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RESEARCH ARTICLE

STABILITY INDICATING ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF AMLODIPINE AND LOSARTAN POTASSIUM BY UPLC

Banothu Bhadru, Arukala Bhavani and Tadikonda Rama Rao
CMR College of Pharmacy, Kandlakoya, Medchal, Hyderabad, Telangana, India-501401.

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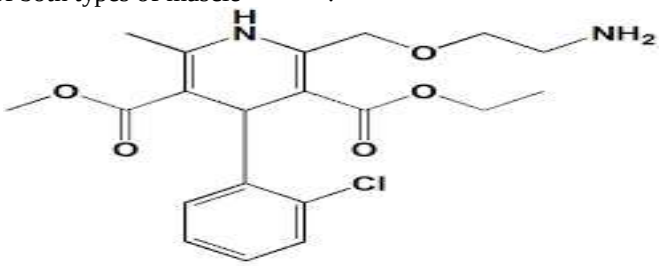
Abstract

A rapid and precise reverse phase high performance liquid chromatographic method has been developed for the validated of Amlodipine and Losartan Potassium, in its pure form as well as in tablet dosage form. Chromatography was carried out on Acquity BEH-shield RP18 UPLC column (3.0 mm × 100) mm, particle size Column using a mixture of Acetonitrile and Acetate buffer (pH-4.3) (35:65% v/v) as the mobile phase at a flow rate of 1.0ml/min, the detection was carried out at 238nm. The retention time of the Amlodipine and Losartan Potassium was found to be 2.179, 3.610 ±0.02min respectively. The method produce linear responses in the concentration range of 20-60µg/ml of Amlodipine and 10-30µg/ml of Losartan Potassium respectively. The method precision for the determination of assay was below 2.0% RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.

Introduction:-

Amlodipine is used alone or in combination with other medications to treat high blood pressure in adults and children 6 years and older [1,2]. It is also used to treat certain types of angina (chest pain) and coronary artery disease (narrowing of the blood vessels that supply blood to the heart) [4, 6]. It is slightly Soluble in water and sparingly soluble in ethanol, crystalline white powder [7, 8]. The chemical formula is $C_{20}H_{25}ClN_2O_5$, pKa value is 8.6 [10, 12].IUPAC name of Amlodipine besylate is 3-ethyl-5-methyl 2(2-aminoethoxy-methyl)-4-(2-chlorophenyl)-6-methyl-1, 4-dihydropyridine-3, 5-dicarboxylate [13, 14, 15]. Amlodipine acts by blocking the angiotensin-specific calcium channel, which prevents calcium ions from entering cardiac and vascular smooth muscle cells and preventing the contraction of both types of muscle [16, 17, 18].



Amplodipine

Fig. 1:- Structure of Amlodipine.

Corresponding Author:- Dr. Banothu Bhadru
Address:- Associate Professor Department of Pharmaceutical Analysis, CMR College of Pharmacy, Kandlakoya, Medchal, Hyderabad, Telangana, India-501401.

Losartan is a medicine called angiotensin receptor blocker[3]. It's widely used to treat high blood pressure (hypertension) and heart failure[4].The chemical formula is $C_{22}H_{22}ClKN6O$, pKa value is 7.8.[5].IUPAC name of Losartan Potassium is [2-Butyl-4-chloro-1-[2-(1Htetrazol-5-yl)-1, 1-biphenyl)-4yl] methyl] 1H-imidazole-5-methanol[9]. It may also be used as an alternative agent for the treatment of systolic dysfunction, myocardial infarction, coronary artery disease, and heart failure [11].It is used for the treatment of high blood pressure and angina. Freely soluble in water, soluble in alcohols and sparingly soluble in ethyl acetate, butyl acetate, and cyclohexane [13].

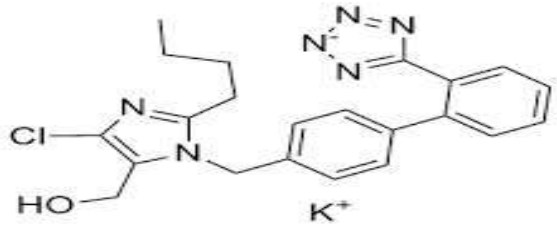


Fig. 2:- Structure of Losartan potassium.

Materials and Method:-

Instruments

The present work was carried out with UPLC Waters ACQUITY, Software: Empower 2, PDA detector. Software: Empower 2. The absorption spectra of reference and test solution were carried in a 1cm quartz cuvette over the range of 200-800nm in Elico SL 164 UV-Visible spectrophotometer, pH meter, Digital ultra Sonicator.

Chemicals

Amlodipine besylate and Losartan Potassium (API) Hetero, Methanol, Acetonitrile and Distill water, all chemicals are analytical grade only.

Method Development:-

Preparation of standard solution

Accurately weigh and transfer 10 mg of Amlodipine and Losartan potassium working standard into a separate 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol. Further pipette 0.4ml of Amlodipine and 0.2ml of Losartan potassium from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Mobile Phase Optimization

Initially the mobile phase tried was Methanol: Water and ACN: Water with varying proportions. Finally, the mobile phase was optimized to Acetonitrile and Acetate buffer (pH-4.3) in proportion 35:65% v/v respectively.

Diluent preparation:

The Mobile phase was used as the diluent.

Table 1:- Optimized Chromatographic Conditions.

UPLC	Waters ACQUITY, Software: Empower 2, PDA detector.	
Column	Acquity BEH-shield RP18 UPLC column (3.0 mm × 100) mm	
Mobile phase	Acetonitrile and Acetate buffer (pH-4.3) (35:65% v/v)	
Wavelength	238nm	
Flow rate	0.8ml/min	
Temperature	40°C	
Injection volume	20µl	
Retention time	Amlodipine	2.179 min
	Losartan Potassium	3.610 min
Run time	6minutes	

$$\%ASSAY = \frac{Sample\ \ area}{Standard\ \ area} \times \frac{Weight\ \ of\ standard}{Dilution\ \ of\ standard} \times \frac{Dilution\ \ of\ sample}{Weight\ \ of\ sample} \times \frac{Purity}{100} \times \frac{Weight\ \ of\ tablet}{Label\ \ claim} \times 100$$

The % purity of Amlodipine and Losartan Potassium in pharmaceutical dosage form was found to be 99.89 and 99.96% respectively.

Mehtod Validation:-

System Suitability

System suitability parameters are essential metrics used to assess the effectiveness of a chromatography system. These parameters encompass: peak retention time, peak area, peak height, peak width at half height, peak symmetry, peak tailing, capacity factor, plate numbers, resolution between peaks, and selectivity in relation to the preceding peak.

Specificity

Specificity in the validation of analytical methods refers to the capability of a method to accurately measure a target analyte while remaining unaffected by other substances present in the sample. These substances may consist of impurities, degradation products, and elements of the sample matrix. The analytical method was tested for specificity to accurately measure quantitated Amlodipine and Losartan Potassium in the formulation.

Accuracy

To determine the accuracy of the developed method, an Amlodipine and Losartan Potassium recovery studies was performed. The method's accuracy was assessed by using the usual addition method to calculate Amlodipine and Losartan Potassium recoveries. Pre-quantified sample solution (10µg/ml) was mixed with a known volume of Amlodipine and Losartan Potassium standard solutions (50%, 100%, and 150%).

Linearity

For Amlodipine and Losartan Potassium linearity studies calibration standards were prepared, and five duplicate assessments were conducted over a period to obtain the linearity range of 20.30, 40, 50, 60 and 10, 15, 20, 25 and 30µg/ml respectively. The calibration curve was plotted with area of peaks and concentration of the drug. The % RSD was calculated

Precision

For several samplings of a homogeneous sample, the precision of the analytical method was determined. Repeatability and intermediate precision measurements of peak area and peak symmetry parameters were used to demonstrate the reproducibility of the method. Single concentration levels were used to test the intermediate precision (for two days) and repeatability (within a day in triplicates).

Limit of Detection

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

$$LOD = 3.3 \times \sigma / s$$

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

Limit of Quantization

The quantization limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined.

$$LOQ = 10 \times \sigma / S$$

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

Robustness

The robustness was performed for the flow rate variations from 0.9 ml/min to 1.1ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Amlodipine and Losartan Potassium. The method is robust only in less flow condition and the method is robust even by change in the Mobile phase ±5%. The standard and samples of Amlodipine and Losartan Potassium were injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor and plate count.

The analysis was performed in different conditions to find the variability of test results. The following conditions are checked for variation of results.

Stability Studies

The method's specificity can be illustrated by subjecting the sample to various stress conditions, including acid, alkaline, peroxide, thermal, photolytic degradation. The primary peak of the drug was analyzed for peak purity, which confirmed that the method successfully distinguished the degradation products from the pure active ingredient.

Results and Discussion:-

System Suitability

System suitability parameters are essential metrics used to assess the effectiveness of a chromatography system.

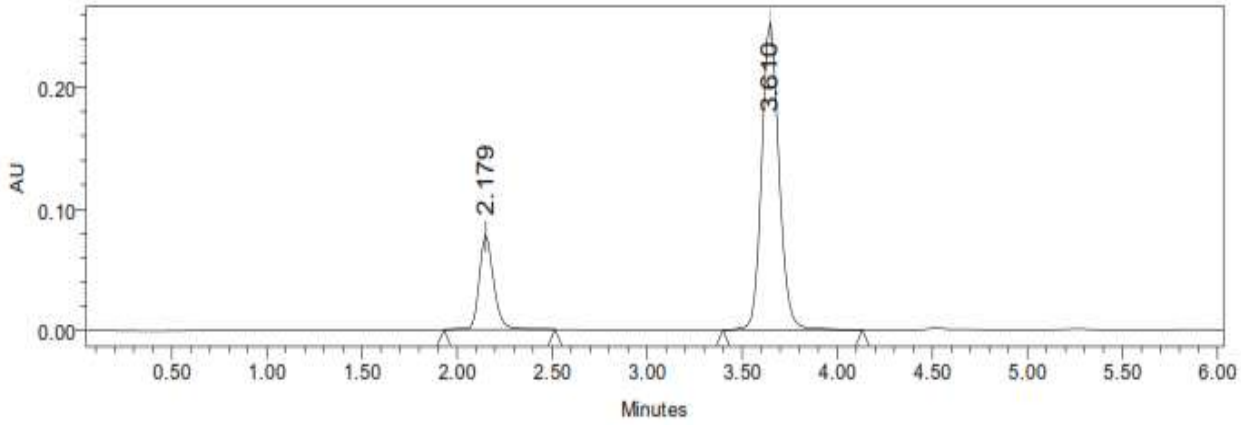


Fig. 3:- Optimized Chromatogram of Amlodipine and Losartan Potassium.

Table 2:- Optimized Chromatogric condition of Amlodipine and Losartan Potassium.

S.No	Name	Rt	Area	Height	USP Tailing	USP Plate Count	Resolution
1	**Amlodipine**	2.179	526389	86756	1.56	5679	
2	**Losartan Potassium**	3.610	1687285	367532	1.79	8685	9.8

Specificity

There was no other components were present at the elution time for Amlodipine and Losartan potassium. As seen in the figure.4, the blank chromatogram is present.

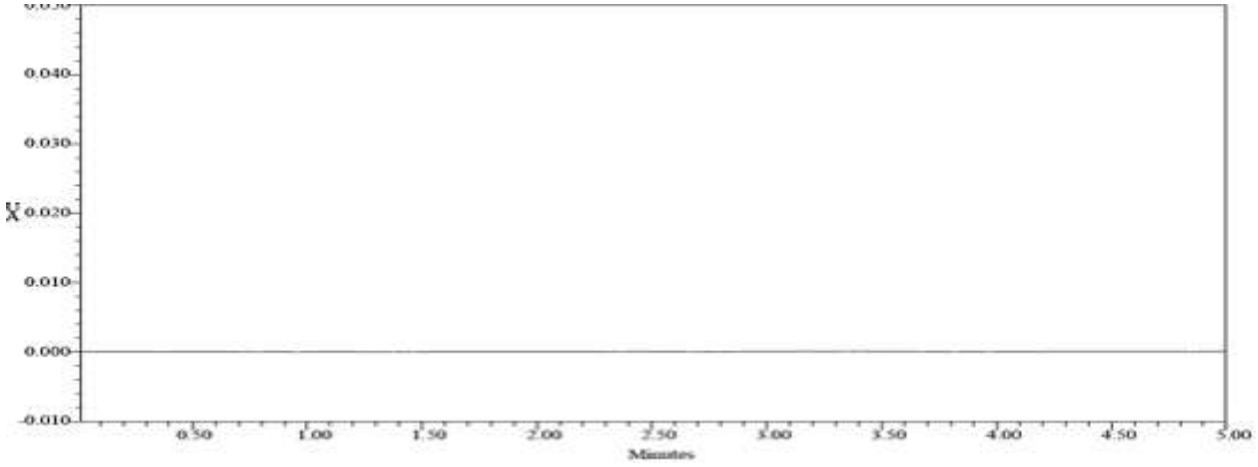


Fig. 4:- Chromatogram of blank.

Linearity

For Amlodipine and Losartan Potassium linearity studies calibration standards were prepared, and five duplicate assessments were conducted over a period to obtain the linearity range of 20.30, 40, 50, 60 and 10, 15, 20, 25 and 30µg/ml respectively. The calibration curve was plotted with area of peaks and concentration of the drug. The % RSD was calculated in table no.3. The linearity graph of Amlodipine and Losartan Potassium are shown in fig.5 and 6 respectively.

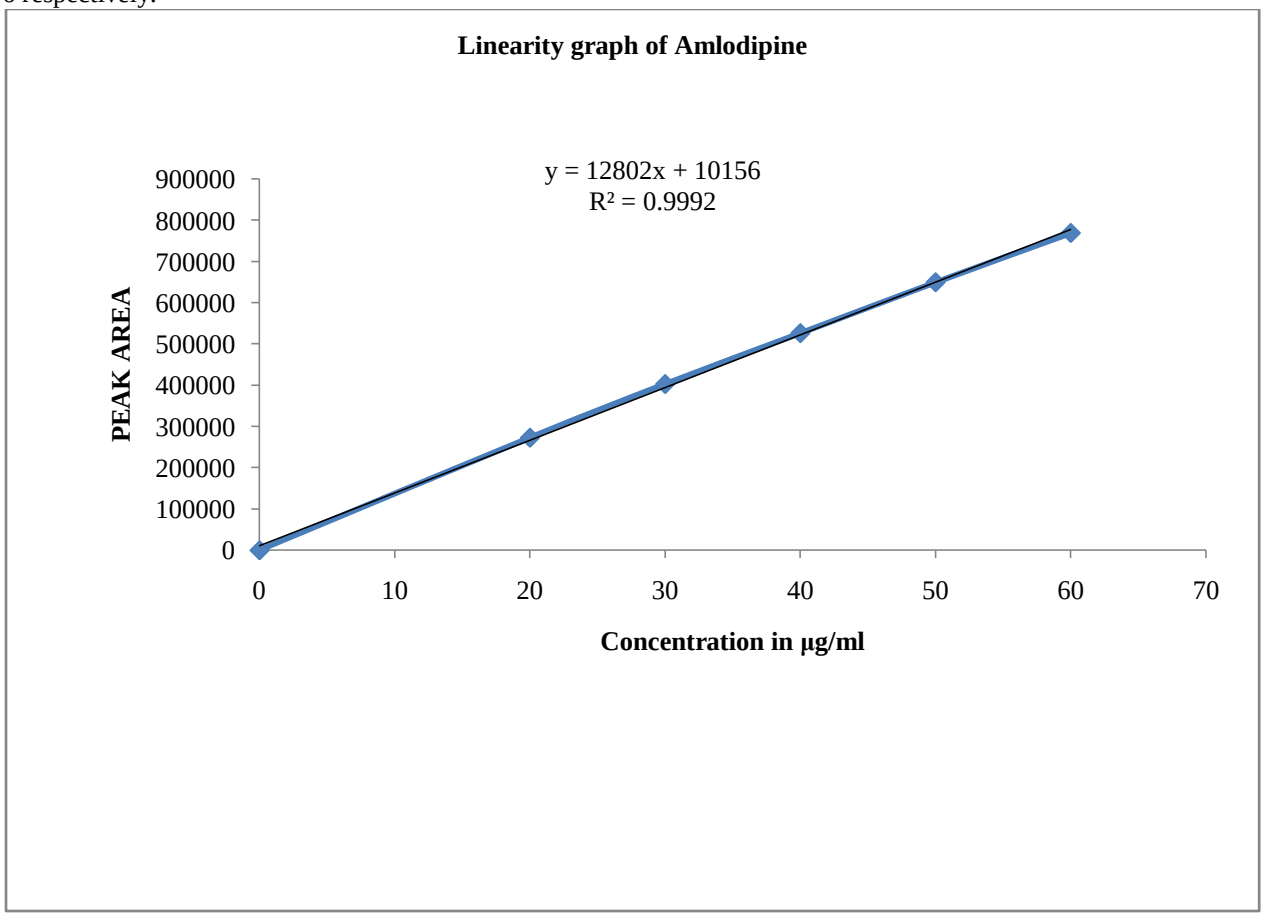


Fig. 5:- Linearity graph of Amlodipine.

Table 3:- Linearity of Amlodipine and Losartan Potassium.

S.No	Amlodipine		Losartan potassium	
	Conc. (µg/ml)	Peak Area	Conc. (µg/ml)	Peak Area
1	20	272897	10	1000237
2	30	402986	15	1448768
3	40	526389	20	1887285
4	50	649785	25	2365897
5	60	769287	30	2826845
Correlation coefficient (r^2)		0.9992		0.9995
Slope		12802		93626
Intercept (c)		10156		27739

Precision

Precision of the method was carried out for both sample and standard solutions as described under experimental work. The results are shown in table no.4

Table 4:- Precision of Amlodipine and Losartan Potassium.

S.No	Amlodipine		Losartan potassium	
	Retention time (min)	Peak area	Retention time (min)	Peak area

1	2.198	546585	3.623	1698587
2	2.196	548758	3.611	1698574
3	2.160	549854	3.696	1698532
4	2.160	548798	3.696	1698574
5	2.160	542659	3.696	1698532
6	2.186	548754	3.642	1698547
Mean		547568		1698558
Std.dev		2631.576		23.77113
%RSD		0.480593		0.001399

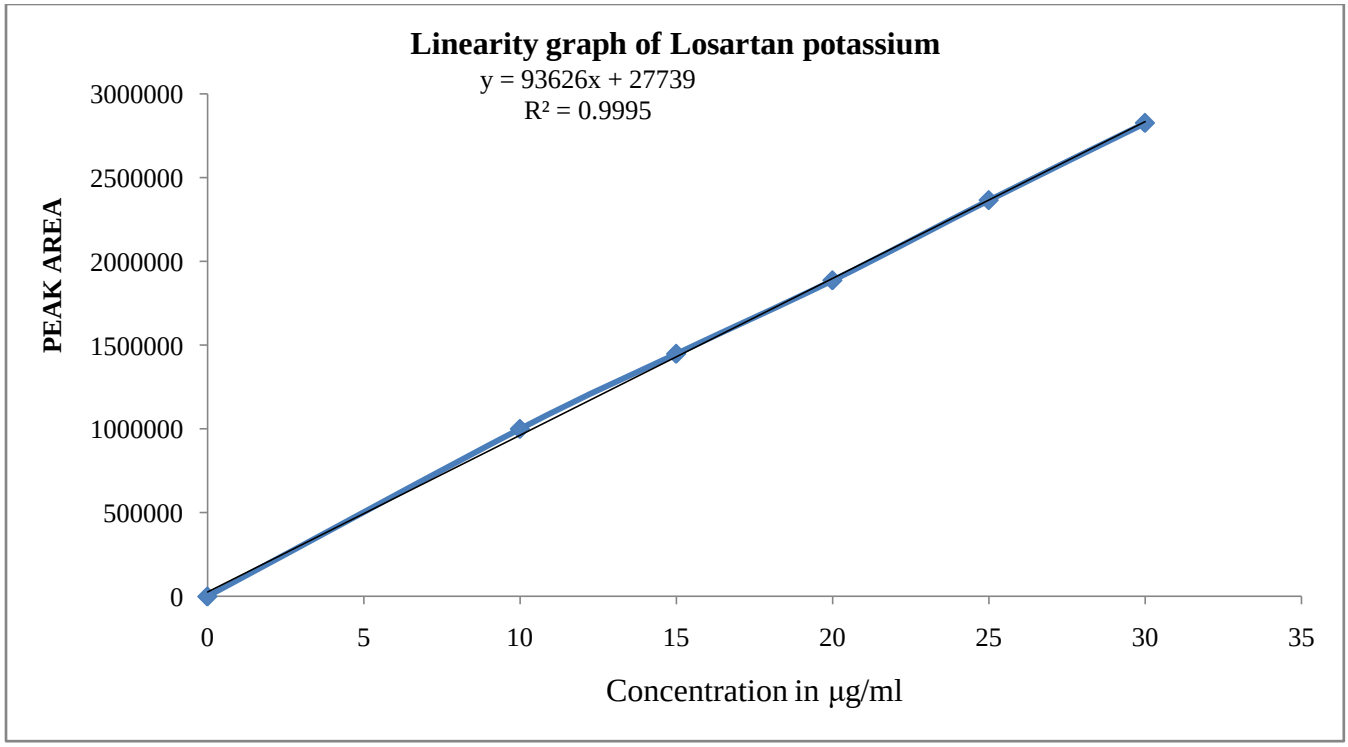


Fig. 6:- Linearity graph of Losartan Potassium.

Accuracy

Sample solutions at different concentrations (50%, 100%, and 150%) were prepared and their % recovery was calculated.

Table 5:- Accuracy results of Amlodipine and Losartan potassium.

Levels of percentage	**Amlodipine**			**Losartan potassium**		
	Amount added (µg/ml)	Amount found (µg/ml)	% Recovery	Amount added (µg/ml)	Amount found (µg/ml)	% Recovery
50	20	20.063	100.315%	10	10.094	100.94%
100	40	40.118	100.295%	20	19.998	99.99%
150	60	60.133	100.221%	30	30.156	100.52%

Limit of Detection and Limit of Quantification (LOD & LOQ)

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. The quantization limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined.

Table 6:- LOD & LOQ data for Amlodipine and Losartan potassium.

Drug	**LOD (µg/ml)**	**LOQ (µg/ml)**
Amlodipine	1.04µg/ml	2.1µg/ml
Losartan potassium	3.12µg/ml	6.3µg/ml

Robustness

The robustness was performed for the flow rate variations from 0.9 ml/min to 1.1ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Amlodipine and Losartan Potassium. The method is robust only in less flow condition and the method is robust even by change in the Mobile phase ±10%.

Table 7:- Robustness data for Amlodipine and Losartan potassium.

Parameter	**Amlodipine**		**Losartan potassium**	
	Retention time	Tailing factor	Retention time	Tailing factor
Actual Flow rate of 1.0 mL/min	2.133	1.56	3.692	1.79
Less Flow rate of 0.9 mL/min	2.210	1.54	4.498	1.68
More Flow rate of 1.1 mL/min	2.184	1.52	3.505	1.59
Less organic phase	2.200	1.57	4.504	1.62
More organic phase	2.172	1.51	3.512	1.63

Stability Studies

The method's specificity can be illustrated by subjecting the sample to various stress conditions, including acid, alkaline, peroxide, thermal, photolytic degradation. The primary peak of the drug was analyzed for peak purity, which confirmed that the method successfully distinguished the degradation products from the pure active ingredient. The amlodipine and Losartan potassium initially taken 30 and 70µg/ml respectively

Table 8:- Stability studies of Amlodipine and Losartan Potassium.

S. No	Type of Stability	Area of sample		Assay content (% w/w)	
		Amlodipine	**Losartan Potassium**	**Amlodipine**	**Losartan Potassium**
1	5N HCl	526358	1682821	92%	95%
2	5N NaOH	526548	1682726	90%	93%
3	30% H_20_2	526854	1687361	92%	91%
4	at 60^0c	526598	1682811	91%	90%
5	Photolytic	524874	1683816	90%	92%

Conclusion:-

The results presented in the tables for the UPLC method were promising. The UPLC method is more sensitive, accurate, and precise than the spectrophotometric methods. This method can be utilized for the routine determination of amlodipine and Losartan potassium in bulk drugs as well as in pharmaceutical dosage forms. The stability study accordingly confirmed the method's specificity. The peak purity study revealed that the peak threshold exceeded the angle, and neither analyte showed any flag. The degradation study showed that amlodipine and Losartan potassium were only degraded under acidic and thermal conditions.

References:-

1. Annapurna S. Gupta, Uday Deokate , Sanjay Vasave , Priya Mijgar, Stability indicating method development for drug stabilization: A review, World Journal of Pharmacy and Pharmaceutical Sciences,2023;12(6): 215-231

2. Ramya Gavini, S.B. Puranik, G.V.S. Kumar1, K.A. Sridha, Development and Validation Of Stability Indicating RP- HPLC method for simultaneous estimation of Amlodipine and Losartan in bulk drug and tablet dosage form, International Research Journal of Pharmacy.2012, 3 (11);92-95
3. Permender Rathee, Sushila Rathee , Hema Chaudhary, Dharmender Rathee , Development and validation of a stability indicating UV spectrophotometric method for the estimation of Losartan Potassium in bulk and tablet dosage form, Journal of Pharmacy Research,2008;1(2):188-192.
4. Anandkumar R. Tengli, G. Shivakumar, B. M. GurupadayyaUPLC-MS Method Development and validation of Amlodipine, Hydrochlorthiazide and Losartan in combined tablet dosage form, American Journal of Analytical Chemistry .2015;6(3):228-238
5. K. Kathiresan, S. Gothandaraman, M. Swamivel Manickam S. Mathan Kumar ,R. Manavalan, Analytical method development and validation of Losartan Potassium tablet by RP-HPLC, Rasayan Journal of Chemistry .2008;1(3):521-525.
6. Kanchan Shelar , Janhavi R. Rao, Chaitali Dhale, Stability Indicating HPTLC method development and validation for simultaneous estimation of Amlodipine Besylate and Losartan Potassium and characterisation of acid degradant product of Losartan. International Journal of Pharmaceutical Sciences and Research. 2019; 10(5): 2456-2464.
7. A.B.M. Helal Uddina, Mohamed Alaamaa, Zaidul I.S.M.A,S.A.Abbasb, Mohamed Awang.c, Fahim T.K. Acurrent analytical methods for Amlodipine and its formulations: A review, Journal Cleanwas.2017; 1(1):17-22.
8. Mr. Mahesh Shinde, Dr. Ashok Pingale, Mr. Ganesh Dhikale , Ms. Vidhyatai Jondhale, Mr.Amol Rathod,Ms.Shravasti Wathore,a Review: method development and validation of Amlodipine and Valsartan, Indo American Journal Of Pharmaceutical Sciences. 2022; 9(6):341-347.
9. M.M.Eswarudu, P.Sakheena,K.Lahari, P. Srinivasa Babu, M.Chinna Eswaraiah, Validated RP-HPLC method for simultaneous estimation of Atenolol, Hydrochlorothiazide and Losartan Potassium in bulk and pharmaceutical dosage form, Asian Journal Of Pharmaceutical Research And Development.2021; 9(1): 60-66.
10. Shubhangi Ramesh Darade, Dr.Amit Kasabe, Dr. Ashok Bhosale,analytical method development and validation for Amlodipine estimation in bulk and pharmaceutical formulation using the Qbd approach, International Journal of Creative Research Thoughts. 2021;9(10): a526-a544.
11. M Sumithra, P Shanmugasundaram, ASK Sankar, Niharika, Method Development and Validation of Losartan Potassium by RP-HPLC,Research Journal of Pharmaceutical, Biological and Chemical Sciences .2012;3(1):463-479.
12. Sumera Kousar, Farhana begum,Stability indicating RP-HPLC method for the determination of amlodipine besylate,International journal of pharmacy and analytical Research.2016;5(1):56-77.
13. Jeeva A S, Meena Chandran, and K Krishna kumar, A Review of analytical methods for estimation of Amlodipin, Hydrochlorthiazide and Losartan Potassium in pharmaceutical formulations.International Research Journal of Pharmacy and Medical Sciences, 2018;1(4):75-77.
14. A.B. M. Helal Uddina, Mohamed Alaamaa,Zaidul I.S.M.a,S. A. Abbasb, Mohamed Awang.c, Fahim T.K. a,current analytical methods for amlodipine and its formulations: a review,Journal Cleanwas .2017;1(1):17-22.
15. Prakash Belure, Paul Richards, Ram Autar Bishwakarma And B.A. Vishwanath, Stability indicating method development and validation for the determination of Olmesartan and Amlodipine by RP-HPLC,World Journal of Pharmacy and Pharmaceutical Sciences. 2022; 11(11):1216-1229.
16. Narvekar V.T.,Kapase A.R., Chavan A.B., Derkar G.K., Bhat M.R.,Chimkode R.M. ,Method development, validation and stability study of amlodipine in marketed formulation by UV Spectrophotometric method.International Journal Of Pharmaceutical Research And Bio-Science.2017;6(5):1-25
17. Rubeen Dadakalandar Nadaf, Sudhahar Dharmalingam, Shweta Harshal Zalte, Shyma Sundar Sahu, Swarnima Pandey, Deepak Sharma, Shanmugam Vippamakula, Awinish Kumar Shrivastaw, Raveesha Peeriga, Ramanlal N. Kachave, Stability indicating assay method development and validation of Amlodipine Besylate in bulk and tablet dosage form by UV Spectroscopy and RP-HPLC, Journal of chemical health risks. 2023; 13(3):1397-1404.
18. Rameshwar Bhausaheb Gholve , Sanjay Sudhakar Pekamwar , Tukaram Mohanrao Kalyankar, Stability-indicating RP-HPLC method development and validation for simultaneous estimation of bisoprolol fumarate and amlodipine besylate in bulk and in tablet dosage form, Journal of Applied Pharmaceutical Science.2021;11(12):121-134.