOQ, 50%, 100% and 150% of the standard concentration and analyzed as per the test method.

Sensitivity

Sensitivity of the method was established with respect to Limit of detection and limit of quantification for Losartan Potassium, Amlodipine, and hydrochlorothiazide impurities. Series of concentration of drug solution and its impurities were injected, LOD and LOQ established by Signal to Noise ratio method. Precision was performed at LOQ level for all the known impurities by injecting six replicate injection of each impurity at the concentration obtained from above method.

Linearity of Detector Response

A series of solutions of all the known impurities in the concentration ranging from limit of quantification level LOQ to 150% of standard concentration were prepared and injected into the UPLC system.

Application of Developed Method

The method suitability was verified by analyzing the finished product. The content of 20 Tablets powder was accurately weighted and transferred equivalent to about to 50 mg of Losartan Potassium (5mg of Amlodipine and 12.5 mg of Hydrochlorothiazide) in 35mL diluent. After sonicating for around 30minutes, volume made up to 50mL.Above solution was filtered through 0.22µ PVDF filter and injected

RESULTS AND DISCUSSION

Selectivity, sensitivity, resolution, and speed of chromatographic separation were optimized for the UPLC method. The retention times of Losartan Potassium at about 9.595 and it impurities - Losartan Impurity J at about 12.143, Losartan Impurity-D at about 6.794, Losartan AG 6 at about 1.044, Losartan AG 12 at about 7.974 and Losartan -PH3COH at about 13.554 , Losartan – 1-H

Dimer at about 14.243, Losartan – 2-H Dimer at about 15.108 , Losartan – MBCT at about 10.822. The retention time of Amlodipine at about 11.604 and its impurities--impurity - A at 15.736, impurity-B at 12.442, impurity-D at 10.430, Amlodipine Methyl Ester at about 9.224 and Amlodipine Ethyl Ester at about 13.686. The retention time of Hydrochlorothiazide at about 1.847 and it impurities Benzochlorothiazide at about 1.211, Chlorothiazide at about 1.311, 5-Chlorothizide impurity at about 3.265., under the chromatographic conditions described, and the total run time was 20 minutes. Chromatograms obtained from blank, diluted standard, controlled sample and Test sample spiked with Degradation impurities and Test sample spiked with all impurities (process as well as Degradation impurities) are shown in Figures 2A, 2B, 2C, 2D, 2E and 2F respectively. UPLC system has been proved to be a promising tool for separation with shorter run time. Use of BEH C18 column (100 mm x 2.6 mm, 1.7µ) as stationary phase enabled optimization of UPLC for both peak selectivity and analysis speed. Losartan Potassium, Amlodipine and Hydrochlorothiazide and its impurities were well separated with good peak shape and resolution. No interfering peaks were observed in blank & placebo, indicating that signal suppression or enhancement by the product matrices was negligible. After satisfactory development of method it was subjected to method validation as per ICH guideline[19]. The method was validated to demonstrate that it is suitable for its intended purpose by standard procedure to evaluate adequate validation characteristics. The result of system suitability parameter was found to be complying acceptable suitability criteria: relative standard deviation of replicate injection is not more than 5.0%. The result of specificity study ascertained the known impurities are separated from each other and also from Losartan Potassium, Amlodipine and Hydrochlorothiazide peak and spectral purity of all exposed samples found spectrally pure [Table 3]. The % RSD of replicate determination was found to be <5 during precision study, which indicates that the method is precise and data of precision study are shown in Table 4. The result obtained in the recovery study were found within the range of 85% to 115% (LOQ to 150%), which indicates that method is accurate and data for the same is given in Table 5 and 6. Sensitivity of method was verified and method is found to be linear, accurate and precise at limit of quantification and data of LOD & LOQ study are given in Table 7. The calibration curve of all impurities were obtained by plotting the peak area of individual impurity versus concentration over the range of LOQ to 150% and were found to be linear (r = 0.999). The data of regression analysis of the calibration curves are shown in Table 8. The applicability of the method was verified by the determination of impurities in In house formulation. The impurity content was found to be satisfactory in the finished product formulations and data shown in Table 9.

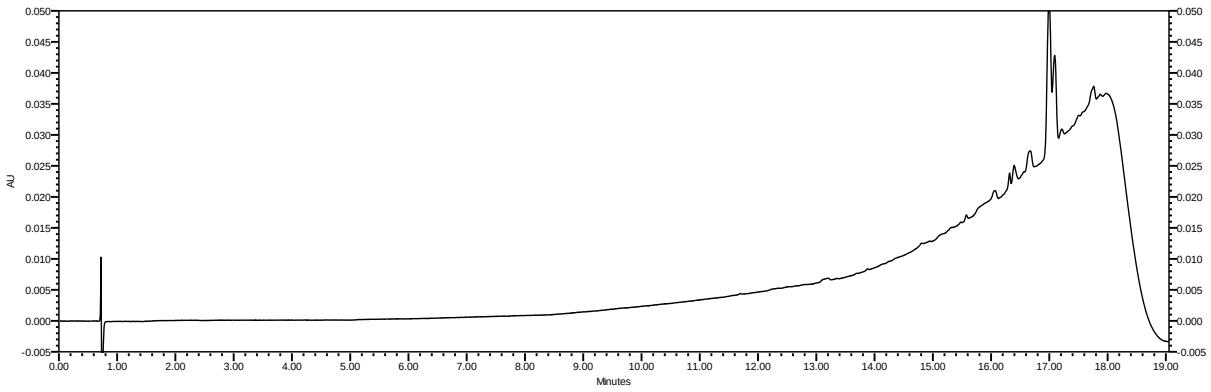

Figure 2A: Typical Chromatogram of Blank

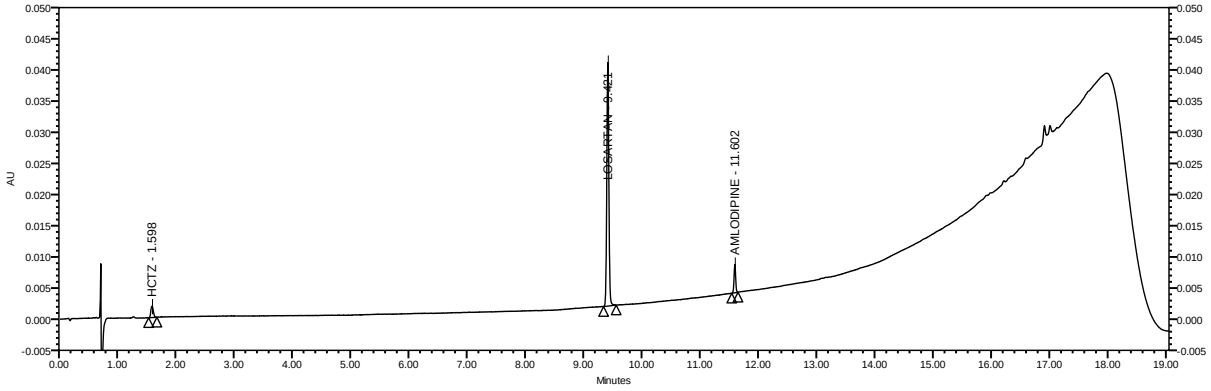

Figure 2B: Typical Chromatogram of Standard Solution

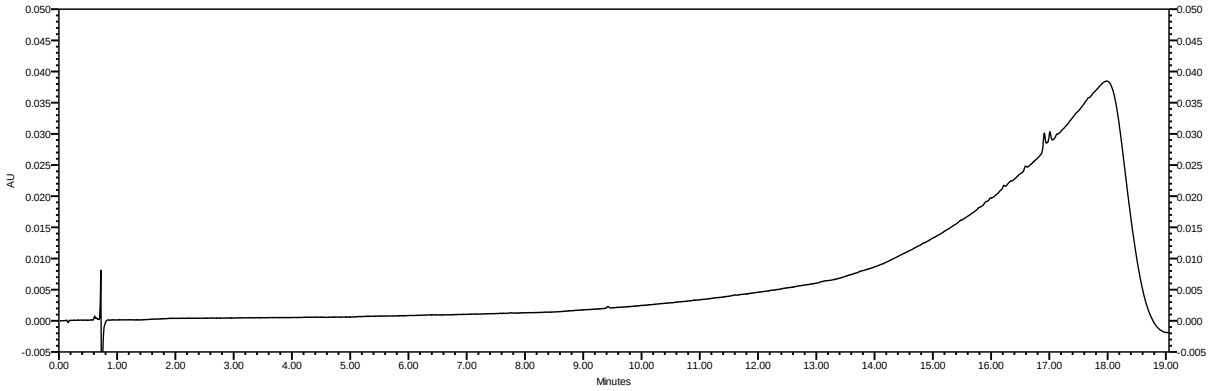

Figure 2C: Typical Chromatogram of Placebo Solution

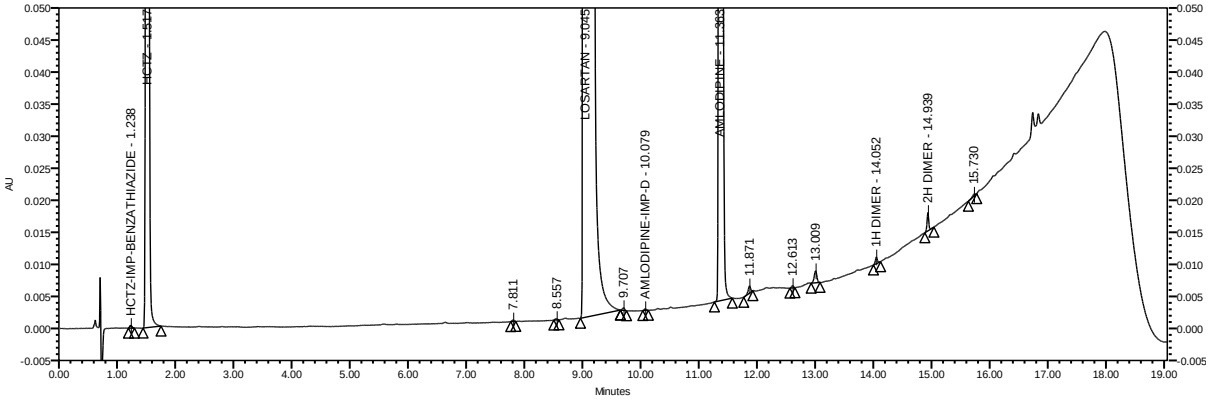

Figure 2D: Typical Chromatogram of Control Sample Solution

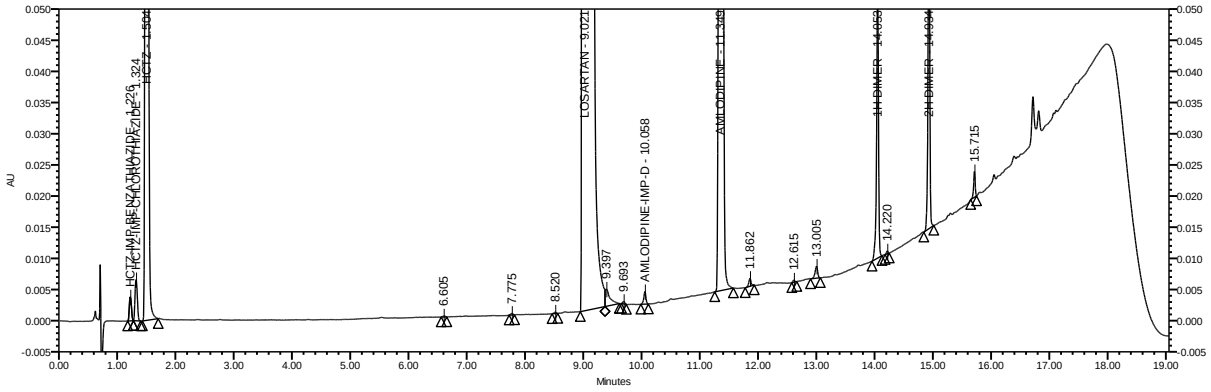

Figure 2E: Typical Chromatogram of Spiked Sample Solution

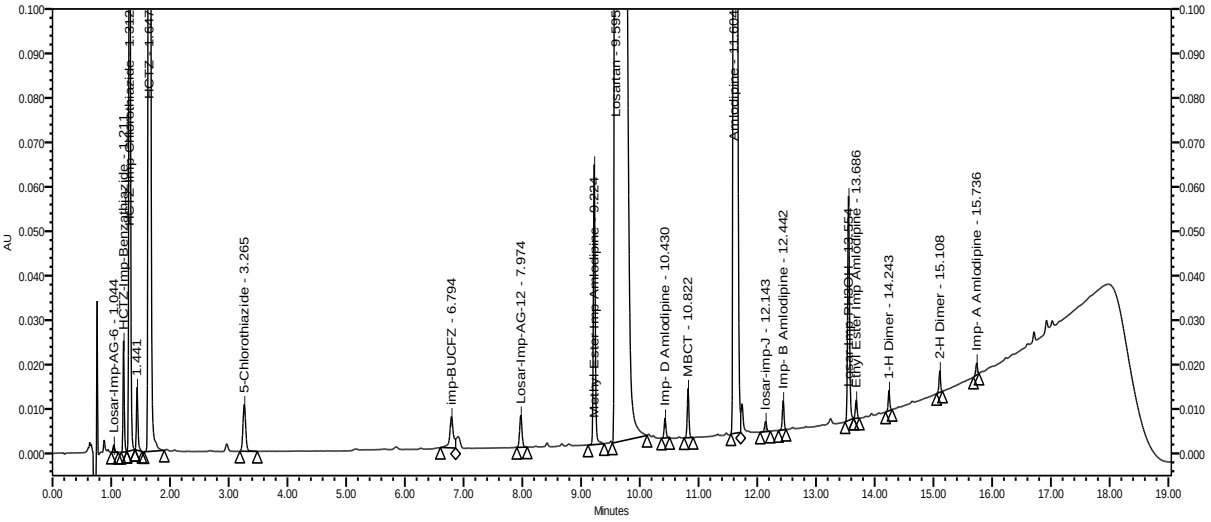

Figure 2F: Typical Chromatogram of Spiked Sample Solution for all 16 impurities

Table 2: Chromatographic Conditions

LC Column	BEH C18 column (100 mm x 2.6 mm, 1.7µ)
Flow Rate	0.35 mL/minutes
Run Time	20 minutes
Wavelength	238nm
Column oven Temperature	25°C

Table 3: Results of Specificity Study

S.No.	Stress conditions	% Degradation	Individual % Degradation Losartan; Amlodipine And Hydrochlorthiazide				
			% Benzathiazide	% Chlorthiazide	% IMP-D Amlodipine	% 1-H-Dimer	% 2-H-Dimer
1	Treated with 1N HCl solution at 55°C temperature for about 30 minutes	12.05	4.31	ND	2.84	1.17	0.98
2	Treated with 1N NaOH solution at 55°C temperature for about 30 minutes	7.90	0.34	ND	0.30	0.03	0.05
3	Treated with 3% H_2O_2 solution at 25°C temperature for about 30 min	10.28	0.57	ND	1.27	0.12	ND
4	Exposed to Heat at 85°C temperature for about 30 minutes	17.33	13.71	ND	3.32	ND	0.05
5	Exposed to Sunlight about 5 hours	2.19	1.83	ND	0.20	ND	0.04
6	Treated with Water at 60°C temperature for about 30 minutes	1.07	0.68	ND	0.27	ND	0.04
7	Treated with UV Light	2.36	1.89	ND	0.25	ND	0.04

Table 4: Percentage of RSD of impurities in precision study

Nominal concentrations (µg mL^{-1})	Precision (RSD, %) (n = 6)
Benzathiazide	1.39
Chlorthiazide	1.08
IMP-D Amlodipine	0.87
1-H-Dimer	0.33
2-H-Dimer	0.96

Table 5: Percentage Recovery of impurities at different level

Nominal concentrations	% Mean Recovery of (n = 3)				
	Benzathiazide	Chlorothiazide	IMP-D Amlodipine	1-H-Dimer	2-H-Dimer
Low@50%		106.2	106.7	103.5	104.8
Midle@100%	108.2	98.3	98.4	100.7	100.4
High@150%	110.3	98.3	100.2	101.6	102.2

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Table 6: Percentage of Recovery & precision at LOQ level

Nominal concentrations	**% Mean Recovery of (n = 3)**				
	Benzathiazide	Chlorothiazide	IMP-D Amlodipine	1-H-Dimer	2-H-Dimer
% Recovery	98.8	93.5	110.6	104.5	102.1
% RSD on precision (n= 6)	2.42	0.84	8.54	0.58	0.45

Table 7: Limit of detection (LOD) and limit of Quantification (LOQ) of impurities in percentage

Nominal concentrations	**Benzathiazide**	**Chlorothiazide**	**IMP-D Amlodipine**	**1-H-Dimer**	**2-H-Dimer**
LOD in %	0.0319	0.0285	0.0176	0.0189	0.0287
LOQ in %	0.0986	0.0842	0.0757	0.0616	0.0891

Table 8: Correlation Coefficient of impurities

Parameters (n=5)	**Benzathiazide**	**Chlorothiazide**	**IMP-D Amlodipine**	**1-H-Dimer**	**2-H-Dimer**
Slope	5640.78	15286.99	8827.36	20635.53	22238.57
Y intercept	103.15	199.98	29.25	318.53	546.81
Correlation Coefficient	0.999	0.999	0.999	0.999	0.999

Table 9: Impurity profile of finished formulation

No.	**Finished Product**	**Losartan, Amlodipine and Hydrochlorthiazide Tablets**				
		% Benzathiazide	**% Chlorthiazide**	**% IMP-D Amlodipine**	**% 1-H Dimer**	**% 2-H-Dimer**
1	Known Impurities	0.45	ND	0.10	0.02	0.04
2	Unknown Impurities	0.09				
3	Total Impurities	0.91				

CONCLUSION

A rapid, specific, sensitive ultra-performance liquid chromatographic method has been developed for determination of Losartan Potassium, Amlodipine and Hydrochlorothiazide and its degradation products in pharmaceuticals preparation. A number of analytical approaches have been previously described to Losartan Potassium, Amlodipine and Hydrochlorothiazide individually in pharmaceutical dosage forms as well as in biological, however, this is the first study reporting a validated reversed phase method for impurity quantification in Losartan Potassium, Amlodipine and Hydrochlorothiazide formulation. Losartan Potassium, Amlodipine and Hydrochlorothiazide drugs are official in the British Pharmacopoeia and United States Pharmacopoeia but their combination is not official. Based on the literature survey, no official method has yet been developed for their separation and its impurities The simple UPLC method developed in this study makes it suitable for separation and estimation of impurities without interference from excipients and other related substances present in the pharmaceutical matrices. The analytical performance and the result obtained from analysis of the formulation demonstrated that the method is reliable and sufficiently robust. In conclusion, the high sensitivity, good selectivity, accuracy and reproducibility of the UPLC method developed in this study makes it suitable for quality control analysis of complex pharmaceutical preparation containing Losartan Potassium, Amlodipine and Hydrochlorothiazide and its impurities.

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