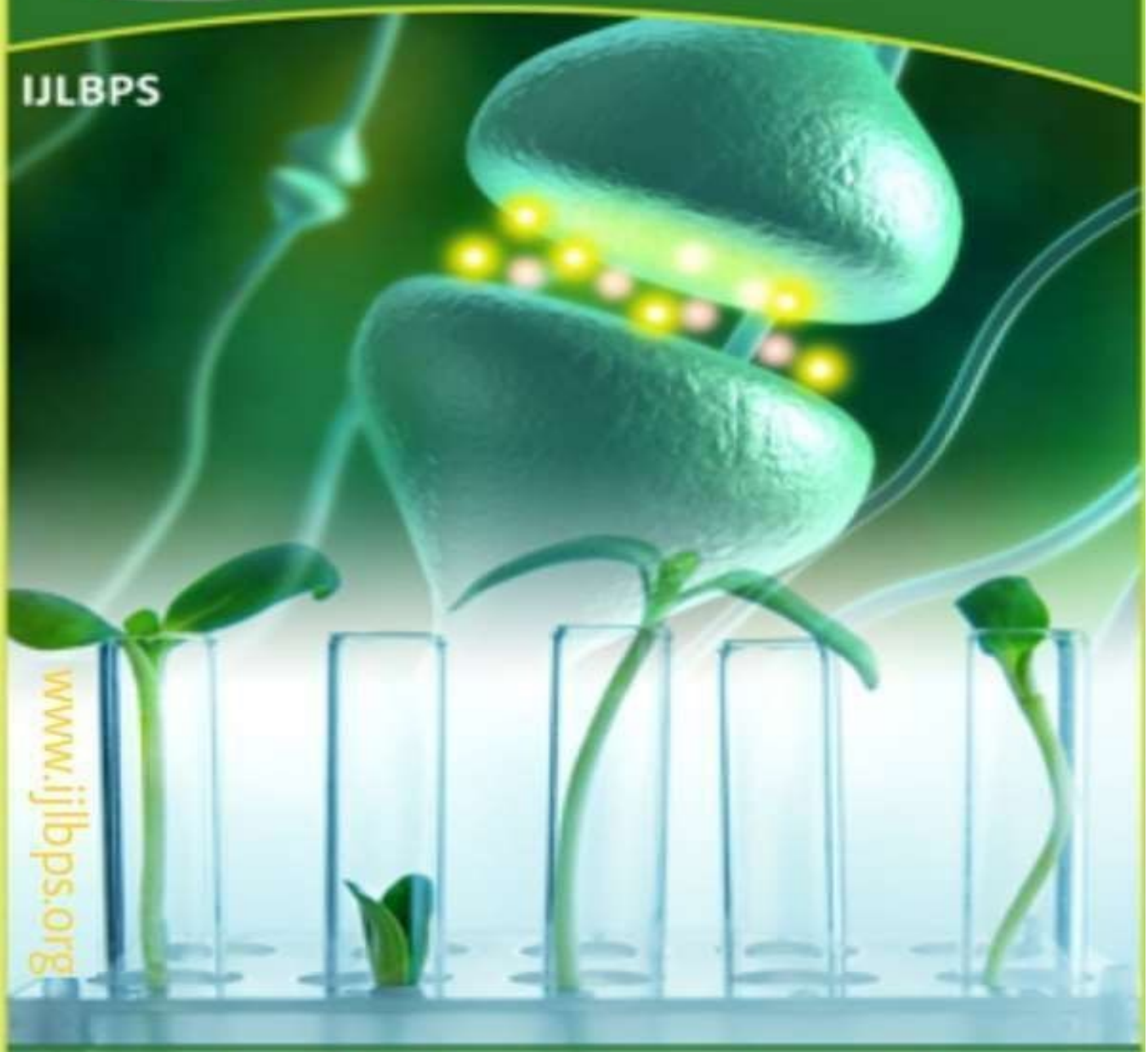
ISSN 2395-650X

International Journal of Life Sciences Biotechnology Pharma Sciences



E-mail: editorijlbps@gmail.com editor@ijlbps.org

International Journal of Life Sciences Biotechnology Pharma Sciences

Volume 20, Issue 3, 2024

Simultaneous Estimation of Antihypertensive Drugs Using Derivative Spectroscopy

Dr. P. Venkateswara Rao, Professor, A. M. Reddy Memorial College of Pharmacy, Petlurivari palem, Narasaraopet, A.P., India, Pin 522601. Email: dr.pvrao2010@gmail.com

Dr. Sk. Abdul Jabbar Basha, Associate Professor, A. M. Reddy Memorial College of Pharmacy, Petlurivari palem, Narasaraopet, A.P., India, Pin 522601. Email: basha.rv20@gmail.com

T. Raja, Professor, Dept. of Ph. Analysis, A. M. Reddy Memorial College of Pharmacy, Petlurivari palem, Narasaraopet, A.P., India, Pin 522601. Email: rajatanneru47@gmail.com

Dr. Himaja Trivedi, Associate professor, A. M. Reddy Memorial College of Pharmacy, Petlurivari palem, Narasaraopet, A.P., India, Pin 522601. Email: himajatrivedi1141@gmail.com

ABSTRACT

For the simultaneous measurement of atorvastatin calcium and amlodipine besylate, a straightforward, accurate, and precise UV spectroscopic approach has been established. The established approach of simultaneous equations is cost-effective and repeatable. 365 and 246 nm were the wavelengths used to measure the absorbance of the two medications, respectively. Amlodipine besylate and atorvastatin calcium showed linearity at their respective λmaxes of 365 and 246 nm as well as at the isoabsorptive point at 238 nm in a concentration range of 2 to 10 µg/mL. The accuracy of the approach was shown by recovery trials, which showed recovery of >99.78% for amlodipine besylate and >99.36% for atorvastatin calcium. The proposed approach is recommended for regular analysis due to its speed, ease of use, accuracy, sensitivity, and specificity. The International Council of Harmonization's (ICH) guidelines were followed for conducting the validation investigations.

Keywords: UV spectroscopy, atorvastatin calcium, amlodipine besylate, and simultaneous equation approach.

INTRODUCTION

High blood pressure that persists for a long time is referred to as hypertension. Increased vascular resistance is the primary cause of hypertension, or persistently elevated blood pressure, even while cardiac output is within the normal range. Vasodilatation is actually how some of the strongest antihypertensive drugs reduce this resistance. One One major problem with treating hypertension is that, in 90% of instances, primary or essential hypertension—a disease in which the reason of the persistently elevated blood pressure is unknown—occurs. Many of the interrelated regulating mechanisms are altered, and often adjusting two or more of these processes is necessary to reduce blood pressure. Hypertension that only sporadically arises from a clinical condition is referred to as secondary hypertension. First and foremost, the ailment must be treated, and doing so will usually lower the secondary hypertension. If not, more direct participation might be investigated. 2. 2 - [methyl ((2-ami noethoxy)] A calcium antagonist is amlodipi ne-4 (2-chlorophenyl)-1,4-dihydro-6-methyl acid carboxylate of 3,5-pyridine 3-ethyl 5-methyl ester. that comes from the chemical dihydropyridine. This drug is often used to treat hypertension and chronic, stable angina. Amlodipine, a calcium channel blocker that belongs to the third generation of dihydropyridines, is categorized as class I in the Biopharmaceutics Classification System (BCS). It is often used to treat hypertension, mostly by causing the smooth muscle in blood arteries to relax, which causes the vasculature to dilate. Inhibiting voltage-gated L-type calcium channels is the mechanism of action, which prevents extracellular calcium from entering cardiac and vascular cells and causes the "slow" influx. 3. Compared to other dihydropyridine derivatives, amlodipine has a decreased incidence of reflex tachycardia and other side effects associated with vasodilation. Additionally, a single daily dosage may be administered due to the drug's slow elimination and extended duration of effect. The gastrointestinal system shows a delayed and almost full absorption profile of amlodipine. The lipid-lowering drug atorvastatin calcium is classified as an HMG-CoA reductase inhibitor and falls within the BCS class II pharmacological category. The creation of mevalonate from HMG CoA, the first and slowest step in the cholesterol manufacturing process, is blocked by statins, such as atorvastatin calcium. It lowers triglyceride and LDL cholesterol levels while increasing HDL cholesterol. 4.

While atorvastatin inhibits the HMG-CoA reductase, amlodipine has a calcium ion antagonist (slow-channel blocker) activity, which accounts for the dual action of the two medications. Transmembrane influx inhibition is the mechanism by which amlodipine, one half of amlodipine/atorvastatin, decreases calcium influx into cardiac muscle and vascular smooth muscle. Amlodipine/atorvastatin functions by selectively and competitively preventing HMG-CoA from forming mevalonate, a precursor of sterols like cholesterol. 5. Only fixed-dose combinations of atorvastatin calcium and amlodipine besylate are marketed as tablets. To the best of our knowledge, no simultaneous technique has been published for their determination. In this message, we describe a novel, rapid, precise, and easy-to-use UV-spectrophotometric technique for concurrently measuring atorvastatin and amlodipine.

MATERIALS AND METHODS

Instrument

A double-beam UV-visible spectrophotometer (Shimadzu-1800) and two matched quartz cells (1-cm) were utilized for recording the absorbance of the solution.

Materials

Amlodipine besylate and atorvastatin calcium were procured as gift samples from Unichem Pharmaceutical and Amoli Labs, respectively.

Solvents

Methanol was used as the solvent. Each and every one of the chemicals and reagents utilized were of analytical quality.

Determination of λ_{max} of Amlodipine Besylate Along With Atorvastatin Calcium

In order to identify the wavelength of maximum absorption for each drug, solutions comprising 10 µg/mL of amlodipine besylate and 10 µg/m L of atorvastatin calcium were appropriately diluted with methanol and then individually screened in the wavelength range of 200 to 400 nm. Amlodipine and atorvastatin both displayed absorbance maxima (Figures 1 and 2). Amlodipine shows at 365 and 238 nm, and Atorvastatin peak was at 246 nm. The overlaid spectra displayed the maximum concentrations of both medicines and iso-absorptive sites at 238 nm.[6,7]

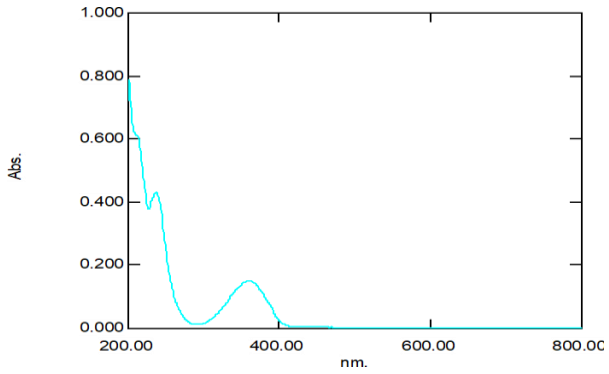


Figure 1: λ_{max} of amlodipine besylate

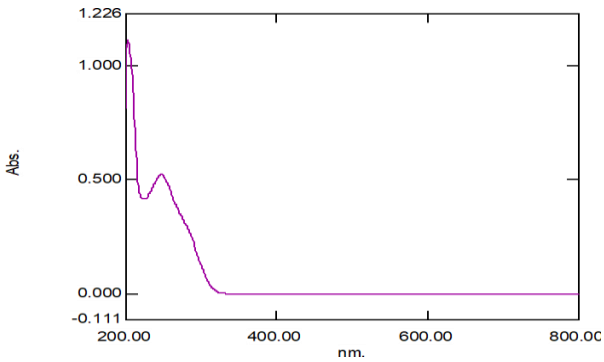


Figure 2: λ_{max} of atorvastatin calcium

Preparation of Standard Drug Solution

Accurately weighed 10 mg of atorvastatin calcium and amlodipine besylate were dissolved in 10 mL methanol in volumetric flasks to obtain a solution concentration as 1000 µg/mL. About 1-mL aliquot from this was diluted 10 mL with methanol in a volumetric flask to obtain 100 µg/mL solution. From these, further dilutions were made so as to obtain solution ranges from 2 to 10 µg/mL.[8-9]

Preparation of Sample Solution

Withdraw 1-mL from the mid-stock solution of amlodipine besylate and atorvastatin calcium in 10 mL volumetric flask and measure the absorbance (Figure 3).[10-11]

Simultaneous Equation Method

The absorption maxima of amlodipine besylate and atorvastatin calcium in methanol were recorded and were found to be at wavelengths of 365 and 246 nm, respectively. These wavelengths were chosen for the simultaneous analysis. By using stock solutions of both drugs, the series of standard solutions having concentrations of 2 to 10 µg/mL were prepared individually using methanol as solvent. The absorbance at each concentration was recorded at the chosen wavelengths, and the absorptivities (A 1%, 1-cm) were calculated for both medicines by taking an average of triplicate determinations.

$$Cx=A_2(ay_1)-A_1(ay_2)/\ ax_2\ ay_1 - ax_1\ ay_2$$
$$Cy=A_2(ax_1)-A_1(ax_2)/\ ax_2\ ay_1 - ax_1\ ay_2$$

Where, A_1 and A_2 are absorbances of mixture at 365 and 246 nm, respectively, ax_1 and ax_2 are absorptivities of atorvastatin calcium at λ_1 and λ_2, respectively and ay_1 and ay_2 are absorptivities of amlodipine besylate at λ_1 and λ_2, respectively. Cx and Cy are concentrations of atorvastatin calcium and amlodipine besylate, respectively.[12]

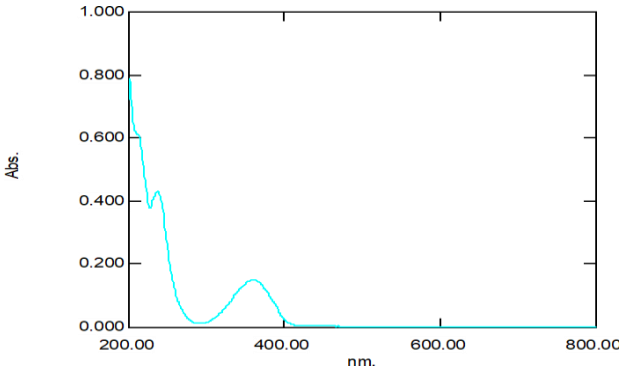


Figure 3: Combined UV spectra

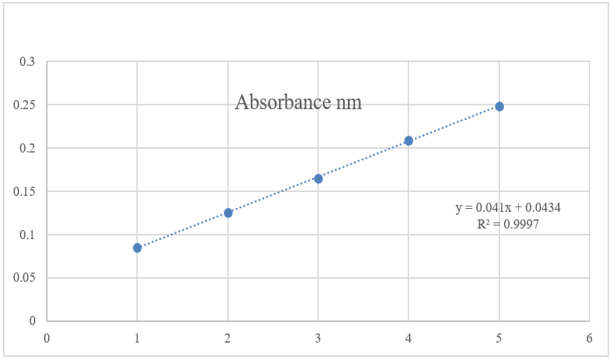


Figure 4: Calibration curve of amlodipine besylate

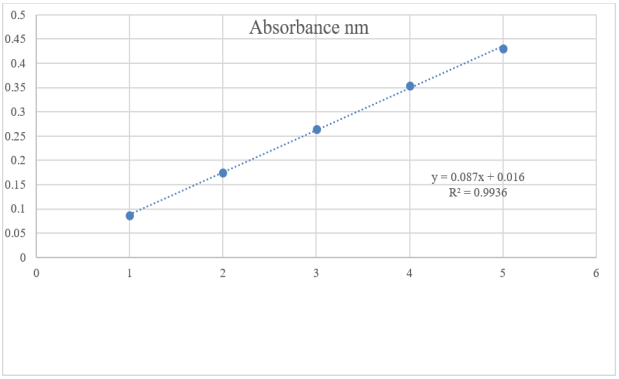


Figure 5: Calibration curve of atorvastatin calcium

RESULT AND DISCUSSION

Method Validation Parameters

Accuracy

Accuracy estimation was carried out by measuring atorvastatin calcium and amlodipine besylate recovery using the traditional addition method. 0.8, 1.0, and 1.2 mL of working standard solution (100 µg/mL) of each drug was added to 1-mL of the test's working sample solution (100 µg/mL of both) in methanol and subsequently diluted to 10 mL. The absorbance of the solution was determined for atorvastatin and amlodipine at a selected wavelength. Triplicate determinations were carried out.

Table 1: Percentage recovery of added substances

Name of drug	*%*	*%recovery of added substances**	*%RSD*
Amlodipine	80	102.23 ± 1.47	1.065
	100	100.56 ± 0.65	
	120	99.78 ± 0.06	
Atorvastatin	80	101.98 ± 0.98	1.208
	100	100.48 ± 0.08	
	120	99.36 ± 0.5	

**Values expressed Mean ± SD, (n = 3)*

Table 2: %Purity of drug of intra-day precision

Name of drug	*Concentration (µg/mL)*	*%Purity of drug*	*%RSD*
Amlodipine	4	99.65 ± 0.47	1.065
	6	101.74 ± 0.52	
	8	98.71 ± 0.08	
Atorvastatin	4	99.78 ± 0.95	1.208
	6	100.87 ± 0.06	
	8	99.58 ± 0.54	

Table 3: %Purity of drug of inter-day precision

Name of drug	*Concentration (µg/mL)*	*%Purity of drug*	*% RSD*
Amlodipine	4	98.54 ± 0.25	0.807
	6	100.25 ± 0.12	
	8	98.561 ± 0.12	
Atorvastatin	4	99.01 ± 0.98	0.351
	6	99.24 ± 0.04	
	8	98.40 ± 0.43	

The simultaneous equation methodology, the absorbance correction method, and the percentage recoveries were used to calculate the dosage of amlodipine and atorvastatin at each level (Table 1).[13]

Accuracy

An adequate statistical analysis was used to establish the procedure's degree of repeatability. For both intra-day and inter-day investigations, the concentrations of both medications were measured three times in a single day. Both the relative standard deviation (RSD) and the standard deviation (SD) were calculated. Tables 2 and 3 provide the findings. 14

The concept of linearity

The quantities of atorvastatin calcium and amlodipine besylate at their respective absorption maxima were plotted against absorbance to create calibration curves and determine the regression equations (Table 4). Plotting of the calibration curves for the medications amlodipine and atorvastatin for solutions with concentrations ranging from 2 to 10 µg/mL was done separately (Figures 4 and 5) and in combination (Figure 6). 15.

Limit of detection (LoD) and limit of quantitation (LoQ)

For an analytical method, LoD is the concentration at which an instrument signal (signal-to-noise ratio of 3) differs

Table 4: Linearity of drugs

Parameters	*Simultaneous equation method (Amlodipine besylate)*	*Simultaneous equation method (Atorvastatin calcium)*	*Simultaneous equation method (Combined)*
λ_{max} (nm)	365	246	238
Beers law limit (µg/mL)	2–10	2–10	2–10
Correlation coefficient	0.9997	0.9936	0.9998
Slope	0.041	0.087	0.2839
Intercept	0.0434	0.016	0.4885
Regression equation	y = 0.041x + 0.0434 R^2 = 0.9997	y = 0.087x + 0.016 R^2 = 0.9936	y = 0.2839x - 0.4885 R^2 = 0.9998

Table 5: LoD and LoQ of drugs

Drug	*LoD*	*LoQ*
Amlodipine besylate	0.145	0.152
Atorvastatin calcium	0.096	0.124

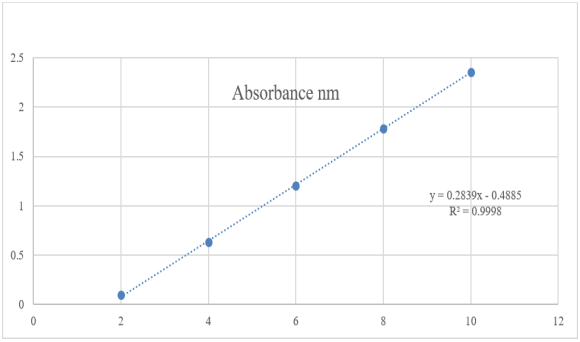


Figure 6: Calibration curve of combined drugs

substantially from the blank (contains a concentration). The LoQ is the concentration that can be accurately and precisely quantifiable reliably with a specific level of signal-to-noise ratio (SNR) (10).

LoD and LoQ were established based on experimental standard deviation and slope (Table 5).[16]

LoD = 3.3 * (σ / S) and

LoQ = 10 * (σ / S)

Where:

σ is the standard deviation

S is the slope of the calibration curve.

Application of developed UV- spectroscopy method

The developed UV- spectroscopy method for simultaneous estimation of amlodipine besylate and atorvastatin calcium can be used to find out the drug content uniformity of drugs and also for the percentage cumulative drug release.[17]

CONCLUSION

The method worked well for calculating the amounts of atorvastatin calcium and amlodipine besylate in a synthetic mixture that included 2.5 mg of amlodipine besylate. and 10 milligrams of calcium atorvastatin. The method's equations were used to directly compute the amounts of both medications. Values for the coefficient of variation and standard deviations were computed. The approaches' accuracy, reproducibility, and repeatability were shown by the decreased standard deviation figures. Reproducibility, dependability, and interference were further validated by recovery trials. As a result, the developed approach was clear-cut, accurate, sensitive, and exact. The results of the analysis of pharmaceutical formulations demonstrate that the proposed approach may be utilized to determine both compounds simultaneously with little interference from typical additives used in pharmaceutical formulations. Therefore, atorvastatin calcium and amlodipine besylate may be estimated concurrently in commercial final goods using the aforesaid approach.

REFERENCES

1. Mali DP, Bhatia NM. Hetero-tricyclic lead scaffold as novel PDE5A inhibitor for antihypertensive activity: *In silico* docking studies. Current Computer-Aided Drug Design. 2019;15(4):318-333.
2. Husseiny RA et al. Fast disintegrating tablet of Valsartan for the treatment of pediatric hypertension: *In vitro* and *in vivo* evaluation. Journal of Drug Delivery Science and Technology. 2018;43:194-200.
3. Naikwade JT, Patil VV, Katkade MH, Thorat VD, Ansari T and Vaidya CR. Formulation and evaluation of fast dissolving tablets of Amlodipine besylate by using co-processed superdisintegrants. Journal of Pharmaceutical Research International. 2013;3(4):865–879.
4. Alshamrani Meshal, et al. Fast dissolving oral films: An approach to co-load and deliver the Atorvastatin and Ezetimibe for better therapeutic response. Pak. J. Pharm. Sci. 2022;35:1229-1239.
5. Messerli FH, et al. Efficacy and safety of coadministered Amlodipine and Atorvastatin in patients with hypertension and dyslipidemia: results of the AVALON trial. The Journal of Clinical Hypertension. 2006;8(8):571-583.
6. Darwish HW, et al. Three different methods for determination of binary mixture of Amlodipine and Atorvastatin using dual wavelength spectrophotometry. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 2013;104:70-76.
7. Kokilambigai KS, Lakshmi KS. Utilization of green analytical chemistry principles for the simultaneous estimation of Paracetamol, Aceclofenac and Thiocolchicoside by UV spectrophotometry. Green Chemistry Letters and Reviews. 2021;14(1):99-107.
8. Srilatha Doddi, et al. Development and validation of UV spectrophotometric method for simultaneous estimation of Hesperidin and Diosmin in the pharmaceutical dosage form. International Scholarly Research Notices. 2013. 2013(2):1-4. Available from: doi.org/10.1155/2013/534830
9. Singh Sunil, Ajit Kumar Yadav, Hemendra Gautam. Simultaneous estimation of Valsartan and Hydrochlorothiazide in solid dosage

form using UV Spectroscopy. Bulletin of Pharmaceutical Research. 2011;1(3):10-2.

10. Sen AK, et al. Analytical method development and validation for simultaneous estimation of Teneligliptin hydrobromide hydrate and Metformin hydrochloride from it's pharmaceutical dosage form by three different UV spectrophotometric methods. Journal of Applied Pharmaceutical Science. 2016;6(9):157-165.
11. Panchale WA, et al. Simultaneous estimation of Salbutamol sulphate and Ambroxol HCl from their combined dosage form by UV-Vis spectroscopy using simultaneous equation method. GSC Biological and Pharmaceutical Sciences. 2020;13(3):127-134.
12. Joshi HV, Patel JK. New spectrophotometric methods for simultaneous determination of Amlodipine besylate and Lisinopril in tablet dosage forms. Journal of Applied Pharmaceutical Science. 2011;1(6):162-164.
13. Patil PR, et al. Simultaneous estimation of Ramipril and Amlodipine by UV spectrophotometric method. Research Journal of Pharmacy and Technology. 2009;2(2):304-307.
14. Haripriya M, Neethu Antony, Jayasekhar P. Development and validation of UV spectrophotometric method for the simultaneous estimation of Cilnidipine and Telmisartan in tablet dosage form utilising simultaneous equation and absorbance ratio method. International Journal of Pharmacy and Biological Sciences. 2013;3(1):343-8.
15. Gholse YN, Chaple DR, Kasliwal RH. Development and validation of novel analytical simultaneous estimation based UV spectrophotometric method for Doxycycline and Levofloxacin determination. Biointerface Research in Applied Chemistry. 2022;12(4):5458-5478.
16. Jani BR, Shah KV, Kapupara PP. Development and validation of UV spectroscopic first derivative method for simultaneous estimation of Dapagliflozin and Metformin hydrochloride in synthetic mixture. J Bioequiv. 2015;1(1):102.
17. Mali DP, Kamble SP, Gaikwad DT, Wadkar GH, Bhutkar MA, Tamboli FA, Pawar VT. Standardization and evaluation of herbal antihypertensive sarpagandha (Reserpine) tablet formulations. International Journal of Pharmaceutical Quality Assurance. 2023;14(1):7-11.