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ABSTRACT: A straightforward, cheapest spectrophotometric technique has been developed to measure both Metoprolol (MET) and Amlodipine (AML) simultaneously in dosage and bulk forms. The technique allows for the collection of data at various wavelengths to produce linear graphs, which is used to calculate concentrations. The absorbance values of the two analytes were linear with the concentration at the wavelengths taken at 10 nm interval over the range of 230 -300 nm. The accuracy and the precision of the new approach demonstrated were very good (RSD < 2%). Developed method was validated in terms of linearity, range, precision, accuracy. The suitability of all methods for quantitative determination of these compounds was confirmed by validation in accordance with the requirements of ICH Guidelines.

KEYWORDS: Spectrophotometry, Multi-wavelength, Regression analysis, Metoprolol, Amlodipine

Introduction[1][2][3]

There are variety of intravenous and oral formulations available in market for the treatment of hypertension, angina, heart failure, myocardial infarction, and atrial fibrillation/flutter. The choice of beta-blockers for the treatment of the aforementioned disorders is a topic of debate. Metoprolol is a cardio selective beta-1-adrenergic receptor inhibitor that competitively blocks beta1 receptors with minimal or no effects on beta-2 receptors and decreases cardiac output by negative inotropic and chronotropic effects. Administration of metoprolol to normal subjects results in a reduction in heart rate and cardiac output; this appears to be related to the dose and concentration of the drug.

Amlodipine inhibits the initial calcium influx by blocking the voltage-dependent L-type calcium channels. Vasodilation is the outcome of enhanced smooth muscle relaxation and decreased vascular smooth muscle contractility caused by reduced intracellular calcium. Amlodipine has also been shown to enhance vascular endothelial function in hypertensive

individuals. Amlodipine lowers blood pressure by relaxing smooth muscles and widening blood vessels.

They work together to improve the heart's efficiency in pumping blood throughout the body.

Literature search revealed that different methods have been developed for the simultaneous determination of MET and AML as mixture as well as in dosage form; absorbance ratio technique and difference spectrophotometric method, derivative spectrophotometric, simultaneous estimation methods as well as chemo metric spectroscopic method. The observed resolution between the individual absorption peaks of the two sample components is the only factor that influences the analytical approach chosen for the simultaneous spectrophotometric measurement of these components. In cases where the absorption peaks can be well resolved, this determination is straightforward. However, chemometric procedures become necessary if there is partial or complete overlap between the signals of the various components. These methods necessitate full-spectrum data and highly specialized software for the processing of the spectral data. Therefore, this research work's goal was to create a straightforward, affordable, and precise spectrophotometric technique based on multi-wavelength regression analysis for the simultaneous measurement of amlodipine and metoprolol in tablet and bulk dose forms.

Theoretical Consideration:[18]

Multi-Wavelength Linear Regression Analysis; enables determination of the composition of a binary mixture with overlapping spectra without determining molar absorptivities or complicated mathematics. The method is very simple it requires only three measurements, the absorbance of a standard solution for each component, and the unknown mixture itself. Assuming additivity, the absorbance of a mixture is the sum of the absorbencies of its components. If we have a mixture consisting of two components, X and Y, with an unknown concentration of Cx and Cy, then the absorbance of the unknown mixture,

However, the absorbencies of standard solutions of the same substances will follow the same Beer’s law relationship and have the same molar absorbance, Є, and one centimetre path length, b, as the unknown solutions under the same conditions.



Cmix = Cx+Cy Csx (standard X) Csy (standard Y)

$A_{mix} = A_x + A_y$ $Asx = €_x bCs_x$ ---------1 $Asy = €_y bCs_y$

$A_{mix} = €_x bC_x + €_y bCy$ -------2 $€_x = Asx/bCs_x$ -------3 $€_y = Asy/bCs_y$---------4

Diving eqn 2 by Asx Diving equation 3 by 4, $\frac{€y}{€x} = \frac{Asy}{Asx} \cdot \frac{Csx}{Csy}$ -------- 5

$\frac{Amix}{Asx} = \frac{€xbCx}{Asx} + \frac{€ybCy}{Asx}$ [substituting Asx from eqn 1]

$\frac{Amix}{Asx} = \frac{€xbCx}{€xbCsx} + \frac{€ybCy}{€xbCsx}$

$\frac{Amix}{Asx} = \frac{Cx}{Csx} + \frac{€y}{€x} \cdot \frac{Cy}{Csx}$ [substituting $\frac{€y}{€x}$ from eqn 5]

$\frac{Amix}{Asx} = \frac{Cx}{Csx} + \frac{Asy}{Asx} \cdot \frac{Csx}{Csy} \cdot \frac{Cy}{Csx}$

$$\frac{Amix}{Asx} = \frac{Cx}{Csx} + \frac{Asy}{Asx} \cdot \frac{Cy}{Csy}$$

Comparing eqn 6 with regression equation, $Y = \frac{Amix}{Asx}$ $slope = \frac{Cy}{Csy}$

$X = \frac{Asy}{Asx}$ $intercept = \frac{Cx}{Csx}$

From eqn 7 and eqn 8, **Cx = Intercept * Csx** and **Cy = Slope * Csy**

$$\frac{Amix}{Asx} = \frac{Cx}{Csx} + \frac{Asy}{Asx} \cdot \frac{Cy}{Csy}$$

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From eqn 7 and eqn 8, **Cx = Intercept * Csx** and **Cy = Slope * Csy**

That is, the concentration of the unknown component Y (Cy) in the mixture, equals the slope times the concentration of the standard solution for component Y. Likewise, the concentration of the unknown component X (Cx) in the mixture equals the product of the intercept times the concentration of the standard solution for component X.

Materials and Methods

Instruments

Spectroscopic analysis was carried out on a UV- VISIBLE 1900 (Shimadzu) double beam UV-Visible spectrophotometer with software of UV probe version 2.70. The absorption spectra were recorded over the wavelength range of 200-400nm, against solvent blank in quartz cuvettes with 1cm diameter. Data analysis was performed using Microsoft Excel Spreadsheet 2016.

Materials

Active Pharmaceutical Ingredient (API) Metoprolol and Amlodipine was received as gift sample from Torrent Pharma. Marketed formulation MPROL AM 25/5 tablet was purchased from local medical store.

Standards and solutions:

Preparation of standard stock solution of Metoprolol:

Weigh 25mg of Metoprolol and dissolved in 25 ml of methanol. Make up the volume up to mark with methanol to get a solution containing 1000ppm Metoprolol. Now take 2.5ml of standard stock solution and dilute up to 25ml with methanol to get a solution containing 100ppm Metoprolol. Take 0.5ml, 1ml, 1.5ml, 2ml, 2.5ml from 100ppm stock solution of Metoprolol and transfer to five different volumetric flask of 10ml to get 5,10,15,20,25 ppm of Metoprolol solution respectively.

Preparation of standard stock solution of Amlodipine by Standard Addition Method:

Weigh 25mg of Amlodipine and dissolved in 25ml of methanol. Make up the volume up to mark with methanol to get a solution containing 1000ppm Amlodipine. Now take 2.5ml of standard stock solution and dilute up to 25ml with methanol to get a solution containing 100ppm Amlodipine. Take 0.1ml, 0.2ml, 0.3ml, 0.4ml, 0.5ml from 100ppm stock solution of Amlodipine and transfer to five different volumetric flask, add additional 1ml of standard stock solution(100ppm) of Amlodipine to each flask and then make up the volume up to 10 ml with methanol to get 11,12,13,14,15 ppm of Amlodipine solution respectively.

Preparation of sample preparation

Twenty tablets were accurately weighed and crushed to a fine powder; the weight of powder equivalent to one tablet was transferred into a 25mL volumetric flask, dissolved with the methanol to get a solution containing 1000ppm Metoprolol and 200ppm Amlodipine. Take 0.05, 0.1, 0.15, 0.2 and 0.25 ml of above solution in five different volumetric flasks of 10ml and make up the volume up to the mark with methanol to get 5,10,15,20,25 ppm metoprolol and 11,12,13,14,15 Amlodipine solution respectively.

For the application of the method, the ratio of the absorbance of mixture and sample absorbance of amlodipine analytical standard solution at each wavelength was calculated and plotted against the ratio of absorbance values obtained for amlodipine analytical standard solution to the same Metoprolol standard solution used in the first instance.The slope and intercept of the straight

line obtained were then used to calculate the concentration of each analyte in the mixture or the sample.

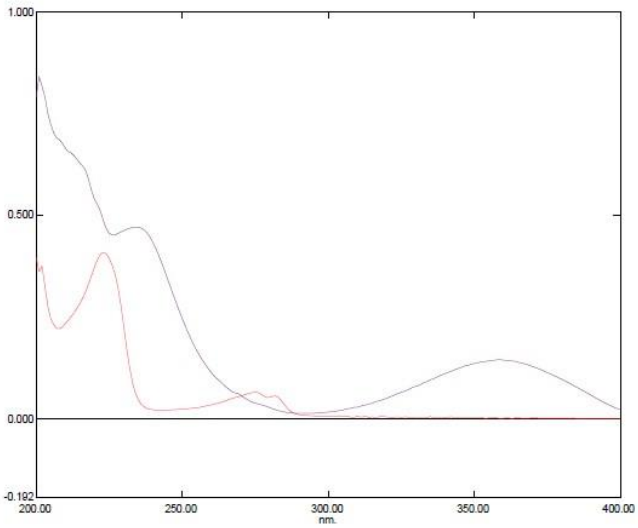

Figure 1: Absorption spectra of (a) Metoprolol 10 µg/ml and (b) Amlodipine 12 µg/ml.

Methods

Spectral characteristics:

The individual spectra of the analytes were obtained by scanning their corresponding working standard solutions over the wavelength range of 230-300 nm.

Linearity

The absorbances of the linearity standard solutions were read at 10 nm intervals over the wavelength range of 230-300 nm

General procedure :

The absorbance values of the two working standards, synthetic mixtures and samples were read at10 nm intervals over the wavelength range of 230-280 nm. The concentrations of MET and AML in the synthetic mixtures and the samples were calculated according to the MLRA principle using the slopes and intercepts of the straight lines obtained.

Results and discussion

Spectral characteristics

The individual spectra of the analytes showed extensive overlapping over the wavelength range of 230-300 nm (Fig 1);hence resolutions of their photometric signals by classical spectrophotmetric methods are not possible.

Linearity

Application of MLRA method requires obeying Beer's law at each wavelength over the selected wavelength range. The two analytes showed linear relation between the concentration and absorbance over the entire wavelengths range investigated (r2 < 0.99).

Table 1 : Linearity data of Metoprolol

PARAMETER	WAVELENGTH					
	220	**230**	**240**	**250**	**260**	**270**
b	0.0389	0.0241	0.0027	0.0027	0.0036	0.0055
a	-0.0124	-0.0122	-0.0045	-0.0026	-0.0026	-0.0026
r^2	0.9984	0.9982	0.9935	0.9947	0.9947	0.9977
S_b	0.0005	0.0009	0.0007	0.0009	0.0008	0.0009
S_a	0.0019	0.0028	0.0007	0.0009	0.0003	0.0008
LOD	0.1612	0.3834	0.8556	1.1000	0.2750	0.4800
LOQ	0.4884	1.1618	2.5926	3.3333	0.8333	1.4545

b slope, a intercept, r^2 correlation coefficient, S_b Standard deviation of slope, S_a Standard deviation of Intercept, LOD limit of detection, LOQ limit of Quantitation.

Table 2 : Linearity data of Amlodipine

PARAMETER	WAVELENGTH					
	220	**230**	**240**	**250**	**260**	**270**
b	0.4545	0.0491	0.0352	0.0156	0.0069	0.0058
a	0.0264	-0.1237	0.0302	0.0792	0.0409	-0.0096
r^2	0.9905	0.9927	0.994	0.9941	0.9853	0.9929
S_b	0.002	0.0035	0.0044	0.0007	0.0005	0.0003
S_a	0.0008	0.0009	0.0007	0.0009	0.0003	0.0004
LOD	0.1454	0.2352	0.4125	0.1481	0.2391	0.1707
LOQ	0.4405	0.7128	1.2500	0.4487	0.7246	0.5172

b slope, a intercept, r^2 correlation coefficient, S_b Standard deviation of slope, S_a Standard deviation of Intercept, LOD limit of detection, LOQ limit of Quantitation.

Determination of synthetic mixtures (Accuracy)

The accuracy of the proposed method was tested by analyzing laboratory prepared synthetic mixtures containing different concentration ratios of METOPROLOL and AMLODIPINE at four different level i.e. at 0%, 80%, 100% and 120%. The results obtained indicated good accuracy of the method supporting the suitability of its application for the analysis of the two drugs in Tablet

Table 3: Accuracy data of Metoprolol and Amlodipine

SAMPLE	**METOPROLOL**			**AMLODIPINE**		
	Theoretical Yield	**Practical Yield**	**% Recovery**	**Theoretical Yield**	**Practical Yield**	**% Recovery**
M1	10	10.1	101	12	12.24	102
M2	18	18.52	101.88	13.6	13.8	101.47
M3	20	20.2	101	14	14.01	100.07
M4	22	22.95	99.77	14.4	14.5	100.28
		Average	100.912		Average	100.955
		SD	0.8673		SD	0.9302
		% RSD	0.8594		% RSD	0.92145

Method precision

a. Repeatability: The standard solution containing 10ppm Metoprolol and 12ppm Amlodipine was prepared six times in a day using same apparatus, instruments and chemical. Then absorbance was noted and %RSD was calculated.

Table 4: Repeatability data

Wavelength	METOPROLOL ABSORBANCE(MEAN)(n=6) ± SD		AMLODIPINE ABSORBANCE(MEAN)(n=6) ± SD	
	10 ppm	**%RSD**	**12 PPM**	**%RSD**
220	0.373±0.0008	0.2019	0.540±0.0043	0.7934
230	0.227±0.0008	0.3319	0.463±0.0014	0.3054
240	0.022±0.0010	0.6625	0.434±0.0005	0.1191
250	0.023±0.0003	1.1308	0.246±0.0009	0.3636
260	0.034±0.0006	1.0114	0.114±0.0012	1.0791
270	0.055±0.0005	1.1499	0.058±0.0008	1.2942

b. Intermediate Precision: Six runs of standard 15ppm Metoprolol and 13ppm Amlodipine was prepared for intermediate precision. Absorbance was noted and %RSD was calculated.

Table 5: Metoprolol and amlodipine intermediate precision data

Wavelength	METOPROLOL ABSORBANCE(MEAN)(n=3) ± SD					
	10 ppm	**%RSD**	**15 ppm**	**%RSD**	**20 ppm**	**%RSD**
220	0.373± 0.0010	0.2268	0.5660± 0.0010	0.1767	0.7477± 0.0006	0.0772
230	0.226± 0.0006	0.2547	0.3437± 0.0006	0.1680	0.4603± 0.0012	0.2508
240	0.021± 0.0010	0.6891	0.3100± 0.0100	0.1854	0.0443± 0.0007	1.3023
250	0.022± 0.0006	1.2642	0.0320± 0.0010	1.7856	0.0467± 0.0006	1.2372
260	0.03± 0.0006	0.8872	0.0467± 0.0058	1.1395	0.0693± 0.0010	0.8327
270	0.055± 0.0010	1.0434	0.0820± 0.0010	1.2195	0.113± 0.0006	0.5186
Wavelength	AMLODIPINE ABSORBANCE(MEAN)(n=3) ± SD					
	12 ppm	**%RSD**	**13 ppm**	**%RSD**	**14 ppm**	**%RSD**
220	0.539± 0.0015	0.0965	0.601± 0.0015	0.2543	0.653± 0.0015	0.2338
230	0.462± 0.0010	0.2165	0.551± 0.0010	0.1815	0.555± 0.0010	0.1802
240	0.434± 0.0010	0.1331	0.471± 0.0010	0.2123	0.514± 0.0010	0.1946
250	0.246± 0.0026	0.4065	0.272± 0.0026	0.9727	0.338± 0.0010	0.2959
260	0.113± 0.0010	1.3478	0.139± 0.0010	0.7194	0.136± 0.0015	1.1259
270	0.059± 0.0010	1.6949	0.063± 0.0010	1.5873	0.07± 0.0010	1.4286

Analysis of commercial sample:

Twenty tablets were accurately weighed and crushed to a fine powder; the weight of powder equivalent to one tablet was transferred into a 25mL volumetric flask, dissolved with the methanol to get a solution containing 1000ppm Metoprolol and 200ppm Amlodipine. Now take 0.05, 0.1, 0.15, 0.2 and 0.25 ml of above solution in five different volumetric flasks and add 1 ml of standard amlodipine (10 ppm) and make up the volume up to the mark with methanol to get 5,10,15,20,25 ppm metoprolol and 11,12,13,14,15 ppm amlodipine solution respectively. **Table 6: Sample Assay Result**

SR. NO.	Concentration (ppm)		Regression Equation (y=mx + c)	Intercept (c)	Slope (m)	Concentration of Metoprolol (ppm) [C_x = Intercept*C_{sx}]	Concentration of Amlodipine (ppm) [C_y = Slope*C_{sy}]
	METO (C_{sx})	AMLO (C_{sy})					
1	**5**	**11**	Y = 0.9988x + 1.0667	1.2667	0.9788	5.333	10.986
2	**10**	**12**	Y = 1.0242x + 1.0095	1.0095	1.0242	10.095	12.290
3	**15**	**13**	y = 1.009x + 0.998	0.998	1.009	14.97	13.117
4	**20**	**14**	y = 1.0344x + 1.0305	1.0305	1.0344	20.61	14.481
5	**25**	**15**	y = 1.031x + 1.0095	1.0095	1.031	25.237	15.465

Conclusion:

Multi-wavelength Linear Regression Analysis is a straightforward procedure allowing the accurate resolution of binary mixtures of compounds with overlapped spectra without prior separation, the method is very simple requires only measurement of absorbance values of the two standards and the sample. The concentrations of the two analytes are calculated using easy to understand simple mathematics. The cost effectiveness in term of time and money renders the method as suitable alternative to other expensive techniques e.g., chromatographic methods for the analysis of binary mixtures of compounds with overlapped spectra in laboratories and countries where such sophisticated equipment are not affordable. The accuracy and simplicity of the method suggest it suitability in cases where quick results are demanded e.g., as in-process analysis procedure during blend analysis in industrial setups.

Reference:

1) Dixit, A., & Dhakad, P. K. "Hypertension: an overview". *European Chemical Bulletin*, **2017,** *6*(9), 393-399
2) Webster, H. "*What types of hypertensions are there?"* **2023, October 27.**

3) Webster, H. "*What types of hypertensions are there?"* https://www.medicalnewstoday.com/articles/types-of-hypertension#other-types. **2023, October 27.**

4) Edwards, J. M. "*12 causes of high blood pressure (and how to prevent it)"*. Healthline, **2022, December 9.**

5) Giles, T. D., Materson, B. J., Cohn, J. N., & Kostis, J. B. "Definition and Classification of Hypertension: An update." *Journal of Clinical Hypertension*, **2009,** *11*(11), 611–614.

6) Harrison, D. G., Coffman, T. M., & Wilcox, C. S.). Pathophysiology of hypertension. *Circulation Research*, **2021,** *128*(7), 847–863.

7) Wikipedia contributors. "Pathophysiology of hypertension*"*, **2023, December 27,** *Wikipedia*.

8) Oparil, S., Acelajado, M. C., Bakris, G. L., Berlowitz, D. R., Cífková, R., Dominiczak, A. F.,

Grassı, G., Jordan, J., Poulter, N., Rodgers, A., & Whelton, P. K. "Hypertension. *Nature Reviews Disease Primers"*, **2018,** *4*(1).

9) Unger, T., Borghi, C., Charchar, F. J., Khan, N., Poulter, N., Prabhakaran, D., Ramiréz, A. J., Schlaich, M. P., Stergiou, G. S., Tomaszewski, M., Wainford, R. D., Williams, B., & Schutte, A. E. 2020 International Society of Hypertension Global Hypertension Practice Guidelines. *Hypertension*, **2020,** *75*(6), 1334–1357.

10) Taler, S. J. "Initial treatment of hypertension". *The New England Journal of Medicine*, **2018,** *378*(7), 636–644.

11) Brodde, O. "Beta-adrenoceptors in cardiac disease". *Pharmacology & Therapeutics*, **1993,** *60*(3), 405–430.

12) Burges, R. A., Dodd, M. G., & Gardiner, D. G. "Pharmacologic profile of amlodipine". *The American Journal of Cardiology*, **1993,** *64*(17), I10–I20.

13) Hallett, L. T. "Modern Instrumental Methods of Analysis - A Symposium." *Analytical Chemistry*, **1948,** *20*(4), 389–391.

14) Lin, S. Y. "Ultraviolet spectrophotometry". In *Springer series in wood science*, **1992,** (pp. 217–232).

15) Limniou, M., Papadopoulos, N., & Roberts, D. "An integrated lecture, virtual instrumentation lab approach to teaching UV-Vis spectroscopy." *Education and Information Technologies*, **2007,** *12*(4), 229–244.

16) Saini, V., Chaudhary, J., & Jain, A. "Simultaneous Estimation of multicomponent formulations by UV-Visible Spectroscopy: An Overview", *International Research Journal of Pharmacy,* **2011** *2*(12):81-83

17) Mukherjee, P., Chakraborty, D. D., Chakraborty, P., Shrestha, B., & Bhuyan, N. R. "Different ultraviolet spectroscopic methods: a retrospective study on its application from the viewpoint of analytical chemistry". *Asian Journal of Pharmaceutical and Clinical Research*, **2021,** 1–11.

18) "International Journal of Advances in Pharmaceutical Analysis". *International Journal of Advances in Pharmaceutical Analysis*, **2017;** 07(02): 16-20.

19) Jindal, D., Kaur, H., Patil, R. K., & Patil, H. C. "Validation – In pharmaceutical industry: Equipment validation: A brief review." *Journal of Medical Science and Research*, **2020,** *2*(2), 94–98.

20) Ermer, J. "Validation in pharmaceutical analysis. Part I: An integrated approach." *Journal of Pharmaceutical and Biomedical Analysis*, **2020,** *24*(5–6), 755–767.

21) *Metoprolol: Uses, interactions, mechanism of action | DrugBank Online*. (n.d.). DrugBank.

22) *Amlodipine: Uses, interactions, mechanism of action | DrugBank Online*. (n.d.). DrugBank.

23) S. A. Pagar, D.M. Shinkar, R.B. Saudagar, "Development and validation of spectrophotometric method for determination of metoprolol succinate", *Pharmaceutical Science*, **2016,** 3(4), 224228.

24) Chaitali Dhale, Suhas Joshi, Shubhangi Shete, "Development and validation of spectrophotometric method for determination of metoprolol tartrate"**,** *J App Pharm*, **July, 2014**, Vol. 6; Issue 3: 280 -285.

25) Jadhav A. S., Tarkase K. N. and Deshpande A.P., "Quantitative Determination of Metoprolol Succinate in bulk and tablet Dosage form through comparative study of UV and derivative Spectroscopy", *Scholars Research Library*, **2012**, 4 (3):763-767

26) Viral Bechara, EVS Subrahmanyam and Ramakrishna Shabaraya, "New analytical methods and their validation for the estimation of metoprolol succinate in bulk and marketed formulation," *International Journal of Pharma Sciences and Research (IJPSR),* **2 Feb 2015,** Vol. 6 No., 425428.

27) "Development and validation of spectrophotometric and RP-HPLC method for determination of metoprolol succinate". *International Journal of ChemTech Research*, **2018,** Vol.11 No.05, pp 179-185.

28) Yadav Sanjay Kumar, Malipatil S. M. and Gupta S. K., "Development and validation of uv spectrophotometric method for estimation of metoprolol in bulk and tablet dosage form", *world journal of pharmaceutical research,* **2014,** *8(9), 919-928.*

29) Donchenko, A. O., & Vasyuk, S. O. "Spectrophotometric determination of metoprolol tartrate in pure and dosage forms". *Ankara Üniversitesi Eczacılık Fakültesi Dergisi*, **2018,** *42*(1), 33–
42.

30) Dhruvin M. Prajapati, Apexa Kadam and Dr. Rajashree Mashru, "Analytical method development and validation for simultaneous estimation of Azelnidipine and metoprolol succinate from the synthetic mixture by three different uv spectrophotometric methods", *WORLD JOURNAL OF PHARMACEUTICAL RESEARCH,* **2016,** *11(10), 785-798.*

31) Vachhani Kevin H and Patel Satish A, "Development and validation of spectrophotometric method for simultaneous estimation of metoprolol succinate and olmesartan medoxomil in tablet", *Journal of Applied Pharmaceutical science,* **2011,** (07) 112-115.

32) Sitaram Atmaji Naik, E.V.S. Subrahmanyam, Paramita Das, A.R. Shabaraya, "New method development and validation for determination of ramipril and metoprolol succinate in bulk and marketed formulation", *International Journal of Chemical and Pharmaceutical Analysis, **2018,** 5(3), 1-12.*

33) K. Suresh kumar, r. Ravikumar, a. Rajasekaran, v. Ravichandran "Simultaneous spectrophotometric determination of Metoprolol tartrate and Ramipril", *Digest Journal of Nanomaterials and Biostructures,* **March 2010,** Vol. 5, No 1, p. 173 – 176.

34) Alekhya, B., Sindhusha, M. D., Raul, S. K., & Padhy, G. K. "A new validated third order derivative spectroscopic method for simultaneous estimation of metoprolol succinate and ramipril in tablet dosage form." *International Journal of Pharmacy and Pharmaceutical Sciences*, **2020,** 54–59.

35) Patel, K., Patel, A., Dave, J. B., & Patel, C. N. "Absorbance correction method for estimation of telmisartan and metoprolol succinate in combined tablet dosage forms." *Pharmaceutical Methods*, **2012,** *3*(2), 106–111.

36) Seema M. Dhole, Dinesh R. Chaple, Madhulika T. Harde, "Validated UV Spectrophotometric

Method for Simultaneous Estimation of Metoprolol Succinate and Amlodipine Besylate in Their Combined Tablet Dosage Form", *International Journal of Analytical and Bioanalytical Chemistry*, **2013**; 3(3): 82-85.

37) Chitlange, S. S., Bhusal, R. D., Nikumbh, M. B., & Bhole, R. P. "Development and validation of spectrophotometric and stability indicating rp-hplc method for the simultaneous estimation of metoprolol succinate and hydrochlorothiazide in tablet dosage form." *International Journal of Pharmacy*, **2012,** *2*(3), 591–597.

38) Tushar k. Kadia1, Mr. Darshil b. Shah2, dr. Dilip G M., development and "Validation of qabsorbance ratio spectrophotometric method for simultaneous estimation of cilnidipine and metoprolol succinate in bulk and combined dosage form," *International Journal of Pharmacy and Pharmaceutical Sciences*, **2014,** Vol 6, Issue 6, *401-407.*

39) Madhuri A. Hinge, Dhwani K. Desai, Ekta S. Patel, Rashmi Singh, Rima Chavda and Dhaval Patel, "Development and validation of UV spectrophotometric method for simultaneous estimation of cilnidipine and metoprolol succinate in bulk drugs and combined dosage form", *Der Pharmacia Lettre*, **2015**, 7 (7):299-306

40) Harshkumar B. Pandya1 and Dr. Parula B. Patel, "Development and validation of analytical methods for the simultaneous estimation of metoprolol succinate, chlorthalidone and cilnidipine in tablet dosage form", *world journal of pharmacy and pharmaceutical sciences*, Volume 7, Issue 5, 945-963**.**

41) BH. Rajesh Varma1, Praveen Kumar Jampana, G. Raveendra Babu, P. Sri Lakshmi Surekha, T.

Kala Praveen and P. Sambhasiva Rao, "Uv spectroscopic method for estimation of amlodipine besylate in tablets", *international journal of pharmaceutical, chemical and biological sciences*, **2014**, 4(1), 69-73.

42) Safila Naveed, Hina Qamar, Wardha Jawaid, Urooj Bokhari. Simple UV Spectrophotometric Assay of Amlodipine. *American Journal of Chemistry and Application.* **2014,** Vol. 1, No. 4, pp. 66-69.

43) Narvekar V T., Kapase A.R., Chavan A b., Derkar G K., Bhat M R., Chimkode R.M., "Method development, validation and stability study of amlodipine in marketed formulation by uv spectrophotometric method", **2017**; Volume 6(5): 1-25.

44) Bina Gidwani, Laxmikant Patel, Anshita Gupta and Chanchal Deep Kaur, "Ultra Violet Spectrophotometric Method for Estimation and Validation of Amlodipine in Bulk and Tablet Formulation", *Journal of Analytical & Pharmaceutical Research***, 2017,** Volume 4 Issue 6, 1-
13.

45)Murat Ozturk Yucel Kadioglu1 "Quantitative determination of Amlodipine Besylate without
Derivatized in Pure Form and Tablet Dosage Forms with UV Spectrophotometric Method," *International Journal of PharmATA*, *1*(1), 1–10.

46)Bernard, S., Mathew, M., & Senthilkumar, K. "Spectrophotometric method of estimation of Amlodipine besylate using hydrotropic solubilization." *Journal of Applied Pharmaceutical Science*, **2011,** *1*(9), 177–180.

47) VP, P., Devdhe, S. J., Angadi, S. S., Shelke, S., Jadhav, V. R., Kawde, R. V., & H, K. S. "New ecofriendly validated spectrophotometric method for the estimation of amlodipine besylate in bulk drug using ninhydrin." *Asian Journal of Biomedical and Pharmaceutical Sciences*, **2013,** *3*(16), 14–19

48) Mishra, P., Gupta, A., & Shah, K. "Spectrophotometric Methods for Simultaneous Estimation of Nebivolol Hydrochloride and Amlodipine besylate in Tablet Dosage Form." *International Journal of Pharmacy and Pharmaceutical Sciences*, **2009,** *1*(2), 55–61.

49) Badran, A., & Hammad, S. "Simultaneous Estimation of Telmisartan and Amlodipine by Second Derivative Spectrophotometric Method and First Derivative Ratio Spectrophotometric Method." *American Journal of Pharmaceutical Research*, **2015,** *5*(2), 172–189.

50) Kavathia, A., Suchak, K., & Misra, M. "Application of absorption correction method and first order derivative spectrophotometry for simultaneous determination of anti-hypertensive combination (amlodipine and benazepril)." *International Journal of Pharmacy and Pharmaceutical Sciences*, **2013,** *5*(3), 371–375.

51) Anandakumar, K., & Jothieswari, D. "Absorption correction method for estimation of amlodipine besylate, valsartan and hydrochlorothiazide in bulk and in combined tablet dosage form." *International Journal of Pharmacy and Pharmaceutical Sciences*, **2011,** *3*(3), 30–34.

52) Pathak, S., Pradan, K., Meshram, B., & Patel, A. "UV spectroscopic method for simultaneous estimation of Celecoxib and Amlodipine." *PHARMAWAVE*, **2016,** *10*(17), 48–55.

53) Kumar, M., Mohit Jindal, Shailendra Bhatt, A. Pandurangan, Anuj Malik, Vichitra Kaushik,
Prabhat Kumar Upadhaya, & G. Arunachalam. "Simultaneous Estimation of Amlodipine Besylate and Ramipril in Tablets Dosage Form by UV Spectrophotometric Method." *Journal of Pharmaceutical Science and Research*, **2019,** *11*(2), 667–670.

54) P. Y. Pawar, B. Y. Mane, Sumit M. Auti, & Vipul V. Trivedi. "Simultaneous estimation of amlodipine besylate and atenolol in combined dosage form by Vierodt's method using U.V. spectroscopy." *Der Pharma Chemica*, **2013,** *5*(2), 97–102.

55) M.S. Kondawar, K.G. Kamble, K.S. Raut, & K.H. Maharshi. "UV Spectrophotometric Estimation of Amlodipine Besylate and Telmisartan in Bulk Drug and Dosage Form by Multiwavelength Analysis." *International Journal of ChemTech Research*, **2009,** *3*(3), 1274– 1278.

56) Prasad, S., & Pallavi, M. P. "Stability Indicating Spectrophotometric Method of Amlodipine and Telmisartan in Bulk and Pharmaceutical Dosage Form." *Int. J. Pharm. Sci. Rev. Res.*, **2014,** *26*(2), 04,19-24.

57) Attimarad, M., Venugopala, K. N., Al-Dhubiab, B. E., Nair, A. B., Sreeharsha, N., Shinu, P., &
Akrawi, S. H. "Development of UV spectrophotometric procedures for determination of amlodipine and celecoxib in formulation: Use of scaling factor to improve the sensitivity." *Journal of Spectroscopy*, **2019**, 1–10.

58) Kavathia, A., & Misra, M. "Development and validation of RP-HPLC and UV-spectrophotometric methods for rapid simultaneous estimation of amlodipine and benazepril in pure and fixed dose combination." *Arabian Journal of Chemistry*, **2017,** *10*, S3021–S3028.

59) Seema M. Dhole, Dinesh R. Chaple, & Madhulika T. Harde. "Validated UV Spectrophotometric
Method for Simultaneous Estimation of Metoprolol Succinate and Amlodipine Besylate in Their Combined Tablet Dosage Form." *International Journal of Analytical and Bioanalytical Chemistry*, **2011,** *3*(3), 82–85.

60) Hirpara, K. P., Davé, V., & Faldu, S. "UV-Spectrophotometric Determination for Simultaneous

Estimation of Amlodipine Besylate and Telmisartan in Combination". **2012**

61) Manish A. Raskar, Ashok B. Chitale, & Pritam D. Giri. "Validated Simultaneous Derivative Spectrophotometric Estimation of Telmisartan, Hydrochlorothiazide and Amlodipine Besylate in Combination Tablet Dosage Form." *International Journal of Pharmacy and Pharmaceutical Research*, **2015,** *4*(1), 39–45.

62) Sasidhar, R. L. C., Vidyadhara, S., Deepti, B., Tejaswi, K., & Suhasini, J. "A validated UV spectrophotometric method for the simultaneous estimation of hydrochlorothiazide, amlodipine besylate and telmisartan in bulk and combined tablet dosage form." *Der Pharmacia Lettre*, **2014,** *6*(4), 449–455

63) Anandakumar, K. "Absorption correction method for estimation of amlodipine besylate, valsartan and hydrochlorothiazide in bulk and in combined tablet dosage form"., **2014,** *3*(3), 1032.

64) Indian Pharmacopoeia **2010**, vol I, 147.

65)Kamal, A. H., Hammad, S. F., & Kamel, D. N. "Chemometric spectroscopic method for simultaneous estimation of metoprolol succinate and amlodipine besylate in their tablet formulation." *ScienceDirect*, **2021,** *254*.

66)Chabukswar, A. R., Tambe, S. D., Sharma, S. N., & Chaudhari, V. P. "Ratio derivative spectrophotometry method for simultaneous estimation of metoprolol and amlodipine in their combined dosage form." *ResearchGate*, **2012,** *5*(7), 950–954.

67) Hapse, S. A., Mogal, S. A., Kamod, A. C., & Bhagat, B. V. "Spectrophotometric estimation and validation of metoprolol succinate and amlodipine besylate by different method from pure and tablet dosage form." *ResearchGate*, **2013,** *5*(1), 126–131.

68) CHAUDHARY, J., JAIN, A., KAUR, N., & SAINI, V. "Development and validation of simultaneous equation method for determination of metoprolol and amlodipine in combined dosage form." *Academic Sciences*, **2011,** *4*(3), 191–194.

69) Dhale, C., Joshi, S., & Shete, S. "Development and validation of spectrophotometric method for determination of metoprolol tartrate." *J Appl Pharm*, **2014**, *6*(3), 280-5.

70) Vora, B. N., Parmar, R. R., Nayak, P. P., & Shah, D. A. "Development and validation of the simultaneous UV spectrophotometric method for estimation of metoprolol succinate

and olmesartan medoxomil in the tablet dosage form. *Pharmaceutical methods",* **2012**, *3*(1), 44-47.

71) Shelar, P., Nazareth, C., Khorjuwenkar, R., Pinto, O., Kamat, G., & Naik, B. "A novel uv spectrophotometric method for simultaneous estimation of metoprolol tartrate and atorvastatin calcium based on absorbance correction principle." *Rasayan Journal of Chemistry,* **(2022)**, *15*(4).

72) Vachhani, K., & Patel, S. A. "Development and Validation of first order derivative spectrophotometric Methods for simultaneous estimation of metoprolol succinate and olmesartan medoxomil in tablets." **(2012).**

73) Shah, R. M., Kakadia, M., Patel, J., Agola, A., & Saisivam, S. "Simultaneous estimation of meoprolol and olmesartan medoxomil by UV spectroscopy in fixed dose combination." *An Int J of Pharm Sci*, **(2012)** *4*, 2516-2532.

74) Vora, B. N., Parmar, R. R., Shah, D. A., & Nayak, P. P. "Absorption correction method for simultaneous estimation of metoprolol succinate and olmesartan medoxomil in combined tablet dosage form." *Journal of pharmaceutical science and bioscientific research*, **(2012),** *2*(2), 54-
7.

75) Gandu, S., Ravinder, M., Gandla, K., & Narmada, G. "Simultaneous Estimation of Metoprolol and Telmisartan in Combined Tablet Dosage Form by Using RP-HPLC and UV Spectrophotometry." *Asian Journal of Pharmaceutical Research and Development*, **(2022),** *10*(4), 22-33.

76) Suryanarayana, L. A. K. A. V. A. T. H. "Sensitive and reproducible study for UVspectrophotometric method for analysis of Clopidogrel and Metoprolol in a combined tablet dosage form." *International Journal of Pharmaceutical Science Invention*, **(2022),** *11*(6), 1-5.

77) Patel, E. S., Hinge, M. A., & Patel, A. A. "Development and validation of UV spectrophotometric methods for simultaneous estimation of trimetazidine hydrochloride and metoprolol succinate in tablet dosage form." *J. Pharm. Sci*. **(2016).**

78) Maheshwari, S., & Srivastav, A. "Development and Validation of Multi-Component Mode UV Spectrophotometric Method for the Estimation of Telmisartan and Amlodipine besylate in Combined Dosage Form." *Asian Journal of Research in Chemistry*, **(2013),** *6*(10), 973-976.

79) Hirpara, K. P., Dave, V. M., Faldu, D. S., & Patel, D. B. "UV-Spectrophotometric determination for simultaneous estimation of amlodipine besylate and telmisartan in combination." *Journal of Pharmaceutical Science and Bioscientific research*, **(2012),** *2*(3), 133-7.