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GREEN SPECTROPHOTOMETRIC METHOD FOR ESTIMATION OF LISINOPRIL AND AMLODIPINE IN COMBINATION

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ABSTRACT

Lisinopril and Amlodipine are used for treatment of hypertension. This research aims to develop a robust and environmentally friendly method for the simultaneous determination of Lisinopril and Amlodipine through spectrophotometric derivatization using absorption correction techniques. The method involves use of ninhydrin as derivatizing agent in presence of $NaHCO_3$. Absorption correction method involves estimation of Lisinopril and Amlodipine at 320 nm after derivatization and estimation of amlodipine without derivatization at 360 nm within the linear range of 20-160 µg/ml. The results demonstrated the feasibility and accuracy of the proposed method, with validation according to ICH guidelines showing precision, accuracy over the linear range. The AGREE software was used to evaluate the greenness of the method, showing alignment with the principles of green analytical chemistry. The findings highlight the potential of the developed method for routine pharmaceutical analysis.

Keywords: Spectrophotometry, Lisinopril, Amlodipine, Derivatization, Absorption correction, Green analytical chemistry

INTRODUCTION

Spectrophotometry, as a quantitative analytical tool, stands as one of the most commonly employed techniques in pharmaceutical analysis. It offers tangible and substantial economic advantages over alternative methods. UV-visible spectrophotometry remains the preferred technique owing to its inherent simplicity, selectivity, sensitivity, precision, accuracy, and cost-effectiveness **[1, 2]**. Nonetheless, many methodologies like spectrophotometric and HPLC methods are restricted in their applications, thus highlighting the necessity for developing robust and cost-effective methods for determining Lisinopril and Amlodipine through spectrophotometric derivatization, employing absorption correction techniques. Compared to current visible spectrophotometric techniques used for quantifying lisinopril and amlodipine, the modified method presented here can be deemed environmentally friendly, as it showcases the potential of visible spectrophotometry without relying on organic solvents.

Lisinopril is1-[N2-[(S)-1-Carboxy-3-phenylpropyl]-L-lysyl]-L-proline dihydrate. It is used as Antihypertensive agent from the groupof angiotensin converting enzyme (ACE) inhibitors.

Amlodipine Besylate is 3,5-Pyridinedicarboxylic acid, 2-[(2-aminoethoxy) methyl]-4 (2-chlorophenyl)-1,4-dihydro-6-methyl-, 3-ethyl 5-methyl ester, (±)-, monobenzenesulfonate. It is used as antihypertensive; antianginal. Amlodipine act by blocking voltage-sensitive calcium channels (L-type).

Numerous techniques have been documented in literature for estimating Lisinopril through spectrophotometric **[2-10]**, fluorometric **[11]** means, employing diverse reagents and reactions, both in its pure state and in tablet formulations. A broad array of methods has been reported for analyzing combinations of Lisinopril and Amlodipine using spectrophotometry **[12-14]**, High performance liquid chromatography **[15-23]**, thin-layer chromatography **[24]**, LC/MS/MS **[25]**, either individually or in conjunction with other medications.

Some methodologies entail measuring combinations at relatively shorter wavelengths using simultaneous equation method and absorbance ratio method **[12]**. Lisinopril shows absorbance at relatively lower wavelength i.e., 219 nm in UV region. Detection at lower UV wavelengths are more susceptible to interference from background noise, solvent effects, and other factors, which

can affect the accuracy and reliability of absorbance measurements.

Therefore, we thought to derivatize the Lisinopril. Lisinopril as a single drug has been evaluated by derivatization **[1]**. Therefore, utilizing the same conditions of derivatization, we analyzed lisinopril and amlodipine in combination by absorption correction method. **Figure 1 and 2** highlights the structure of Lisinopril and Amlodipine.

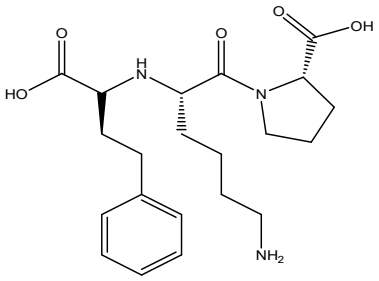


Figure 1: Structure of Lisinopril

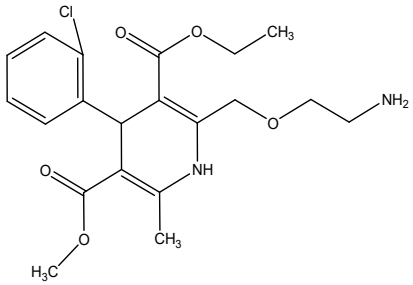


Figure 2: Structure of Amlodipine

MATERIALS AND METHODS

Instruments and equipment:

UV- Visible spectrophotometer (JASCO, V730), Digital balance (Shimadzu, ATX 224R), magnetic stirrer

Chemicals:

Lisinopril and Amlodipine were received as gift sample from Emcure Pharmaceuticals and Lupin Limited. Ninhydrin (AR Grade) was purchased from Laboworld private limited, Pune. Sodium bicarbonate (AR grade) and Methanol (AR Grade) were purchased from Loba Chemie Pvt ltd.

Preparation of Reagents:

1. 0.5 % Ninhydrin: - 500 mg of ninhydrin was weighed and dissolved in 100 ml distilled water.
2. Sodium bicarbonate (saturated solution): - 25 g of sodium bicarbonate was taken in beaker and dissolved in 100 ml water and was stirred for 20 minutes on magnetic stirrer. Then the saturated solution was filtered using filter paper.

Standard stock solution:

Standard stock solutions of Lisinopril and Amlodipine was prepared by weighing 10 mg drug and transferred in two different 10 ml of volumetric flasks and dissolved in water and methanol respectively and then volume was

made up with respective solvents to get 1000 µg/ml standard stock solution.

Absorption Correction method:

Various volumes (ranging from 0.0 to 1.6 mL) of a 1000 µg/mL LNP and AML solution were precisely measured and transferred into a series of 10 mL volumetric flasks. The volume in each flask was then adjusted to 5 mL with water. Subsequently, 1 mL of 0.5% ninhydrin solution and 1 mL of a saturated $NaHCO_3$ solution were added to each flask. Afterward, the volume in each flask was brought to 9 mL with water. Following a 10-minute immersion in a boiling water bath, the flasks were cooled to room temperature. Post-cooling, the volumes in all flasks were adjusted to the mark with water. Absorbance readings were taken at 320 nm against a reagent blank. A calibration curve was constructed by plotting absorbance against concentration, from which the concentration of the unknown solution was determined from the curve. Similarly, Amlodipine was measured at 360 nm without treatment with ninhydrin as the method employs absorption correction method. **Figure 3 and 4** shows UV spectrum of Amlodipine and Lisinopril.

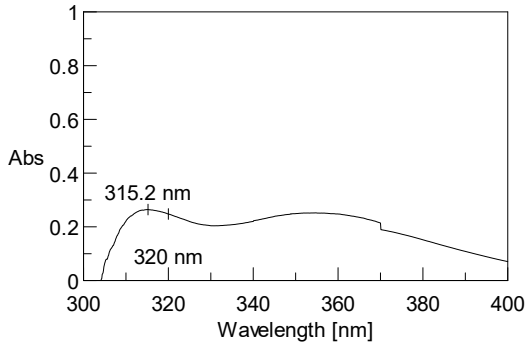


Figure 3: UV spectrum of Amlodipine

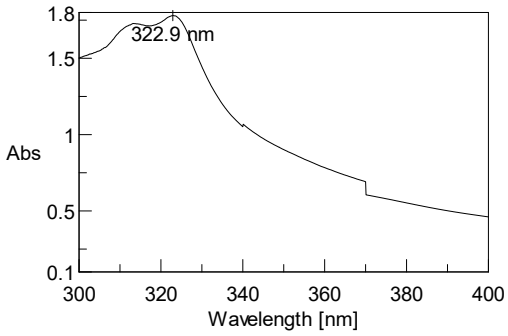


Figure 4: UV spectrum of Lisinopril after derivatization after derivatization

Assay:

Marketed formulation (Amlovas- L) was weighed and crushed, powder equivalent to 5 mg of drug was weighed and transferred to 10 ml to volumetric flask and dissolved in methanol to get stock solution of 1000 µg/ml. Then it was sonicated and filtered. Aliquot of this solution was diluted and treated with ninhydrin and NaHCO3 to produce the concentration of 20µg/ml for AML and LIS. The absorbances are measured at 320nm and 360 nm respectively.

Greeness evaluation using AGREE:

The alignment of the developed method with the 12 principles of green analytical chemistry (GAC) was deliberated using the AGREE software. The spectrophotometric method proposed in this work is compared with other

spectrophotometric method involving use of different organic solvents. AGREE assesses the performance of the proposed analytical method for each principle by assigning a score, predetermined by a defined model ranging from 0 to 1, reflecting the method's alignment with the principle. The score is contingent upon the input parameters estimated for insertion into the AGREE software. Equal weighting has been applied to all 12 principles, implying that each principle of GAC is considered equally important for the AGREE analysis **[26]**

RESULT AND DISCUSSION:

Ninhydrin has been known as reagent for detection of amino acids which when reacts with primary amines produces a purple-colored product, providing a foundation for quantifying substances containing primary amino groups. As both Lisinopril and Amlodipine contain amino group in its structure makes the reaction with ninhydrin more challenging when used for combination.

Optimization of Reaction Conditions:

In the absence of $NaHCO_3$, even with prolonged heating, the reaction between ninhydrin and LNP did not yield any colored product. Four parameters were optimized: the volume of NaHCO3, pH effect, concentration of ninhydrin, and boiling time. Upon using more than 1 mL of saturated NaHCO3 solution, a violet-colored product was formed. However, the violet-colored product proved unstable, gradually turning yellow over time. Thus, 1 mL of saturated NaHCO3 solution, yielding a stable yellow-colored product, was determined to be the optimal volume in a total volume of 9 mL and reaction was found to be specific in $NaHCO_3$ medium. When a heating duration of 10 minutes was extended the violet-colored product started to destabilize, transitioning into yellow. Hence, a heating period of 10 minutes in a boiling water bath was identified as optimal for producing a stable yellow-colored product; this remained stable for 90 minutes.

Method validation:

The spectrophotometric technique used to quantify lisinopril and amlodipine in combination was validated in accordance with the International Conference on Harmonization (ICH) guidelines Q2 (R1). Validation was conducted concerning parameters such as linearity range, limit of detection (LOD), limit of quantification (LOQ), Assay and accuracy, precision.

Linearity:

The calibration curve was obtained using data of the spectrophotometric analysis of standard stock solution of both drugs ranging from 20-160 µg/mL. The correlation coefficient was found to be 0.9925 for lisinopril at 320 nm and

0.982 for amlodipine at 320 and at 360 nm for amlodipine it was found to be 0.9803.

Precision:

The precision method was established by repeatability and intermediate precision studies. The RSD (%) values of intra-day and inter-day showed good precision **Table 1**.

Assay and Accuracy:

The method was suitably employed for assaying both drugs in formulation as per the above stated procedure. The results of the marketed formulations were found to be100.72% for Amlodipine and 102.08 %for Lisinopril. To verify the accuracy of the proposed methods, recovery studies were conducted using the standard addition method at three distinct levels (80%, 100%, and 120%) and the results are listed in **Table 2 and 3.**

LOD and LOQ:

LOD and LOQ were calculated using the following equations (Eq 1 and Eq 2) respectively, from the linear regression data of the calibration curve and the results are listed in **Table 1**.

$LOD = 3.3\ \sigma/S$ (1)

$LOQ = 10\ \sigma/S$ (2)

Table 1: Validation parameters

Parameters	Lisinopril	Amlodipine
Working λmax	320nm	360nm
Beer's law limit	20-160 µg/mL	20-160 µg/mL
Regression equation	y = 0.0109x - 0.0438	y = 0.0111x + 0.1926
Regression coefficient(R^2)	0.9925	0.9803
LOD* (µg/mL)	1.022	0.891
LOQ* (µg/mL)	3.091	2.702
% RSD intra-day precision	1.89	0.72
% RSD inter-day precision	1.31	0.95

Table 2: Assay results of Marketed formulation (Amlovas- L)

Tablet Brand name	Drug	LABEL CLAIM (mg/tablet)	Amount found (mg/tablet)	% Label claimed
Amlovas- L	Lisinopril	5	5.10	102.1
	Amlodipine	5	5.03	100.7

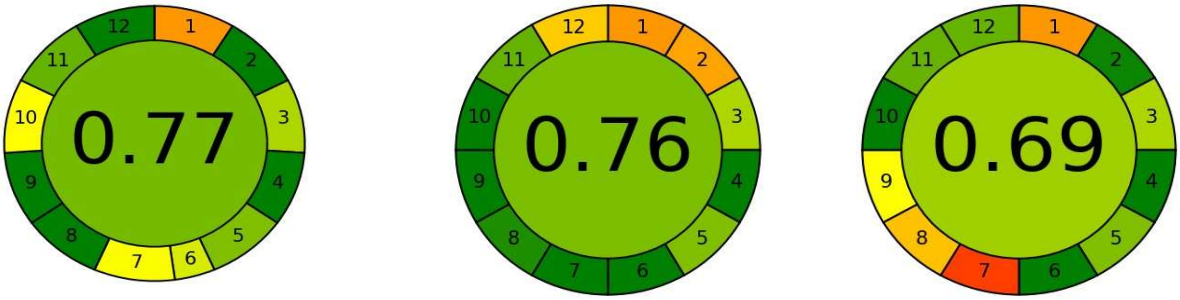
Table 3: Results of recovery study by Standard-addition method

Level Recovery (%)	Drug	Conc of drug in µg/ml		% Recovery
		Drug taken	Std drug added	
80	Lisinopril	20	16	98.42
100		20	20	99.44
120		20	24	98.99
80	Amlodipine	20	16	98.21
100		20	20	98.08
120		20	24	99.59

AGREE

The developed method was compared with other method involving organic solvents and HPLC method used for the determination of this combination on the basis of their accordance with the 12 principles of green analytical chemistry using the AGREE software. The results are summarized in **Figure 4**.

The AGREE evaluation's ultimate score underlines the increased eco-friendliness achieved with the proposed spectrophotometric method. This is primarily attributed to the quicker and simpler sample derivatization process without use of organic solvents for its estimation. **Figure 5 a, b and c** highlights the comparison between three methods i.e. proposed method involving use of ninhydrin, UV method without derivatization and HPLC method for this combination. The results of proposed method show appreciable improvement in the score associated with 8th principle i.e. the number of analytes determined in 1hr was compared to HPLC method already reported.



(a)Proposed UV method **(b) Reported UV method** **(c) Reported HPLC method**

Figure 5: Comparison of output graphical results of AGREE analysis for (a) proposed UV method (b) Reported UV method (c)Reported HPLC method

CONCLUSION

A simple robust, cost-effective spectrophotometric derivatization method for combination of Lisinopril and Amlodipine was developed and validated. Validation according to ICH guidelines confirmed good linearity, precision, accuracy, and acceptable limits of detection and quantification. Finally, the proposed method and reference method have been compared according to the 12 principles of GAC using the software AGREE. Hence it can be concluded that the developed spectrophotometric methods are accurate, precise and can be employed successfully for the estimation of Amlodipine besylate and Lisinopril dihydrate.

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REFERENCES

[1] Basavaiah K, Tharpa K, Hiriyanna SG, Vinay KB. Spectrophotometric determination of lisinopril in pharmaceuticals using ninhydrin- a modified approach. Journal of Food and Drug Analysis. 2020 Jul 14;17(2) https://doi.org/10.38212/2224-6614.2294.

[2] Nataliia Shulyak, Kateryna Budzivula, Kucher T, Liubomyr Kryskiw, Poliak O, Liliya Logoyda. Spectrophotometric methods for the determination of lisinopril in medicines. Pharmacia. 2021 Oct 18;68(4):811–8 https://doi.org/10.3897/pharmacia.68.e72991.

[3] Sbârcea L, Trandafirescu C, Zoltán Szabadai, bojiţă m. Spectrophotometric method for lisinopril determination using ninhydrin. Farmacia. 2014 jan 1;62(1).

[4] Rahman N, Azmi SNH. Spectrophotometric method for the determination of amlodipine besylate with ninhydrin in drug formulations. Il Farmaco. 2001 Sep;56(10):731–5 https://doi.org/10.1016/S0014-827X(01)01093-X.

[5] Rahman N, Singh M, Hoda MdN. Optimized and validated spectrophotometric methods for the determination of lisinopril in pharmaceutical formulations using ninhydrin and ascorbic acid. Journal of the Brazilian Chemical Society. 2005 Oct;16(5):1001–9 https://doi.org/10.1590/S0103-50532005000600018

[6] Jamakhandi CM, Disouza J, Chougule US, Mahanthesh MC. Newer spectrophotometric method of determination for lisinopril dosage forms research article. International Journal of Pharmacy and Pharmaceutical Sciences. 2011 Jun 26;3.

[7] Rahman N, Masoom Raza Siddiqui, Syed Najmul Hejaz Azmi. Spectrophotometric determination of lisinopril in commercial dosage forms using N-bromosuccinimide and chloranil. Chemia Analityczna. 2007;52(3):465–80.

[8] D Noel Preetham, K Sujana, P D S Sankar . Development and Validation of UV Spectrophotometric Method for the Estimation of Lisinopril in Bulk and Pharmaceutical Formulation. 2014.

[9] Fawzy A. El-Yazbi, Heba H. Abdine, Rasha A. Shaalan. Spectrophotometric and Spectrofluorometric Methods for the Assay of Lisinopril in Single and Multicomponent Pharmaceutical Dosage Forms. Journal of Pharmaceutical and Biomedical Analysis. 1999;819–27 https://doi.org/10.1016/S0731-7085(98)00110-1.

[10] Raza A, Tariq Mahmood Ansari, Atta-Ur-Rehman. Spectrophotometric Determination of Lisinopril in Pure and Pharmaceutical Formulations. Journal of the Chinese Chemical Society. 2005 Oct 1;52(5):1055–9 https://doi.org/10.1002/jccs.200500149.

[11] Jamkhandi CM, Javali C, Kumar S, Kumar S, Ds SK. New fluorimetric method of determination for lisinopril dosage forms. International Journal of Pharmaceutical Sciences and Drug Research. 2010 Jul 1;2(3):182–7.

[12] Masih M, Mittal A, Nandy BC. Spectrophotometric simultaneous estimation of amlodipine besylate and lisinopril dihydrate in tablet dosage forms. Journal of Biomedical and Pharmaceutical Research. 2014 Jul 3;3(4).

[13] Joshi H.V, Patel J.K. New Spectrophotometric Methods for Simultaneous Determination of Amlodipine Besylate and Lisinopril in Tablet Dosage Forms. Journal of Applied Pharmaceutical Science. 2011 Aug 3;01(06):162–4

[14] Omayma Abdel Razak, Belal SF, Bedair MM, Barakat NS, Haggag RS. Spectrophotometric and polarographic determination of enalapril and lisinopril using 2,4-dinitrofluorobenzene. Journal of Pharmaceutical and Biomedical Analysis. 2003 Mar 26;31(4):701–11 https://doi.org/10.1016/S0731-7085(02)00654-4.

[15] Chauhan V, Prajapati S, Patel C. A validated rp-hplc method for simultaneous estimation of amlodipine and lisinopril in pharmaceutical dosage form. IJPSR.

2011;2(7):1712–5http://dx.doi.org/10.13040/IJPSR.0975-8232.2(7).1712-15.

[16] Shelke S, Patil B, Kukade V, kewari C. Analytical method development and validation of rp-hplc for amlodipine besylate and lisinopril in combined tablet dosage form by using simultaneous estimation method. Journal of Advanced Scientific Research. 2023 Aug 31;63–8 https://doi.org/10.55218/JASR.2023 14710.

[17] Peikova L, Boyka Tsvetkova, D. Kostova, Dimitar Rachev. Investigations and HPLC Assay of Model Formulations Containing Amlodipine Besylate and Lisinopril. International Journal of Pharmaceutical Sciences Review and Research. 2013 Jan 1;

[18] Pawar VT, More HN, Bhatia MS. Development and Validation of RP-HPLC Method for the Determination of Lisinopril and Amlodipine in Bulk and Multicomponent Pharmaceutical Cardiovascular Dosage Form. Nat Volatiles & Essent Oils. 2021;

[19] Verma A, Sharma R, Chauhan J, Dahiya M, Sharma N. Development and Validation of RP-HPLC Method Using Photodiode Array or Diode-Array Detection Detector for Simultaneous Estimation of the Amlodipine Besylate and Lisinopril in Fixed-Dose Formulation. International Journal of Pharmaceutical Quality Assurance. 2020 Sep 25;11(3) https://doi.org/10.25258/IJPQA.11.3.4.

[20] Piponski M, Stoimenova TB, Serafimovska GT, Stefova M. Development and Validation of Fast, Simple, Cost-effective and Robust RP-HPLC Method for Simultaneous Determination of Lisinopril and Amlodipine in Tablets. Analytical Chemistry Letters. 2019 May 4;9(3):385–402 https://doi.org/10.1080/22297928.2019.1650108.

[21] V. Leela Padmini, Firdose SK, S. Madhavi, M. Javed Mohammed, Nalluri BN. Simultaneous Estimation of Lisinopril and Hydrochlorthiazide in bulk, Pharmaceutical Dosage Forms and in Dissolution Samples by RP-HPLC-PDA Method. Journal of Chemical

and Pharmaceutical Research. 2013 Jan 1;5(8).

[22] Chander V, Mohan M, Seth R, Singh P, Singh R, Gupta S. Development and Validation of RP-HPLC Method for the Estimation of Lisinopril in Tablet Dosage Form. Analytical Chemistry Letters. 2012 Jan;2(5):309–13 https://doi.org/10.1080/22297928.2012.10648282.

[23] Adam EHK. Stress Degradation Studies on Lisinopril Dihydrate Using Modified Reverse Phase High Performance Liquid Chromatography. American Journal of Analytical Chemistry. 2014;05(05):316–22 http://dx.doi.org/10.4236/ajac.2014.55039.

[24] Pandya JJ, Sanyal M, Shrivastav PS. Simultaneous densitometric analysis of amlodipine, hydrochlorothiazide, lisinopril, and valsartan by HPTLC in pharmaceutical formulations and human plasma. Journal of Liquid Chromatography & Related Technologies. 2017;40(9):467–78 https://doi.org/10.1080/10826076.2017.1324482

[25] Tsakalof A, Kyriaki Bairachtari, Manolis Georgarakis. Development of a Liquid Chromatography–mass Spectrometry Method for Monitoring the angiotensin-converting Enzyme Inhibitor Lisinopril in Serum. Journal of Chromatography B. 2003 Jan 1;783(2):425–32 https://doi.org/10.1016/S1570-0232(02)00704-3.

[26] Prete P, Iannaccone D, Proto A, Marek Tobiszewski, Cucciniello R. Development and Validation of an eco-compatible UV–Vis Spectrophotometric Method for the Determination of Cu2+ in Aqueous Matrices. Analytical and Bioanalytical Chemistry. 2023 Jun 14;415 https://doi.org/10.1007/s00216-023-04785-6.