Int. J. Pharm. Investigation, 2024; 14(1):151-159.
https://www.jpionline.org

Original Article

Simultaneous Estimation of Amlodipine and Enalapril Maleate by Stability Indicating UHPLC Method

Neha Gaikwad[1,*], Charushila Bhangale[2], Sangita Bhandare[3]

[1]Department of Pharmaceutical Quality Assurance, PRES's College of Pharmacy (For Women), Chincholi, Nashik, Maharashtra, INDIA.
[2]Department of Pharmaceutical Chemistry, PRES's College of Pharmacy (For Women), Chincholi, Nashik, Maharashtra, INDIA.
[3]Department of Pharmaceutical Pharmacology, PRES's College of Pharmacy (For Women), Chincholi, Nashik, Maharashtra, INDIA.

ABSTRACT

Objectives: The key objective of this work is to establish a stability-indicating UHPLC technique for measuring amlodipine and enalapril maleate in bulk and pharmaceutical formulations **Materials and Methods:** The mobile phase consisted of 0.02M KH_2PO_4 buffer: Methanol (70:30 v/v). The chromatographic separation was carried out using an Analytical Technologies UHPLC-3000 equipment in conjunction with a variable wavelength programmable UV identifier and a Rheodyne injector equipped with a 5 µL fixed circle. Detection was done at 224 nm with the flow rate of 0.3 mL/min. **Results:** Amlodipine's retention time was determined to be 0.7 min, while Enalapril's was 1.2 min. The linearity range for Amlodipine and Enalapril was discovered to be 5–25 µg/mL. The technique has been examined in accordance with ICH (International Conference on Harmonisation) standards. A procedure is precise and accurate if the %RSD is less than two. Amlodipine's limit of detection and quantitation ranges are 0.12–0.37 µg/mL and Enalapril's range is 0.10–0.30 µg/mL, respectively. The procedure proved to be simple, linear, swift, exact, accurate, repeatable, and reliable. The drug was prone to acid, basic, and oxidation conditions, according to the stress degradation research. **Conclusion:** Even in actual samples, the techniques clearly distinguished between the drugs and degradation products. The method was proved as a stability indicating method and can be used in practice for bulk and the dosage forms and also to evaluate the shelf life.

Keywords: Amlodipine, Enalapril, UPLC method, Method validation, Stabilty study.

Correspondence:
Ms. Neha Gaikwad
Research Scholar, PRES'S College of Pharmacy (For Women), Chincholi, Nashik-422102, Maharashtra, INDIA.
Email: charushila.bhangale@pravara.in

Received: 07-07-2023;
Revised: 28-08-2023;
Accepted: 07-10-2023.

INTRODUCTION

Amlodipine (ADP) is a medication often used to treat high blood pressure and angina. Amlodipine has antioxidant properties as well as the ability to stimulate the generation of Nitric Oxide (NO), a vasodilator that reduces blood pressure.[1] It is an antihypertensive medicine that belongs to the class of pharmaceuticals known as dihydropyridine calcium channel blockers. ADP is 3-ethyl 5-methyl 2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate; benzene sulfonic acid.[2-4] The chemical structure are shown in Figure 1.

Enalapril (ELP) is a prodrug of the Angiotensin-Converting Enzyme (ACE) inhibitor family of drugs. It operates on the renin-angiotensin-aldosterone pathway, which regulates blood pressure as well as fluid and electrolyte balance. It is a non-sulphydryl anti-hypertensive medication that is used orally. It was developed using molecular modelling to focus targeted research. Enalapril lowers blood pressure by relaxing and expanding blood arteries, making it simpler for the heart to flow blood throughout the body. Enalapril maleate has the chemical formula (2S).-1-[(2S)-2-[(1S)-1-(Ethoxycarbonyl)-3 -phenylpropyl] amino propanoyl Z-pyrrolidine-2-carboxylic acid-butenedioate.[5] The chemical structure of ELP is shown in Figure 2.

Hypertension is a medical condition in which the blood exerts a high pressure on the blood vessel walls. Because of this condition, the heart has to work harder to pump blood throughout the body. Stroke, heart failure, heart attack, and kidney failure are all major health concerns that can be caused by hypertension. Amlodipine and enalapril are used together to treat hypertension and successfully lower blood pressure. It works by relaxing blood arteries, allowing blood to flow more freely and allowing the heart to pump blood more efficiently. A review of the literature reveals that several UV,[6-13] HPTLC,[14-16] HPLC,[13,16,17-27] and UPLC[28] techniques have been described for the ADP and ELP alone or in combination with other medicines. To the best of our knowledge, no UHPLC method for determining stability has been published for ADP and ELP combination dose form. The study's purpose is

DOI: 10.5530/ijpi.14.1.19

to establish a simple, robust, and precise technique for quantifying ADP and ELP in bulk and dosage form at the same time. The method has been verified according to ICH guidelines.[29,30] The formulation sample recoveries were in good agreement with their label claims, and no formulation excipients interfered with the estimate. As a result, this approach is straightforward and practical for regular examination of bulk medicines in pure form and combinations.

MATERIALS AND METHODS

Instruments

Analytical Technologies UHPLC-3000 equipment, a variable wavelength programmable UV identifier, and a Rheodyne injector with a 5l fixed circle were used for the chromatographic operation. The opposing stage was a Cosmosil C_{18} (100mm x 2.1ID, particle size: 1.7 micron). For spectrophotometer judgements and gauging, the UV-3000-M spectrophotometer and the Wenser High Precision Balance Model: PGB 100 electronic equilibrium were employed separately.

Chemicals and reagents

Amlodipine and Enalapril was procured from Pharma Tech Solutions. The fixed-dose combination of tablet containing Amlodipine and Enalapil (AMTAS-E 5+5 mg) is FDA-approved for the treatment of hypertension purchased from local market; Merck Specialities Private Limited, Mumbai, provided UHPLC grade Methanol and water.

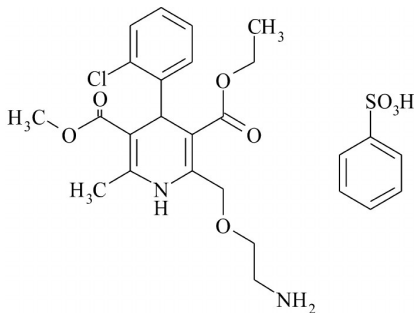

Figure 1: Structure of Amlodipine besylate.

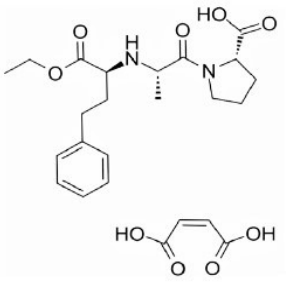

Figure 2: Structure of Enalapril Maleate.

Chromatographic conditions

Cosmosil C_{18} (100mm x 2.1ID, particle size: 1.7 micron) was used for the chromatographic technique at 224 nm. The mobile phase for elution was 0.02M KH_2PO_4 buffer: methanol, and the standard and sample solutions were prepared in the same solvent. By infusing 5µL and increasing the flow rate to 0.3 mL/min, the elution was examined.

Preparation of (0.02M KH_2PO_4 buffer)

Dissolve 0.5 g of Sodium Chloride and 0.5 g of 1-Hexane Sulphonic Acid Sodium salt in 800 mL water, sonicated to dissolve fully, then diluted to volume 1000 mL with water. The pH was adjusted to 3.2 using dilute Orthophosphoric acid, then filtered and degassed via a 0.45 m filter.

UHPLC Method Development

Preparation of Standard Stock solutions

Accurately Weighed and placed 25 mg ADP and 25 mg ELP working standards into a 100 mL clean dry volumetric flask, sonicated for 5 min, and made up to final volume using diluents. The ultimate concentration of amlodipine is 250 µg/mL, while the final concentration of enalapril is 250 µg/mL. The working standard solutions for these drugs were created by diluting the corresponding stock solution with mobile phase.

Validation of RP-UHPLC method[29,30]

The optimised RP-UHPLC technique was verified in accordance with ICH Q^2 (R) standards.

Linearity

Different concentrations of test solutions were injected individually, and chromatograms were obtained. A series test preparation of Amlodipine and Enalapril were made by placing 1 mL - 5 mL of the stock solution containing ADP (250 µg/mL) and ELP (250 µg/mL) in five 10 mL volumetric flasks and filling to the mark with mobile phase. Under the optimised chromatographic conditions, a 5 µL volume of each concentration was injected into UHPLC three times.

Accuracy

The accuracy is expressed as a percentage of recovery. A known amount of ADP and ELP standard drugs powder equivalent to 50% to 150% of the label claim was added, mixed, and analysed through chromatograms in optimised mobile phase.

Precision

The intraday precision study consisted of developing a test solution of the same concentration and assessing it three times during the day. On two distinct days, the same procedure was applied to assess interday precision. The result was given as %RSD.

Table 1: Optimized Chromatographic Conditions.

Mobile phase	0.02M KH_2PO_4 buffer: methanol
Selection of column	Cosmosil C_{18} (100 mm x 2.1 mm ID, Particle size: 1.7 µm)
Injection volume	5 µL
Flow rate	0.3 mL/min
Column temperature	Room Temperature
Detection wavelength	224 nm
Run Time	2.5 min
Retention time	Amlodipine (0.7 min) and Enalapril (1.2 min)

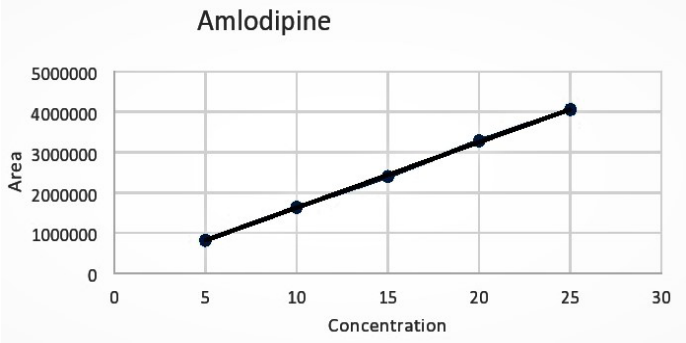

Figure 3: Calibration curve for Amlodipine.

Limit of Quantitation (LOQ) and Limit of Detection (LOD)

The LOD and LOQ were estimated using the slope(s) of the calibration curve and the Standard Deviation (SD) of the peak areas. It was determined by using the formulae LOD = 3.3 s/s and LOQ = 10 s/s.

Robustness

Robustness was assessed by changing the chromatographic conditions such as mobile phase composition, detection wavelength, flow rate, and so on, and the percentage RSD should be reported. Small modifications in the ideal circumstances were allowed, and the method's robustness was determined. Individual variations in detecting wavelength of 2 nm and flow rate of 0.1 mL/min were tested. In the ideal circumstances, solutions of 100% test concentration with the required changes were injected into the system in triplicate.

Ruggedness

Ruggedness is the investigation of the effect of external parameters on the approach. To assess the robustness of the suggested approach, factors were purposefully altered. These parameters included system variation, various analysts, and atmospheric fluctuations. Two distinct analysts prepared the test solution

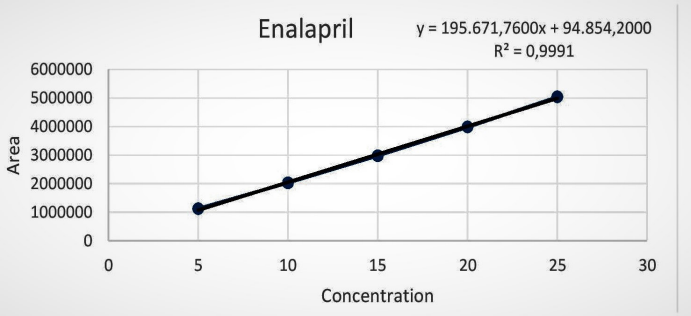

Figure 4: Calibration curve for Enalapril.

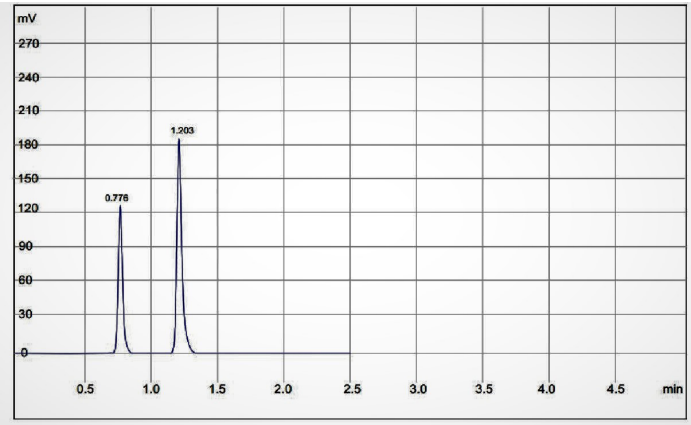

Figure 5: Chromatograph of Amlodipine and Enalapril.

according to the test procedure and injected three concentrations of test solution into the UHPLC system at a flow rate of 0.3 mL/min.

Assay of marketed formulation

20 tablets of the commercial formulation (AMTAS-E 5+5 mg) were ingested, weighed individually, and powdered. The average weight of the tablet determined and powder equivalent to one tablet was weighed and transferred to a 100 mL volumetric flask, where mobile phase was added to make up the volume. Sonicate for 10 min, occasionally spinning. The aforementioned solution was filtered via a 0.45 m membrane filter, yielding a stock solution containing 200 µg/mL of amlodipine and 200 µg/mL of enalapril. 0.75 mL of solution was withdrawn for analysis, diluted to 10 mL, and fed into the system.

System suitability

To validate the system, method and column performance, system suitability characteristics were examined. The system was tested for system applicability by injecting a standard solution of Amlodipine and Enalapril five times.

Force degradation study[31,32]

The ADP and ELP drugs combinations were subjected to forced degradation experiments. The principal stress conditions were 1N HCl (1 hr at 60°C), 1N NaOH (1 hr at 60°C), 3% H_2O_2 (24 hr), dry heat (24 hr), and UV radiation (24 hr).

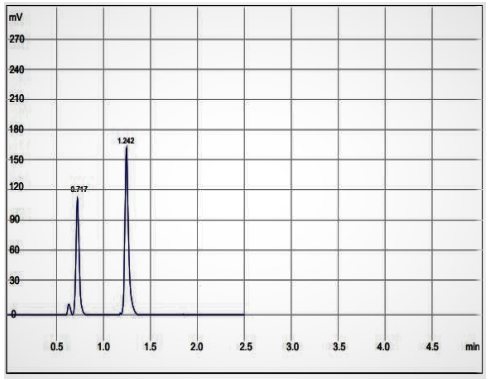

Figure 6: Acid Stressed Chromatogram.

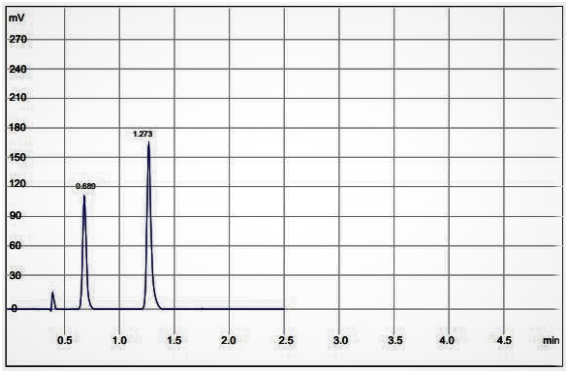

Figure 7: Alkali Stressed Chromatogram.

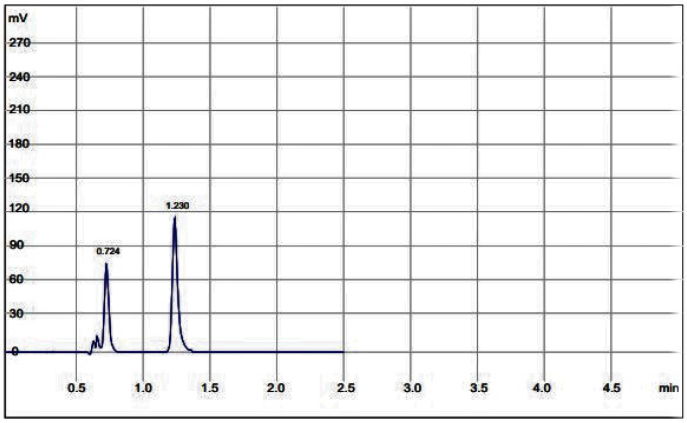

Figure 8: Peroxide Stressed Chromatogram.

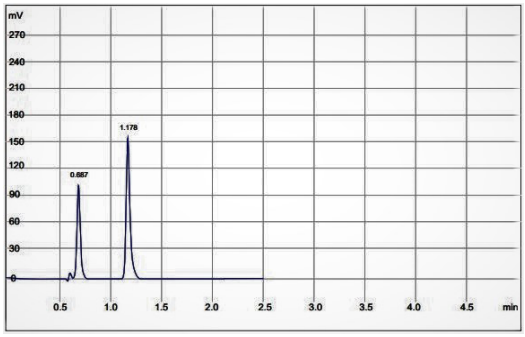

Figure 9: Thermal Stressed Chromatogram.

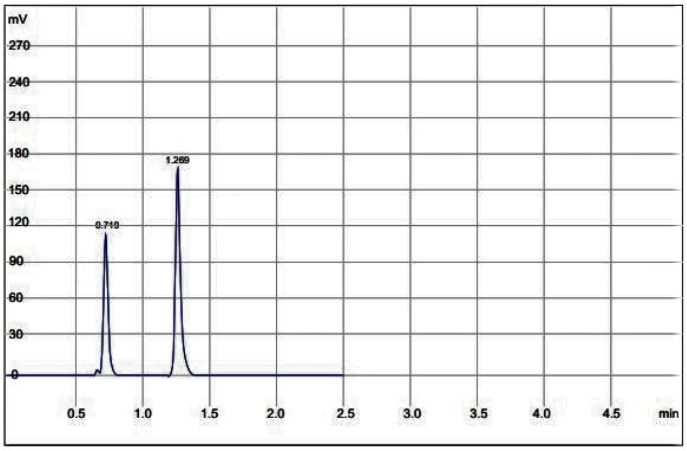

Figure 10: Photolytic Stressed Chromatogram.

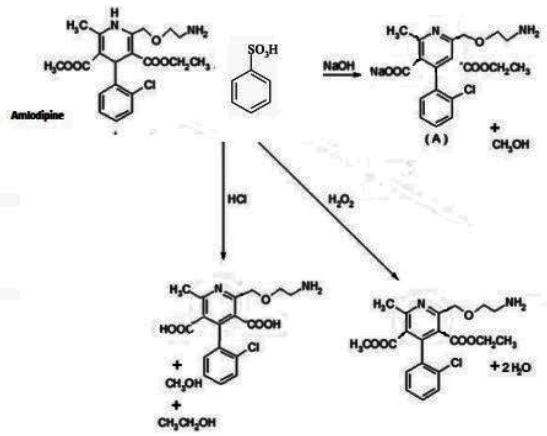

Figure 11: Degradation Pathway of ADP.

RESULTS

Optimization of RP-UHPLC method

The UHPLC technique was established for evaluating Amlodipine and Enalapril. Various mobile phases were investigated for technique optimisation, but suitable retention time, hypothetical plates, and good resolution were obtained with 0.02 M KH_2PO_4 buffer: methanol utilising Cosmosil C18 (100 mm x 2.1 mm ID, particle size: 1.7 m) using gradient method. (Table 1).

Linearity

The linearity of an analytical method was defined as the technique's ability to deliver test results that are directly proportional to analyte concentration within a particular range. The graph of calibration

Table 2: Recovery studies.

Level of addition	% Mean recovery*		SD		% RSD	
	ADP	ELP	ADP	ELP	ADP	ELP
50%	99.65	99.71	0.16	1.03	0.16	1.03
100%	99.70	99.99	0.22	0.81	0.22	0.81
150%	99.88	100.12	0.23	0.37	0.23	0.37

* Mean of three estimations.

Table 3: Precision studies (Intra-day and Inter-day).

Drug	Conc. [g/mL]	Intra- day Amount found [g/mL]		Inter- day Amount found [g/mL]	
		SD [*n*= 3]	% RSD	SD [*n*= 3]	% RSD
ADP	5	10393.36	1.28	6542.69	0.80
	15	12178.68	0.51	6062.04	0.25
	25	11290.12	0.28	14250.39	0.35
ELP	5	11307.53	1.00	5841.79	0.53
	15	34989.18	1.17	12471.05	0.42
	25	23612.47	0.47	6804.75	0.14

Table 4: Data for Robustness study of Amlodipine and Enalapril.

Sl. No.	Parameter	Amlodipine				Enalapril			
		Area	Mean	SD	% RSD	Area	Mean	SD	%RSD
1	Change in Flow rate (mL/min)	2394142	2390202	13782	0.58	2978784	2988690	9642	0.32
2		2401587				2998046			
3		2374879				2989241			
1	Change in Wavelength (nm)	2401023	2392473	12280	0.51	3001205	2992453	12496	0.42
2		2397995				2998013			
3		2378401				2978142			

was constructed by graphing the UHPLC chromatograph peak area against the relevant concentrations. Figure 3 shows that ADP is linear in the 5-25 µg/mL concentration range, whereas ELP is linear in the 5-25 µg/mL concentration range (Figure 4).

Accuracy

The method's accuracy defines how close the method's results are to the true value. The results of the accuracy testing revealed that the technique is accurate within acceptable ranges. When the % RSD for ADP and ELP is calculated, all of the results are within acceptable bounds. A maximum RSD of 2.0% indicated acceptable accuracy within the range. The results are shown in Table 2.

Precision

The repeatability of test results is ensured by intraday and interday precision. Amlodipine and Enalapril both had % RSD values less than 2. Results are shown in Table 3.

Robustness

Robustness was investigated using various deliberate alterations in chromatographic settings, such as changes in flow rate and wavelength. RSD was shown to be less than 2% in the Amlodipine and Enalapril robustness studies. As a result, it is strong and adheres to ICH criteria. Results are shown in Table 4.

Ruggedness

Ruggedness was studied by different analyst. From ruggedness study % RSD was found to be within limit of 2% for the ADP and ELP. Hence it is complying as per ICH guidelines.

Specificity

The specificity of the approach was determined by analysing reference drugs and sample. Excipients and contaminants had no effect on the standard drugs. As a result, the procedure is distinct (Figure 5).

% Assay of Marketed formulation

The % Assay of Intas Pharmaceutical's AMTAS - E (5 mg+5 g) commercial formulation was computed. The percentage assay was discovered to be ADP (98.40%) and ELP (99.31%), which was in good accord with the label claim.

System Suitability Parameters

The system, method, and column performance were validated by testing system suitability features. Five times, a standard solution of ADP and ELP was injected into the system, and the system's suitable features were evaluated. Results are shown in Table 5.

Degradation studies[31,32]

Stress assessment of the drug material can aid in identifying the anticipated degradation products as well as the analytical technique's stability and specificity. Degradation of the materials with acid, alkali, oxidation, thermal heat and photo light showed some new peaks with different R_t values., as shown in Figures 6, 7, 8, 9 and 10.

Degradation tests were carried out on solutions containing Amlodipine (15 g/mL) and Enalapril (15 g/mL). Results are shown in Table 6.

DISCUSSION

The UHPLC method for simultaneous quantification of amlodipine and enalapril was developed and validated. The mobile phase was a 70:30 v/v mixture of 0.02M KH_2PO_4 buffer and methanol. The linearity ranges of Amlodipine and Enalapril were found to be 5-25 µg/mL and 5-25 µg/mL, respectively. The calibration curve was plotted, and the ADP regression equation was found to be y = 1,62,671.3400x - 3,976.3000 with a correlation coefficient (r^2) of 0.9996, while the ELP regression equation was found to be y = 1,95,671.7600x + 94,854.2000 with a correlation coefficient (r^2) of 0.9991. The method's appropriateness for quantitative measurement of the compounds is demonstrated through validation in line with ICH principles. The proposed methods' reliability and analytical performance, including linearity, range, precision, accuracy, detection, and quantitation limitations, were statistically validated.

According to the Accuracy research, the percent recovery of Amlodipine is 99.46-100.11% and Enalapril is 98.68-100.73%, both of which are within the ICH standards. Intraday and interday precision ensure that % RSD remained within ICH criteria, i.e., NMT 2 for both Amlodipine and Enalapril. Amlodipine has a detection limit of 0.12 g/mL - 0.37 g/mL and Enalapril has a detection limit of 0.10 g/mL - 0.30 g/mL. Robustness was examined using deliberate variation, i.e., a change in flow rate and a change in wavelength that was within 2% of RSD according to ICH norms. The ruggedness testing yields results within 2% of the variance in Analyst examined. Amlodipine (98.40%) and Enalapril (99.31%) were reported to have the highest % assay of AMTAS-E (5 mg+5 mg). When ADP and ELP were treated to various stress conditions, the proposed methods were able to efficiently separate the medication from its degradation products and were thus regarded as good stability-indicating procedures. The degradation pathway[33,34] of DTV, LMV and TDF are shown in Figures 11 and 12.

Figure 11 shows the degradation pathway of ADP in acid, base and oxidation condition. The ADP was found to be prone to HCl, NaOH and H_2O_2 and shows degradation. In acidic condition leads to formation of acid as a degradation product. In basic condition

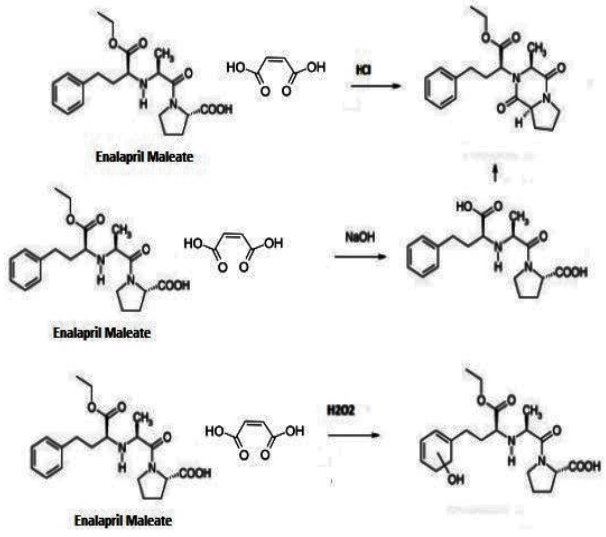

Figure 12: Degradation Pathway of ELP.

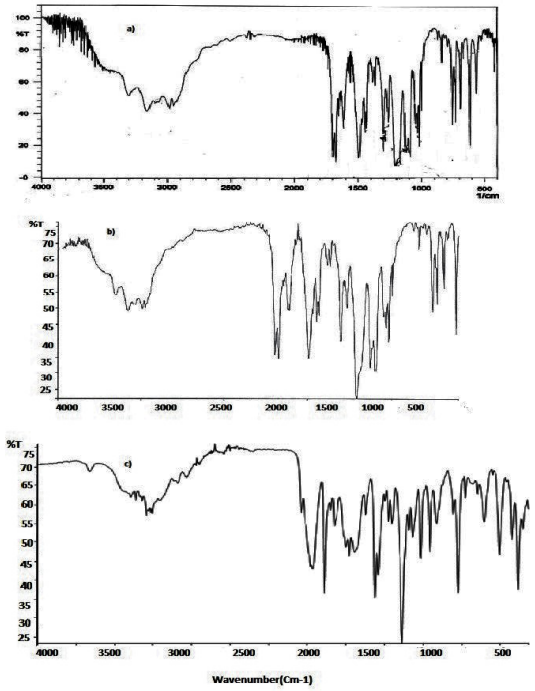

Figure 13: IR Spectra of ADP (a) its acidic degradate (b) Its alkaline degradate and (c) Its oxidation degradate.

Table 5: System suitability parameter.

Parameter	ADP	ELP
Retention Time (min)	0.744	1.253
Theoretical plates	9878	10552
Asymmetry Factor	1.08	1.11
Tailing factor	1.12	1.05

Table 6: Results of Forced Degradation Studies for ADP and ELP.

Sl. No.	Condition	Drugs	Area of sample	Area of Standard	% Drug recovered	% Degradation
1	Acid stress	ADP	2301254	2394765	96.10	3.90
		ELP	2923464	2977653	98.18	1.82
2	Alkali Stress	ADP	2271415	2394765	94.85	5.15
		ELP	2836540	2977653	95.26	4.74
3	Oxidative Stress	ADP	2102448	2394765	87.79	12.21
		ELP	2631448	2977653	88.37	11.63
4	Thermal Stress	ADP	2168035	2394765	90.53	9.47
		ELP	2910236	2977653	97.74	2.26
5	Photolytic Stress	ADP	2365410	2394765	98.77	1.23
		ELP	2948993	2977653	99.04	0.96

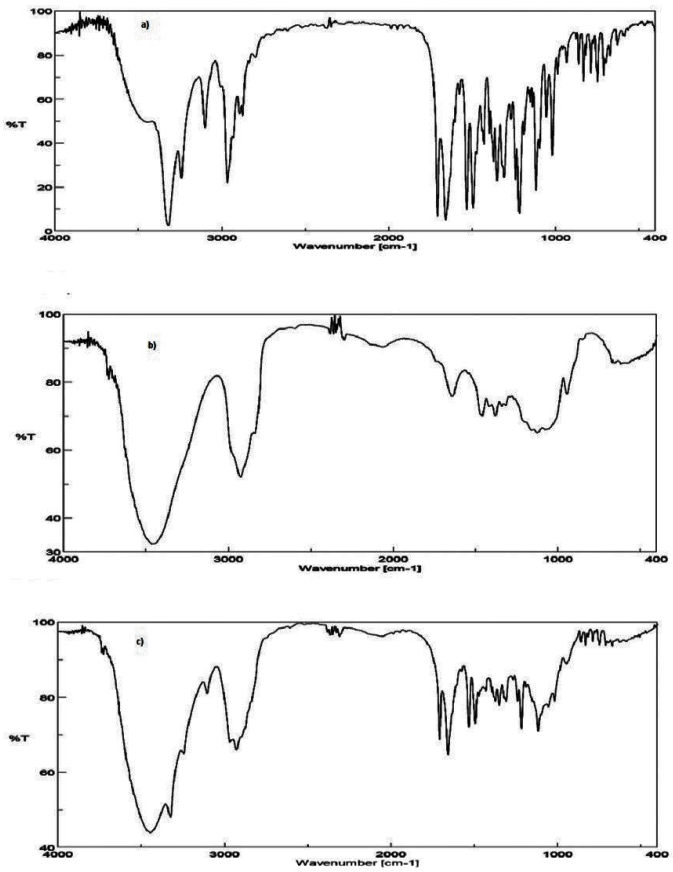

Figure 14: IR Spectra of ELP (a) its acidic degradate (b) Its alkaline degradate and (c) Its oxidation degradate.

it leads to form a sodium salt. In oxidation condition it forms esters.

Figure 12 shows the degradation pathway of ELP. The drug showed instability in acid and alkali and oxidation while it remained stable in dry heat and photolightic conditions. When ELP treated with alkali and peroxide the hydroxyl radical was generated.

Studies on forced degradation provide data on potential degradation pathways, active component degradation products, and can help to clarify the composition of the degradants. However, they aid in the creation of a stability indicating strategy. The degradation products generated by forced degradation studies are potential degradation products that may or may not emerge under pertinent storage circumstances. With IR, the degradation products were verified. The degraded sample spectra of ADP and ELP are shown in Figures 13 and 14.

Figure 13 depicts the ADP's deterioration. The ADP acidic degradate's IR spectra revealed a wide band of the (OH) group at 3400 cm^{-1}. It appears as a broad band of the (OH) group at 3400 cm^{-1} in the IR spectrum of an alkaline degradate, and it also appears as a broad band of the (OH) group at 3400 cm^{-1} in an oxidative degradate.

Figure 14 depicts the decline of ELP. The band of the ester moiety's C=O group appeared in the IR spectrum of the acidic degradate at 1740 cm^{1} and the wide band of the (OH) group appeared at 3400 cm^{1}. In the IR spectrum of an alkaline degradate, a broad band

of the (OH) group can be seen at 3400 cm^{1} and a CH aromatic band can be seen at 3179 cm^{1}, but in the spectrum of an oxidative degradate, a broad band of the (OH) group can be seen at 3400 cm^{1}.

CONCLUSION

The recommended chromatographic technique for identifying Amlodipine and Enalapril from pure and dosage forms was found to be straightforward, precise, accurate, fast, and specific. The mobile phase used in method development is easy and inexpensive to prepare. In the formulation, sample recoveries were excellent. Among all the established methods, this approach is the most cost-effective and has the shortest run time, allowing for rapid analysis. As a result, this method may be simply and conveniently used for *in vitro* dissolution and routine analysis of Amlodipine and Enalapril in Pharmaceutical dosage form. The degradation products generated during the stability investigation were well isolated from the pure drugs, revealing the uniqueness of the developed technique.

ACKNOWLEDGEMENT

The authors extend they're thanks to PRES's College of Pharmacy for Women in Nashik for providing the essential facilities for this research.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

mg: Milligram; **ml:** Mililitre; **µg:** Microgram; **AUC:** Area Under Curve; **ICH:** International Conference on Harmonization; R^2: Coefficient of Molar; **Rt:** Retention time; **N:** Theoretical Plates; **SD:** Standard Deviation; **% RSD:** % Relative Standard Deviation; **LOD:** Limit of Detection; **LOQ:** Limit of Quantitation; **RP-UHPLC:** Reverse Phase-Ultra High Performance Liquid chromatography.

REFERENCES

1. Available from: https://go.drugbank.com/salts/DBSALT001054.
2. Available from: https://go.drugbank.com/drugs/DB00381.
3. 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;3(2):e000473. doi: 10.1136/openhrt-2016-000473, PMID 27752334.
4. Meredith PA, Elliott HL. Clinical pharmacokinetics of amlodipine. Clin Pharmacokinet. 1992;22(1):22-31. doi: 10.2165/00003088-199222010-00003, PMID 1532771.
5. Available from: https://go.drugbank.com/drugs/DB00584.
6. Chaudhari BG. Development and validation of dual wavelength spectrophotometric method for simultaneous estimation of amlodipine besylate and enalapril maleate in combined dosage form. Ijpcbs. 2012;2(4):626-32.
7. Garg GG, Saraf SS. Development and validation of simultaneous estimation of enalapril maleate and amlodipine besylate in combined dosage forms. Trends Appl Sci Res. 2008;3(3):278-84. doi: 10.3923/tasr.2008.278.284.
8. Naveed S, Qamar H. Simple UV spectrophotometric assay of amlodipine. American journal of chemistry and application. J Chem Appl. 2014;1(4):66-9.
9. Roshanara MA, Bhavani MI, Prabahar AE, Rao RN. Development and validation of UV spectrophotometric method for analysis of enalapril maleate in bulk and pharmaceutical dosage form. Indo Am J Pharm Sci. 2019;6(2):4049-53. doi: 10.528 1/Zenodo. 256 7 493.
10. Gidwani B, Patel L, Gupta A, Kaur CD. Ultra-violet spectrophotometric method for estimation and validation of amlodipine in bulk and tablet formulation. J Anal Pharm Res. 2017;4(6):00125. doi: 10.15406/japlr.2017.04.00125.
11. Dhake AS, Kasture VS, Sayed MR. Spectrophotometric method for simultaneous estimation of amlodipine besylate and enalapril maleate in tablets. Indian Drugs. 2002;39(1):14-7.
12. Prasad CVN, Saha RN. Simultaneous determination of amlodipine-enalapril maleate and amlodipine-lisinopril in combined tablet preparations by derivative spectrophotometry. J Pharm Pharmacol. 2010;5(6):383-8. doi: 10.1211/1460808991 28735027.
13. Masih M. Method development and validation for simultaneous estimation of amlodipine besylate and enalapril maleate in solid dosage form. Int J Pharm Chem Anal. 2021;8(3):129-33. doi: 10.18231/j.ijpca.2021.025.
14. Dangi M, Chaudhari D, Sinker M, Racha V, Damle MC. Stability indicating HPTLC method for estimation of nebivolol hydrochloride and amlodipine besylate in combination. Eur Asian J Anal Chem. 2010;5:161-9.
15. Mennickent SC, Rivas C, Vega MA, De Diego MG. A stability indicating HPTLC method for quantification of enalapril maleate in tablets. J Chil Chem Soc. 2013;58(2):1737-40. doi: 10.4067/S0717-97072013000200021.
16. Jain PS, Patel MK, Bari SB, Surana SJ. Development and validation of HPTLC method for simultaneous determination of amlodipine besylate and metoprolol succinate in bulk and tablets. Indian J Pharm Sci. 2012;74(2):152-6. doi: 10.4103/0250-474X.1038 49, PMID 23325996.
17. Logoyda L. Efficient validated method of HPLC to determine amlodipine in combinated dosage form containing amlodipine, enalapril and bisoprolol and *in vitro* dissolution studies with *in vitro/in vivo* correlation. Pharmacia. 2020;67(2):55-61. https:. doi: 10.3897/pharmacia.67.e48220.
18. Chaudhari BG. Development and validation of RP-HPLC method for simultaneous estimation of enalapril maleate and amlodipine besylate in combined dosage form. J App Pharm Sci. 2012;2(9):54-7. doi: 10.7324/JAPS.2012.2911.
19. Koppala S, Ranga Reddy V, Anireddy JS. User-Friendly HPLC method development and validation for determination of enalapril maleate and its impurities in enalapril tablets. J Chromatogr Sci. 2017;55(10):979-88. doi: 10.1093/chromsci/bmx060, PMID 28985272.
20. Masih M, Mittal A. Development and validation of HPLC method for simultaneous estimation of amlodipine besylate and enalapril maleate in solid dosage form. World J PharmSci. 2014;2(10):1401-5.
21. Naveed S, Sultana N, Arayne MS. HPLC-UV method for the determination of enalapril in bulk, pharmaceutical formulations and serum. J Anal Bioanal Techniques. 2012;03(1):130. doi: 10.4172/2155-9872.1000130.
22. Logoyda L, Ahmed M. Megied A, Kondratova Y, Trofimenko O, Korobko D, Dakhym I. Development and validation of HPLC method for the simultaneous determination of enalapril maleate in present of their impurities: Application to tablet analysis. Int J App Pharm. 2018;10(1):98-102. DOI: http://dx.doi.org/10.22159/ijap.2018v10i1.22 805.
23. Çelebier M, Kaynak MS, Altınöz S, Sahin S. HPLC method development for the simultaneous analysis of amlodipine and valsartan in combined dosage forms and *in vitro* dissolution studies. Braz J Pharm Sci. 2010;46(4):761-8. doi: 10.1590/ S1984-82502010000400018.
24. Halder D, Dan S. LC–MS/MS assay for quantitation of enalapril and enalaprilat in plasma for bioequivalence study in Indian subjects, Future Sci. 2017;3(1):FSO165. doi: 10.4155/fsoa-2016-0071.
25. Yasodha A, Goud GA, Surekha G, Bhargavi L. Method development and validation for the determination of amlodipine and enalapril maleate in active pharmaceutical ingredient and combined tablet dosage form by RP-HPLC. Eur J Pharm Med Res. 2023;10(4):360-7.
26. Sarısaltık Yaşın D, Arslantürk Bingül A, Karaküçük A, Teksin ZŞ. Development and validation of an HPLC method using an experimental design for analysis of amlodipine besylate and enalapril maleate in a fixed-dose combination. Turk J Pharm Sci. 2021;18(3):306-18. doi: 10.4274/tjps.galenos.2020.89725, PMID 34157820.
27. Chan ML, Chun MW. Determination of amlodipine in human plasma by LC-MS/ MS and Its bioequivalence study in healthy Chinese subjects. Pharmacol Pharm. 2013;4(2):191-200. doi: 10.4236/pp.2013.42027.
28. Vuyyala G. Reddy DRS. UPLC method development and validation for simultaneous determination of amlodipine besylate and enalapril maleate in drug products. Int J Health Sci. 2022; 6(S4);11965: 12343-54. doi: 10.53730/ijhs.v 6nS4.
29. ICH Guideline Q2. Validation of analytical procedures: text and methodology. Available from: http://www.ich.org/LOB/media/MEDIA417.pdf. 2005;1.

30. ICH. Q2B validation of analytical procedures: methodology, consensus guidelines ICH harmonized tripartite guidelines; 1996.
31. Bakshi M, Singh S. Development of validated stability indicating assay methods critical review. J Pharm Biomed Anal. 2002;28(6):1011-40. doi: 10.1016/s0731-7085(02)00047-x, PMID 12049968.
32. Kats R. Forced degradation studies: regulatory considerations and implementation. Bio Pharm Int. 2005;18:1-7.
33. Chandramore K, Sonawane S. Overview on Development and Validation of Force degradation studies with Stability Indicating Methods. Biosci Biotechnol Res Asia. 2022;19(4):881-94. doi: 10.13005/bbra/3039.
34. Blessy M, Patel RD, Prajapati PN, Agrawal YK. Development of forced degradation and stability indicating studies of drugs—a review. J Pharm Anal. 2014;4(3):159-65. doi: 10.1016/j.jpha.2013.09.003, PMID 29403878.

Cite this article: Gaikwad N, Bhangale C, Bhandare S. Simultaneous Estimation of Amlodipine and Enalapril Maleate by Stability Indicating UHPLC Method. Int. J. Pharm. Investigation. 2024;14(1):151-9.