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DEVELOPMENT AND VALIDATION OF A NOVEL RP-HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF TELMISARTAN AND AMLODIPINE IN TABLET FORMULATION

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Abstract:
Analytical Method Development and Validation for Telmisartan and Amlodipine in Tablet Dosage Form by RP-HPLC. New method was established for simultaneous estimation of Telmisartan and Amlodipine by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Telmisartan and Amlodipine by using Inertsil C18 (4.6mm ×250mm, 5µm particle size), flow rate was 1.0 ml/min, mobile phase ratio was (55:45% v/v) Methanol: Phosphate buffer pH 4.8 (pH was adjusted with ortho phosphoricacid), detection wavelength was 282nm. The instrument used was WATERS Alliance 2695 separation module, Software: Empower 2, 996 PDA detector. The retention times were found to be 1.688mins and 3.282mins. The % purity of Telmisartan and Amlodipine was found to be 99.86%. The system suitability parameters for Telmisartan and Amlodipine such as theoretical plates and tailing factor were found to be 7586, 1.69 and 6235 and 1.58, the resolution was found to be 10.85. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study of Telmisartan and Amlodipine was found in concentration range of 100µg-500µg and 30µg-70µg and correlation coefficient (r2) was found to be 0.999 and 0.999, % recovery was found to be 100.112% and 100.16%, %RSD for repeatability was 0.1702 and 0.043 respectively. The precision study was precise, robust, and repeatable. The LOD value was found to be 2.1µg/ml and 1.28µg/ml, and LOQ value was 6.3µg/ml and 3.84µg/ml for Telmisartan and Amlodipine respectively. Hence the suggested RP-HPLC method can be used for routine analysis of Telmisartan and Amlodipine in Tablet dosage form.

Keywords: *Telmisartan and Amlodipine, Method Development, Validation, Accuracy, Precision.*

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INTRODUCTION:

Telmisartan is an ARB used to treat hypertension, diabetic nephropathy, and congestive heart failure. Telmisartan is an angiotensin II receptor antagonist (ARB) used in the management of hypertension1. Generally, angiotensin II receptor blockers (ARBs) such as telmisartan bind to the angiotensin II type 1 (AT1) receptors with high affinity, causing inhibition of the action of angiotensin II on vascular smooth muscle, ultimately leading to a reduction in arterial blood pressure. Recent studies suggest that telmisartan may also have PPAR-gamma agonistic properties that could potentially confer beneficial metabolic effects2.IUPAC name of Telmisartan is 2-[4-[[4-methyl-6-(1-methylbenzimidazol-2-yl)-2-propylbenzimidazol-1-yl] methyl] phenyl] benzoic acid. Molecular Formula is C33H30N4O2. Molecular Weight is 514.6. Telmisartan is soluble in organic solvents such as DMSO and dimethyl formamide (DMF), which should be purged with an inert gas. The solubility of telmisartan in DMSO is approximately 1 mg/ml and approximately 1.6 mg/ml in DMF. Telmisartan is sparingly soluble in aqueous buffers.

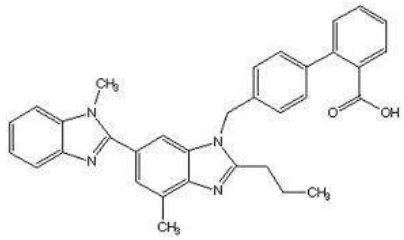

Figure 1: Structure of Telmisartan

Amlodipine is a popular antihypertensive drug belonging to the group of drugs called dihydropyridine calcium channel blockers. Due to their selectivity for the peripheral blood vessels, dihydropyridine calcium channel blockers are associated with a lower incidence of myocardial depression and cardiac conduction abnormalities than other calcium channel blockers 3. Amlodipine is commonly used in the treatment of high blood pressure and angina. Amlodipine has antioxidant properties and an ability to enhance the production of nitric oxide (NO), an important vasodilator that decreases blood pressure 4. The option for single daily dosing of amlodipine is an attractive feature of this drug. IUPAC Name is 3-ethyl 5-methyl 2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5 dicarboxylate; benzenesulfonic acid. Molecular formula is C26H31ClN2O8S. Molecular weight is 567 g/mol. It is slightly soluble in water and sparingly soluble in ethanol.

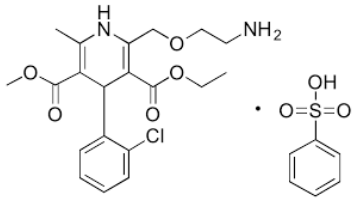

Figure 2: Structure of Amlodipine

The literature survey revealed that There are very few methods reported in the literature for analysis of Telmisartan and Amlodipine alone or in combination with other drugs in the pure form and pharmaceuticals formulations [5-13]. In view of the need for a suitable, cost-effective HPLC method for routine analysis of Telmisartan and Amlodipine Simultaneous estimation of in pharmaceutical dosage form. Attempts were made to develop simple, precise, accurate and cost-effective analytical method for the estimation of Telmisartan and Amlodipine. The proposed method will be validated as per ICH guidelines. The objective of the proposed work is to develop a new, simple, sensitive, accurate and economical analytical method and validation for the Simultaneous estimation of Telmisartan, Amlodipine in pharmaceutical dosage form by using HPLC. To validate the developed method in accordance with ICH guidelines for the intended analytical application i.e., to apply the proposed method for analysis of the drug in its dosage form.

MATERIALS AND METHODS:

Chemicals and Reagents:

Telmisartan and Amlodipine were Purchased from Hetero drugs. NaH_2PO_4 was analytical grade supplied by Finerchem limited, Orthophosphoric acid (Merck), and Water and Methanol for HPLC (Lichrosolv (Merck).

Equipment and Chromatographic Conditions:

The chromatography was performed on a Waters 2695 HPLC system, equipped with an auto sampler, UV detector and Empower 2 software. Analysis was carried out at 282 nm with column Inertsil C18 (4.6mm ×250mm, 5µm particle size), dimensions at 35^0C temperature. The optimized mobile phase consists of Phosphate Buffer (pH-4.8): Methanol (55:45% v/v). Flow rate was maintained at 1 ml/min.

Preparation of solutions:

Preparation of mobile phase: Accurately measured 500 ml (50%) of HPLC Methanol and 350 ml of

Acetonitrile (35%) and 150 ml of Water (15%) were mixed and degassed in a digital ultrasonicater for 10 minutes and then filtered through 0.45 µ filter under vacuum filter.

Diluent Preparation:
Accurately measured 450 ml (45%) of HPLC Methanol and 550 ml of Phosphate Buffer (55%) were mixed and degassed in a digital ultra sonicater for 15 minutes and then filtered through 0.45 µ filter under vacuum filter.

Assay:
Preparation of the Telmisartan and Amlodipine standard solution:
Preparation of standard solution: (Telmisartan)
Accurately weigh and transfer 40 mg of Telmisartan, working standard into a 10ml of clean dry volumetric flasks add about 7ml of diluent and sonicate to dissolve and removal of air completely and make volume up to the mark with the diluent.

Preparation of standard solution: (Amlodipine)
Accurately weigh and transfer 5 mg of Amlodipine working standard into a 10ml of clean dry volumetric flasks add about 7ml of diluent and sonicate to dissolve and removal of air completely and make volume up to the mark with the diluent.
Further pipette 3ml of Telmisartan, 0.5ml of Amlodipine from stock solutions in to a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure:
Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Preparation of Sample Solution:
Take average weight of Tablet and crush in a mortar by using pestle and weight 10 mg equivalent weight of Telmisartan, Amlodipine sample into a 10ml clean dry volumetric flask and add about 7ml of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.
Procedure:
Further pipette 1.2ml of Telmisartan, Amlodipine from above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

RESULTS AND DISCUSSION:

METHOD:

The developed chromatographic method was validated for system suitability, linearity accuracy, precision, ruggedness and robustness as per ICH guidelines.

System suitability parameters:
To evaluate system suitability parameters such as retention time, tailing factor and USP theoretical plate count, the mobile phase was allowed to flow through the column at a flow rate of 1.0 ml/min to equilibrate the column at ambient temperature. Chromatographic separation was achieved by injecting a volume of 20 µL of standard into Inertsil C18 (4.6mm ×250mm, 5µm particle size), the mobile phase of composition Phosphate Buffer (pH-4.8): Methanol (55:45% v/v) was allowed to flow through the column at a flow rate of 1.0 ml per minute. Retention time, tailing factor and USP theoretical plate count of the developed method are shown in table 1.

Table 1: System suitability parameters

S. NO	Parameter	Telmisartan	Amlodipine
1.	Retention Time (min)	1.688	3.282
2.	Theoretical Plates	7586	6235
3.	Tailing factor	1.69	1.58
4.	Area	1658768	426589
5.	Resolution	10.89	

Assay of pharmaceutical formulation: The proposed validated method was successfully applied to determine Telmisartan and Amlodipine in their tablet dosage form. The result obtained for was comparable with the corresponding labeled amounts and they were shown in Table-2.

Table 2: Assay results for Telmisartan and Amlodipine

	Label Claim (mg)	% Assay
Telmisartan	40	99.86
Amlodipine	5	98.86

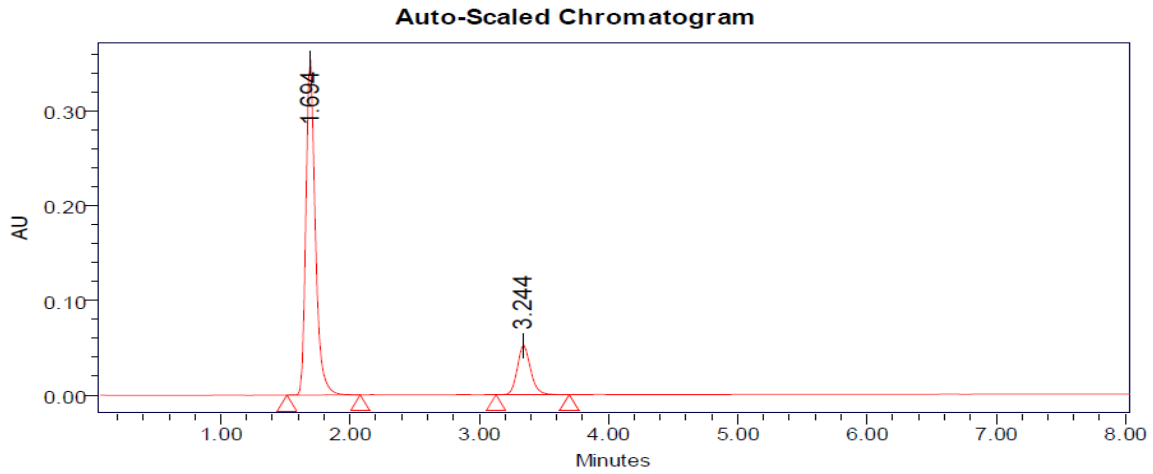

Figure 3: Standard chromatogram

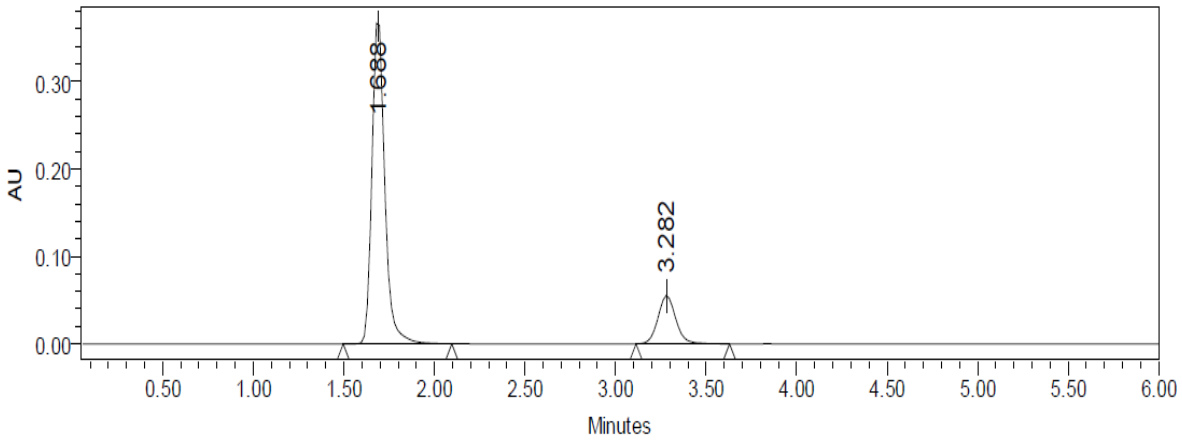

Figure 4: Sample chromatogram

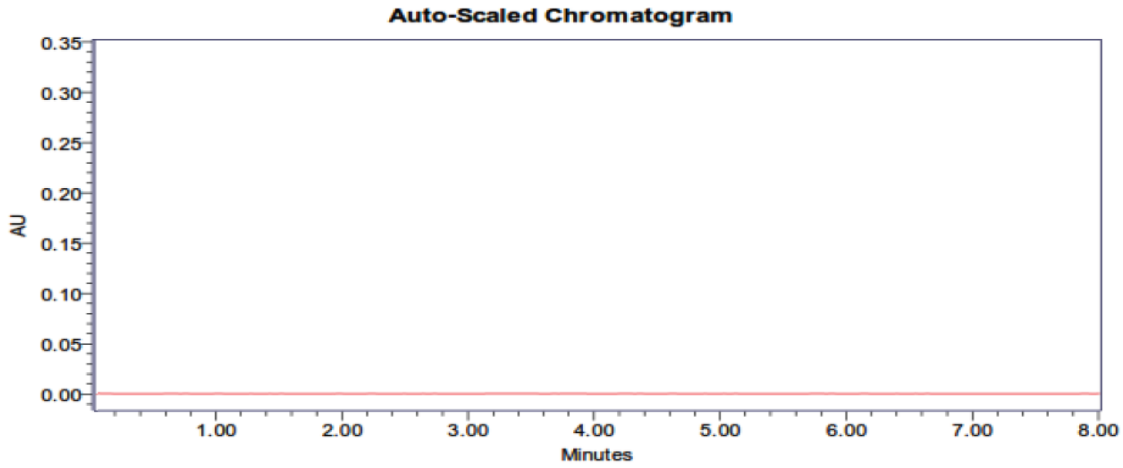

Figure 5: Blank chromatogram

Validation of Analytical method:

Linearity: The linearity study was performed for the concentration of 100 ppm to 500 ppm and 100 ppm to 500 ppm level. Each level was injected into chromatographic system. The area of each level was used for calculation of correlation coefficient. Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient. The results are shown in table 3,4.

Table 3: Linearity results of Telmisartan

S. No	Concentration Level (%)	Concentration µg/ml	Average Peak Area
1.	I	100	585985
2.	II	200	1182468
3.	III	300	1768785
4.	IV	400	2326852
5.	V	500	2856874
Correlation coefficient			0.999

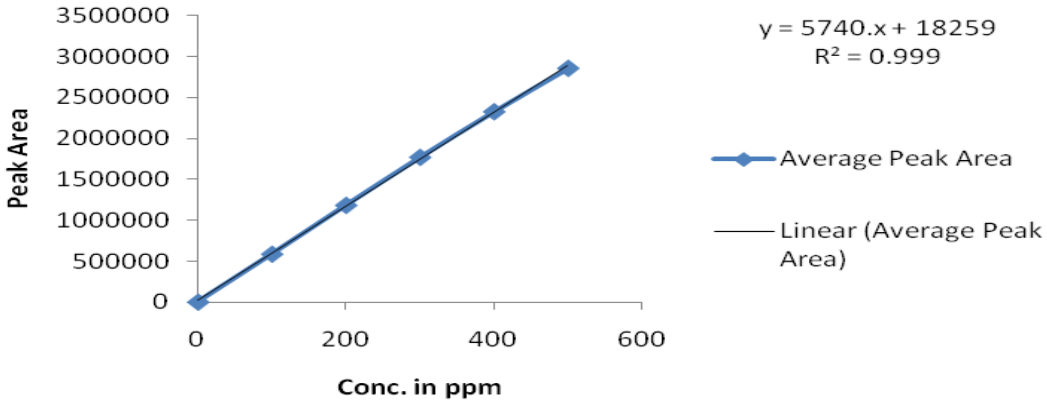

Figure 6: Linearity graph for Telmisartan

Table 4: Linearity results of Amlodipine

S. No	Concentration Level (%)	Concentration µg/ml	Average Peak Area
1.	I	100	585985
2.	II	200	1182468
3.	III	300	1768785
4.	IV	400	2326852
5.	V	500	2856874
Correlation coefficient			0.999

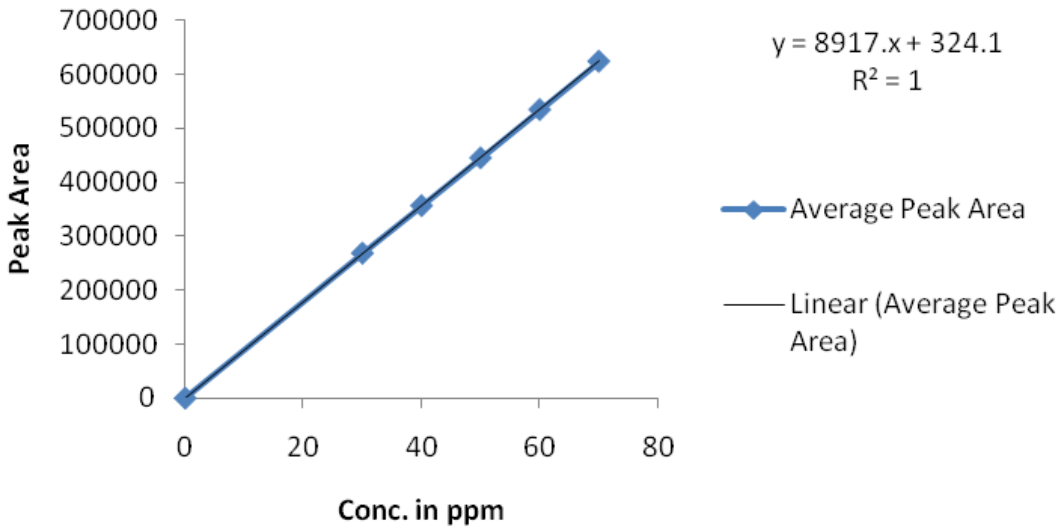

Figure 6: Linearity graph for Amlodipine

Accuracy studies:

The accuracy was determined by help of recovery study. The recovery method carried out at three level 50%, 100%, 150% and 50%, 100%, 150% Inject the standard solutions into chromatographic system. Calculate the Amount found and Amount added for Telmisartan and Amlodipine and calculate the individual recovery and mean recovery values. The results are shown in table 5,6.

Table 5: Showing accuracy results for Telmisartan

%Concentration (at specification Level)	Average Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	879537	150	150.048	100.032	100.112%
100%	1743252	300	300.521	100.172	
150%	2609693	450	450.598	100.132	

Table 6: Showing accuracy results for Amlodipine

%Concentration (at specification Level)	Average Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	224271	25	25.114	100.456%	100.16%
100%	445748.3	50	49.952	99.904%	
150%	670006.3	75	75.101	100.134%	

Precision Studies:

Precision was caliculated from Coefficient of variance for five replicate injections of the standard. The standard solution was injected for five times and measured the area for all five Injections in HPLC. The %RSD for the area of five replicate injections was found. The resulte are shown in table 7.

Table 7: Precision results for Telmisartan and Amlodipine

S. No	Sample Area 1	Sample Area 2
1	1658254	426598
2	1658952	426589
3	1654857	426985
4	1659854	426587
5	1653298	426515
Mean	**1657043**	**426654.8**
Std.dev	**2820.29**	**187.5692**
%RSD	**0.1702**	**0.043963**

Ruggedness:

To evaluate the intermediate precision of the method, Precision was performed on different day. The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found. The resulte are shown in table 8.

Table 8: Ruggedness results of Telmisartan and Amlodipine

S. No	Sample Area 1	Sample Area 2
1	1648598	415985
2	1642587	415267
3	1649852	415986
4	1648754	415265
5	1645289	415874
6	1647581	415632
Mean	**1647110**	**415668.2**
Std. Dev.	**2699.291**	**337.2106**
% RSD	**0.16388**	**0.081125**

Robustness:

As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method. The flow rate was varied at 0.8 ml/min to 1.2 ml/min. The results are shown in table 9,10,11,12.

Table 9: Flow variation results for Telmisartan

Flow Rate (ml/min)		System suitability Results		
		USP Plate Count	USP Tailing	Retention Time (min)
Less Flow rate	0.8	7365	1.62	1.868
Actual Flow rate	1	7586	1.69	1.688
More Flow rate	1.2	7254	1.61	1.544

Table 10: Flow variation results for Amlodipine

Flow Rate (ml/min)		System suitability Results		
		USP Plate Count	USP Tailing	Retention Time (min)
Less Flow rate	0.8	6284	1.51	3.621
Actual Flow rate	1	6235	1.58	3.282
More Flow rate	1.2	6168	1.56	2.998

Table 11: Change in Organic Composition in the Mobile Phase for Telmisartan

Organic phase		System suitability Results		
		USP Plate	USP Tailing	Retention Time (min)
Less organic phase	50:50	7269	1.61	1.868
Actual organic phase	55:45	7586	1.69	1.688
More organic phase	60:40	7496	1.64	1.675

Table 12: Change in Organic Composition in the Mobile Phase for Amlodipine

Organic phase		System suitability Results		
		USP Plate Count	USP Tailing	Retention Time (min)
Less organic phase	50:50	6182	1.54	3.621
Actual organic phase	55:45	6235	1.58	3.282
More organic phase	60:40	6322	1.56	2.302

LOD and LOQ: The sensitivity of RP-HPLC was determined from LOD and LOQ. Which were calculated from the calibration curve using the following equations as per ICH guidelines. The results are shown in table 13.

LOD = 3.3σ/S and

LOQ = 10 σ/S, where

σ= Standard deviation of y intercept of regression line,

S = Slope of the calibration curve

Table 13: LOD, LOQ of Telmisartan and Amlodipine

Drug	LOD	LOQ
Telmisartan	2.1	6.3
Amlodipine	1.28	3.84

CONCLUSION:

The validated HPLC method developed for the quantitative quality control determination of Telmisartan and Amlodipine in combination was evaluated for system suitability, specificity, sensitivity, linearity, range, accuracy (recovery), precision (repeatability and intermediate precision), and robustness. All the validation results were within the allowed specifications of ICH guidelines. The developed method has proven to be rapid, accurate, and stability-indicating for the simultaneous determination of combined Telmisartan and Amlodipine in tablet dosage form in the presence of excipients and the degradation products. There was always a complete separation of both ingredients from their degradation products and from the placebo. As a result, the proposed HPLC method

could be adopted for the quantitative quality control and routine analysis of the tablet dosage form.

REFERENCES:

1. Karlberg BE, Lins LE, Hermansson K: Efficacy and safety of telmisartan, a selective AT1 receptor antagonist, compared with enalapril in elderly patients with primary hypertension. TEES Study Group. J Hypertens. 1999 Feb;17(2):293-302.
2. Balt JC, Mathy MJ, Nap A, Pfaffendorf M, van Zwieten PA: Effect of the AT1-receptor antagonists losartan, irbesartan, and telmisartan on angiotensin II-induced facilitation of sympathetic neurotransmission in the rat mesenteric artery. J Cardiovasc Pharmacol. 2001 Jul;38(1):141-8.
3. Meredith PA, Elliott HL: Clinical pharmacokinetics of amlodipine. Clin Pharmacokinet. 1992 Jan;22(1):22-31.
4. Fares H, DiNicolantonio JJ, O'Keefe JH, Lavie CJ: Amlodipine in hypertension: a first-line agent with efficacy for improving blood pressure and patient outcomes. Open Heart. 2016 Sep 28;3(2)
5. Malesuik MD, Cardoso SG, Bajerski L, Lanzanova FA. Determination of amlodipine in pharmaceutical dosage forms by liquid chromatography and ultraviolet spectrophotometry. J AOAC Int. 2006;89:359–364. [PubMed] [Google Scholar]
6. Lima DM, Dos Santos LD, Lima EM. Stability and in vitro release profile of enalapril maleate from different commercially available tablets: possible therapeutic implications. J Pharm Biomed Anal. 2008;47:934–937. [PubMed] [Google Scholar]
7. Bhardwaj SP, Singh S. Study of forced degradation behavior of enalapril maleate by LC and LC-MS and development of a validated stability-indicating assay method. J Pharm Biomed Anal. 2008;46:113–120. [PubMed] [Google Scholar]
8. Naidu KR, Kale UN. Stability indicating UPLC method for simultaneus determination of amlodipine and benazepril hydrochloride from their combination drug product. J Pharm Biomed Anal. 2005;39:147–155. [PubMed] [Google Scholar]
9. Kurbanoglu S, Gumustas M, Uslu B, Ozkan SA. A sensitive and selective RP-LC method for the simultaneous determination of the antihypertensive drugs, enalapril, lercandipine, nitrendipine and their validation. Chromatographia. 2013;76:1477–1485. [Google Scholar]
10. Al-Mahmud A, Bhadra S, Haque A, Al-Mamun E, Haider SS. Development and validation of HPLC method for simultaneous determination of gliclazide and enalapril maleate in tablet dosage form. Dhaka Uni J Pharm Sci. 2014;13:51–56. [Google Scholar]
11. Wankhede SB, Wadkar SB, Raka KC, Chitlange SS. Simultaneous estimation of amlodipine besylate and olmesartan medoxomil in pharmaceutical dosage form. Ind J Pharm Sci. 2009;71:563–567. [PMC free article] [PubMed] [Google Scholar]
12. Sharma M, Kothari C, Sherikar O, Mehta PJ. Concurrent estimation of amlodipine besylate, hydrochlorothiazide and Enalapril maleate by UPLC, HPTLC and UV spectrophotometry. J Chromatogr Sci. 2013;52:27–35. [PubMed] [Google Scholar]
13. Elsebaei F, Zhu Y. Fast gradient high performance liquid chromatography method with UV detection for simultaneous determination of seven angiotensin converting enzyme inhibitors together with hydrochlorothiazide in pharmaceutical dosage forms and spiked human plasma and urine. Talanta. 2011;85:123–129. [PubMed] [Google Scholar]