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Research

Stability indicating analytical method development and validation for amlodipine & losartan potassium by uplc method

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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.

Keywords: Amlodipine and Losartan Potassium, UPLC, Validation.

INTRODUCTION

Science & Art of determining the composition of materials in terms of molecules/compounds contained within, is known as "Analytical Chemistry" encompassing Qualitative and Quantitative information's. Qualitative information deals with the identity of atoms, molecular species or functional groups in the sample, whereas the Quantitative information provides numerical values on relative amount of components.

Analytical techniques, either Chemical or Instrumental, are used in routine analyses of drugs and drug related substances. Chemical techniques, such as Gravimetry and Titrimetry, are basic ones used but are less precise and time consuming, thus are not recommended for routine analysis nowadays. Instrumental techniques, which includes Electrochemical methods such as conductometry, polarography, potentiometry, electrogravimetry etc., Absorption/Emission methods such as Ultraviolet spectrophotometry, Visible spectrophotometry, Infrared spectrophotometry, Fluorimetry, Atomic Absorption spectrophotometry, Atomic Emission spectrophotometry,

Flame Photometry etc., and Adsorption/Partition methods of separation such as Chromatography[1] viz. TLC, HPTLC, HPLC, GC etc. Chromatography, an effective technique of separation finding applications in most fields of science. The term "Chromatography" was coined after the turn of the last century by the Russian botanist Mikhali Tswett. Tswett used this technique to isolate different plant pigments, such as chlorophylls and xanthophylls by moving solutions of these compounds through a glass column filled with finely divided calcium carbonate[2]. The separated species appeared on the column as coloured bands, accounting for the Greek "Chroma" meaning "colour" and "Graphein" meaning "writing.

In the last few decades, applications of chromatographic technique has developed explosively not only due to emergence of new forms of this technique but also due to the increasing need for better methods for characterization of complex mixtures. Chromatography involves diverse and significant techniques allowing the separation, identification and determination of components of complex mixtures that are closely related; many of these separations are impossible by other separations.

Samples intended to be separated is dissolved in mobile phase, can be a gas, liquid or a supercritical fluid, and forced into a immiscible stationary phase fixed in a column or on a solid surface.[3] The two phases are chosen such that the sample components disperse themselves to different degrees between each mobile and stationary phase. These strongly held components of the stationary phase only shift slowly with the flow of the mobile phase. Components that are weakly held by the stationary phase, on the other hand, move quickly. As a result of these variations in migration rates, the components of the sample are separated into different bands or zones that can be quantitatively and qualitatively analyzed further.

Chromatographic technique, non-destructive method for separating mixture of components into individual components, most frequently used until 2004 was HPLC. But due to certain limitation of HPLC, a new technique popularly known as "Ultra Performance Liquid Chromatography" [4] was introduced with high efficiency and also to address the limitation of HPLC technique.

Ultra Performance Liquid Chromatography (UPLC)

UPLC refers to chromatographic method with improvised performance namely in three areas, Chromatographic resolution, Speed of analysis and Sensitivity of analysis. The instrumentation design supports the operations at much higher pressure than that used in HPLC, uses finely sized particles of less than 2.5µm 3 decreases column length and the decreased solvent consumption & save time at high linear velocities. As the particle size decreases to less than 2.5 µm, there is a major efficiency benefit, according to the van Deemter equation, whereas the efficiency does not decrease at increased flow rates or linear velocities[5]. Therefore, by using smaller particles, velocity and peak capacity (number of peaks resolved in gradient separations per unit time) can be extended to new limits, called Ultra Performance Liquid chromatography.

Pharmaceutical industries and analytical laboratories are now searching for innovative ways to reduce the expenses, time of drug research and improve the quality of their product. With improved resolution, assay sensitivity and high sample throughput, UPLC makes it possible to conduct a greater number of tests in a shorter period of time and also offers a cost-effective advantage over HPLC analysis. So the traditional assay for the UPLC method was transferred and optimized.

MATERIALS AND METHODS

Amlodipine (Pure)-Local Market, Losartan Potassium (Pure)-Local Market, Water and Methanol for UPLC-LICHROSOLV (MERCK), Acetonitrile for UPLC- Merck, Acetic Acid-Merck.

UPLC method development

Preparation of standard solution: Accurately weigh and transfer 10 mg of Amlodipine and Losartan Potassium working standard into a 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.

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.

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.

Optimization of Column: The method was performed with various C18 columns like Kromacil column C18 (50×2.1mm, 3.5µ), Acquity UPLC CHS C18 (50 × 2.1 mm, 1.7 m), Zorbax Eclipse Plus C18 (2.1×50mm 1.8µm),

BEH C18 (2.1 × 50 mm, 1.7 µm), and Acquity BEH-shield RP18 UPLC column (3.0 mm × 100) mm. Waters ACQUITY, Software: Empower 2, PDA detector Column was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

Optimized chromatographic conditions

Instrument used	:	Waters ACQUITY, Software: Empower 2, PDA detector.
Temperature	:	40 °C
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)
Flow rate	:	1ml/min
Wavelength	:	238nm
Injection volume	:	20µl
Run time	:	6minutes

Method validation

Preparation of mobile phase

Preparation of mobile phase: Accurately measured 350ml of Acetonitrile (35%) of and 650ml of Acetate buffer (65%) were mixed and degassed in digital ultrasonicater for 20 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation: The Mobile phase was used as the diluent.

RESULTS AND DISCUSSION

Optimized Chromatogram (Standard)

Mobile phase ratio	: Acetonitrile and Acetate buffer (pH-4.3) (35:65% v/v)
Column	: Acquity BEH-shield RP18 UPLC column (3.0 mm × 100) mm,
Column temperature	: 40°C
Wavelength	: 238nm
Flow rate	: 1ml/min
Injection volume	: 20µl
Run time	: 6minutes

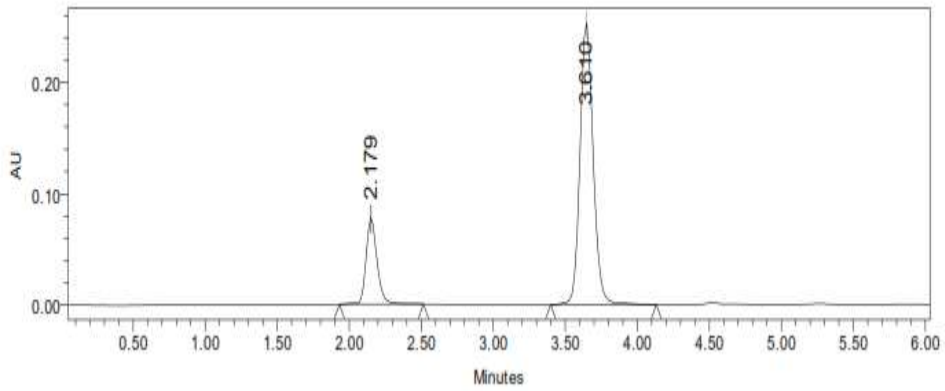

Fig 1: Optimized Chromatogram (Standard)

Table 1: Optimized Chromatogram (Standard)

S.No	Name	RT	Area	Height	USPTailing	USPPlate Count	Resolution
1	Amlodipine	2.179	526389	86756	1.56	5679	
2	Losartan Potassium	3.610	1687285	367532	1.79	8685	9.8

From the above chromatogram it was observed that the Amlodipine and Losartan Potassium peaks are well separated and they shows proper retention time, resolution, peak tail and plate count. So it's optimized trial.

Optimized Chromatogram (Sample)

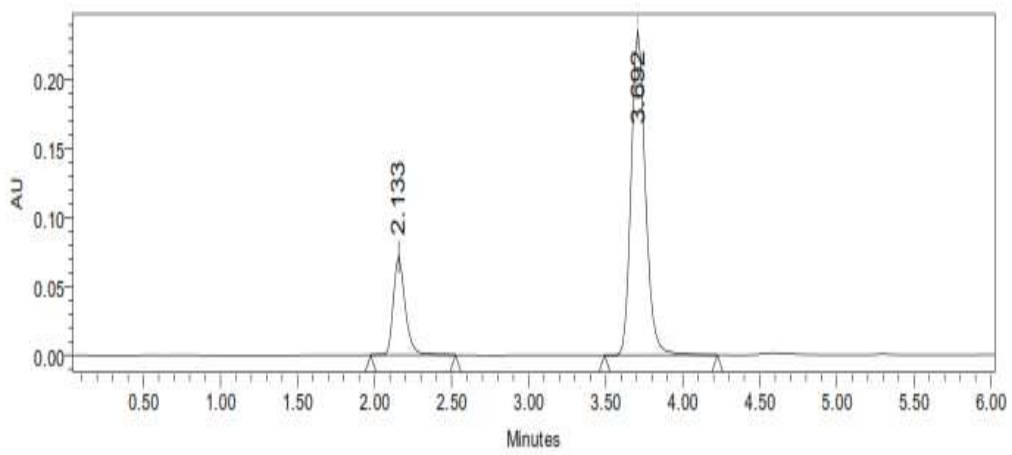

Fig 2: Optimized Chromatogram (Sample)

Table 2: Optimized Chromatogram (Sample)

S.No	Name	RT	Area	Height	USPTailing	USPPlate Count	Resolution
1	Amlodipine	2.179	534514	87568	1.61	5786	
2	Losartan Potassium	3.610	1796854	375424	1.82	8769	10.01

- *Resolution between two drugs must be not less than 2.*
- *Theoretical plates must be not less than 2000.*
- *Tailing factor must be not less than 0.9 and not more than 2.*
- *It was found from above data that all the system suitability parameters for developed method were within the limit.*

System suitability

Table 3: Results of system suitability for Amlodipine

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Amlodipine	2.152	526358	86598	5695	1.56
2	Amlodipine	2.157	526548	86254	5652	1.57
3	Amlodipine	2.141	526854	86598	5627	1.56
4	Amlodipine	2.133	526598	86245	5692	1.57
5	Amlodipine	2.166	524874	86521	5641	1.56
Mean			526246.4			
Std.Dev.			787.353			
%RSD			0.149617			

- *%RSD of five different sample solutions should not more than 2.*
- *The %RSD obtained is within the limit, hence the method is suitable.*

Table 4: Results of system suitability for Losartan Potassium

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing	Resolution
1	Losartan Potassium	3.674	1682821	1686958	8659	1.56	9.8
2	Losartan Potassium	3.631	1682726	1685745	8675	1.57	9.9
3	Losartan Potassium	3.625	1687361	1685421	8692	1.56	9.8

4	Losartan Potassium	3.692	1682811	1685242	8642	1.57	9.8
5	Losartan Potassium	3.629	1683816	1685364	8635	1.58	9.8
Mean			1683907				
Std.Dev.			1982.03				
%RSD			0.117704				

- *%RSD of five different sample solutions should not more than 2.*
- *The %RSD obtained is within the limit, hence the method is suitable.*

Assay (Standard)

Table 5: Peak results for assay standard of Amlodipine

S.No	Name	RT	Area	Height	USPTailing	USP Plate Count	Injection
1	Amlodipine	2.152	526358	86598	1.56	5698	1
2	Amlodipine	2.198	526584	86784	1.57	5687	2
3	Amlodipine	2.179	529658	86253	1.56	5639	3

Table 6: Peak results for assay standard of Losartan Potassium

S.No.	Name	RT	Area	Height	USPTailing	USP Plate Count	Injection
1	Losartan Potassium	3.646	1687589	365879	1.80	8659	1
2	Losartan Potassium	3.604	1685987	365854	1.79	8697	2
3	Losartan Potassium	3.610	1685974	369854	1.80	8675	3

Assay (Sample)

Table 7: Peak results for Assay sample of Amlodipine

S.No	Name	RT	Area	Height	USPTailing	USP Plate Count	Injection
1	Amlodipine	2.152	536859	87584	1.58	5789	1
2	Amlodipine	2.150	532654	87965	1.59	5784	2
3	Amlodipine	2.187	532685	87465	1.58	5769	3

Table 8: Peak results for Assay sample of Losartan Potassium

S.No	Name	RT	Area	Height	USPTailing	USPPlateCount	Injection
1	Losartan Potassium	3.646	1698568	378562	1.81	8759	1
2	Losartan Potassium	3.651	1698574	375847	1.80	8795	2
3	Losartan Potassium	3.601	1698547	376584	1.81	8745	3

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

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

Linearity

Chromatographic data for linearity study of amlodipine

Table 9: Chromatographic Data for Linearity Study of Amlodipine

Concentration µg/ml	Average Peak Area
20	272897

30	402986
40	526389
50	649785
60	769287

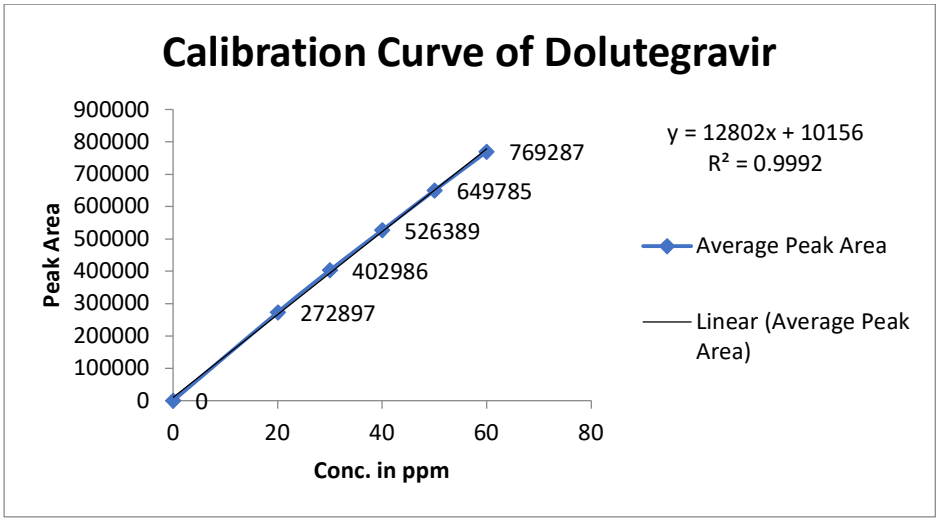

Fig 3: Calibration Curve of Amlodipine

Chromatographic data for linearity study of losartan potassium

Table 10: Chromatographic Data for Linearity Study of Losartan Potassium

Concentration µg/ml	Average Peak Area
10	1000237
15	1448768
20	1887285
25	2365897
30	2826845

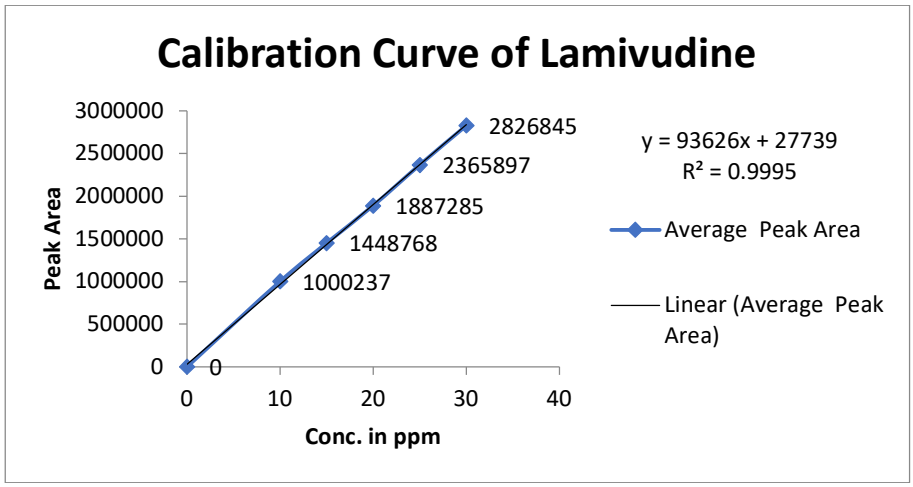

Fig 4: Calibration Curve of Losartan Potassium

Precision
Repeatability

Table 11: Results of repeatability for Amlodipine

S. No.	Peak Name	Retention time	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Amlodipine	2.157	526358	86598	5689	1.56
2	Amlodipine	2.159	524856	86542	5687	1.57
3	Amlodipine	2.186	526985	86578	5684	1.56
4	Amlodipine	2.160	528654	86354	5689	1.56
5	Amlodipine	2.170	528457	86958	5639	1.56
Mean			527062			
Std.dev			1569.114			
%RSD			0.297709			

- *%RSD for sample should be NMT 2*
- *The %RSD for the standard solution is below 1, which is within the limits hence method is precise.*

Table 12: Results of Repeatability for Losartan Potassium:

S. No.	Peak Name	Retention time	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Losartan Potassium	3.603	1687589	367859	8659	1.79
2	Losartan Potassium	3.608	1685987	368547	8679	1.80
3	Losartan Potassium	3.600	1685987	367985	8645	1.80
4	Losartan Potassium	3.696	1685754	365874	8695	1.79
5	Losartan Potassium	3.629	1685985	364589	8625	1.79
Mean			1686260			
Std.Dev			749.493			
%RSD			0.044447			

Intermediate precision
Day 1

Table 13: Results of Intermediate precision for Amlodipine

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing	%Assay
1	Amlodipine	2.198	546585	87589	5898	1.58	100%
2	Amlodipine	2.196	548758	87985	5879	1.59	100%
3	Amlodipine	2.160	549854	87452	5868	1.58	100%
4	Amlodipine	2.160	548798	87421	5847	1.59	100%
5	Amlodipine	2.160	542659	87963	5896	1.58	100%
6	Amlodipine	2.186	548754	87254	5874	1.59	100%
Mean			547568				
Std.Dev.			2631.576				
%RSD			0.480593				

- *%RSD of five different sample solutions should not more than 2*

Table 14: Results of Intermediate precision for Losartan Potassium

S.No.	Peak Name	Rt	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing	Resolution
1	Losartan Potassium	3.623	1698587	385482	8789	1.81	9.8
2	Losartan Potassium	3.611	1698574	385698	8759	1.80	9.8
3	Losartan Potassium	3.696	1698532	385748	8754	1.81	9.9
4	Losartan Potassium	3.696	1698574	386958	8754	1.81	10.01
5	Losartan Potassium	3.696	1698532	385755	5798	1.80	9.98

6	Losartan Potassium	3.642	1698547	386558	8762	1.80	10.02
Mean			1698558				
Std.Dev.			23.77113				
%RSD			0.001399				

- *%RSD of five different sample solutions should not more than 2.*

Table 15: Results of Intermediate precision Day 2 for Amlodipine

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Amlodipine	2.198	536854	8758	5789	1.58
2	Amlodipine	2.196	536985	8795	5726	1.59
3	Amlodipine	2.178	536587	8746	5742	1.58
4	Amlodipine	2.142	532546	8754	5746	1.59
5	Amlodipine	2.177	534587	8725	5798	1.58
6	Amlodipine	2.177	538598	8726	5785	1.59
Mean			536026.2			
Std.Dev.			2131.492			
%RSD			0.397647			

- *%RSD of five different sample solutions should not more than 2.*

Table 16: Results of Intermediate precision Day 2 for Losartan Potassium

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing	Resolution
1	Losartan Potassium	3.611	1678598	356875	8875	1.82	9.9
2	Losartan Potassium	3.623	1678985	358985	8856	1.83	10.01
3	Losartan Potassium	3.684	1678984	358754	8862	1.82	9.9
4	Losartan Potassium	3.697	1678985	352412	8849	1.83	10.01
5	Losartan Potassium	3.684	1678549	358987	8873	1.82	9.9
6	Losartan Potassium	3.684	1678984	358986	8842	1.83	10.01
Mean			1678848				
Std.Dev.			212.8048				
%RSD			0.012676				

- *%RSD of five different sample solutions should not more than 2.*

Accuracy

Table 17: The accuracy results for Amlodipine

% Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	267011.3	20	20.063	100.315%	100.28%
100%	523752.3	40	40.118	100.295%	
150%	778457.3	60	60.133	100.221%	

- *The percentage recovery was found to be within the limit (98-102%).*

Table 18: The accuracy results for Losartan Potassium

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	972876.3	10	10.094	100.94%	100.48%
100%	1900122	20	19.998	99.99%	
150%	2851152	30	30.156	100.52%	

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

Robustness

Table 19: Results for Robustness Amlodipine

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	526389	2.133	5679	1.56
Less Flow rate of 0.9 mL/min	542685	2.210	5264	1.54
More Flow rate of 1.1 mL/min	526483	2.184	5426	1.52
Less organic phase	516854	2.200	5163	1.57
More Organic phase	506898	2.172	5098	1.51

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

Table 20: Results for Robustness Losartan Potassium

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	1687285	3.692	8685	1.79
Less Flow rate of 0.9 mL/min	1725468	4.498	8265	1.68
More Flow rate of 1.1 mL/min	1652847	3.505	8415	1.59
Less organic phase	1687485	4.504	8326	1.62
More organic phase	1674524	3.512	8415	1.63

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

Stability studies

The specificity of the method can be demonstrated by applying stress conditions using acid, alkaline, peroxide, thermal, UV, water degradations. The sample was exposed to these conditions the main peak of the drug was studied for peak purity that indicating the method effectively separated the degradation products from the pure active ingredient.

Table 21: Results of Forced Degradation Studies

S.No.	Stress Condition	Peak Area	% of Degraded Amount	% of Active Amount	Total % of Amount
1	Standard	526389	0	100%	100%
2	Acidic	371683.27	29.39	70.61	100%
3	Basic	411794.11	21.77	78.23	100%
4	Oxidative	480645.79	8.69	91.31	100%
5	Thermal	327045.48	37.87	62.13	100%
6	Photolytic	477118.99	9.36	90.64	100%

Table 22: Results of Forced Degradation Studies

S.No.	Stress Condition	Peak Area	% of Degraded Amount	% of Active Amount	Total % of Amount
1	Standard	1687285	0	100%	100%
2	Acidic	1359614.25	19.42	80.58	100%
3	Basic	1445497.05	14.33	85.67	100%
4	Oxidative	1644427.96	2.54	97.46	100%
5	Thermal	1297353.43	23.11	76.89	100%
6	Photolytic	1632954.42	3.22	96.78	100%

CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate UPLC method was developed for the quantitative estimation of Amlodipine and Losartan Potassium in bulk drug and pharmaceutical dosage forms. This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps. Amlodipine was found to be freely soluble in methanol, Acetonitrile, slightly soluble in water. Losartan Potassium is soluble in organic solvents such as ethanol, DMSO, and dimethyl formamide (DMF), which should be purged with an inert gas. The solubility of Losartan Potassium in ethanol is

approximately 0.5 mg/ml and approximately 20 mg/ml in DMSO and DMF. Acetonitrile: Acetate Buffer (pH-4.3) (35:65 v/v)was chosen as the mobile phase. The solvent system used in this method was economical. The %RSD values were within 2 and the method was found to be precise. The results expressed in Tables for UPLC method was promising. The UPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods. This method can be used for the routine determination of Amlodipine and Losartan Potassium in bulk drug and in Pharmaceutical dosage forms. Stability study correspondingly confirmed the specificity of the method. As a part of peak purity study, peak threshold was found to be higher than angle and no flag for both the analytes was observed. Degradation study revealed that Amlodipine and Losartan Potassium were degraded in acidic and thermal condition only. The results displayed in Table 44 and 45 and the chromatograms are given in figures 65 and 68.

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