Research Article:

Novel Spectrophotometric Multicomponent Analysis of a Ternary Mixture of Perindopril, Amlodipine and Indapamide by Simultaneous Equation Method

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ABSTRACT:
A new, simple, accurate, precise and reproducible UV-Spectrophotometry method is being developed for the simultaneous estimation of Perindopril, Amlodipine and Indapamidein tablet dosage form. It is smart, more time & money saving novel method because this method does not require any derivatization step or complex algorithms. The stock solutions were prepared in methanol. The λmax for Perindopril, Amlodipine and Indapamide were 232 nm, 244 nm and 258 nm resp. The Perindopril, Amlodipine and Indapamide obeyed Beer's law in concentration range of 0.4-1.2 µg/ml, 1-3 µg/ml and 0.5-2.5 µg/ml respectively. Results of analysis of simultaneous equation method were analysed and validated for various parameters according to ICH guidelines for Accuracy, Precision, Linearity, Robustness, LOD and LOQ. The proposed method is highly sensitive, precise and accurate, therefore can be used for intended purpose.

KEYWORDS: Simultaneous Equation Method; Validation; Perindopril; Amlodipine; Indapamide; ICH.

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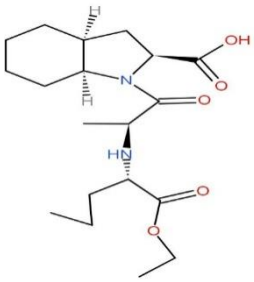
1. INTRODUCTION:

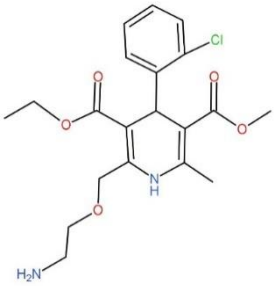
Perindopril is anangiotensin-converting enzyme (ACE) inhibitor class used to treat Hypertension which associated by high blood pressure. It reduces blood pressure by inhibiting the enzyme which catalyzes the conversion of angiotensin I to angiotensin II [1, 5, 10]. It has molecular formula $C_{23}H_{43}N_3O_5$ and chemical structure of Perindopril is given in figure 1.

Amlodipine is calcium channel blocker used in management of Hypertension as well as in Angina Pectoris. It is an angioselective calcium channel blocker and it acts by inhibiting the movement of calcium ions into vascular smooth muscle cells as well as cardiac muscle cells which inhibits the contraction of cardiac muscle and vascular smooth muscle cells [3, 4, 7]. It has molecular formula $C_{26}H_{31}ClN_2O_8S$ and chemical structure of Amlodipine is given in figure 2.

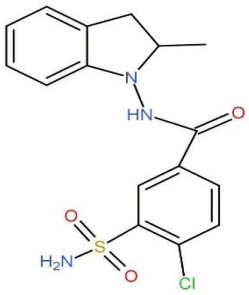
Indapamide is thiazide like diuretic drugs used in treatment of Hypertension by inhibiting reabsorption of sodium (Na+) and chloride (Cl^-) ions from distal convoluted (D. C. T.) In the kidney they are blocking the Na+ & Cl^- symporter [5, 10-12]. It has molecular formula $C_{16}H_{16}ClN_3O_3S$ and chemical structure of Indapamide is given in figure 3.



(Figure 1: Perindopril)



(Figure 3: Indapamide)



(Figure 2: Amlodipine)

2. MATERIALSANDMETHODS:

Chemicals pharmaceutically pure sample of Amlodipine was obtained from Hetero Drugs Limited (Telangana), Perindoprilwas obtained from Immense Culture Pvt. Ltd. (Narhe) and Indapamide was obtained from Supra Chemicals Pvt. Ltd. (Thane) as gift samples. Methanol AR grade was available in college Laboratory and Commercial tablet of Perindopril (4 mg), Amlodipine (5 mg), Indapamide (1.25 mg), Triplixam (Serdia Pharmaceuticals Pvt. Ltd. India) were procured from the local drug market.

2.1 Instrument:

A double beam UV-visible spectrophotometer model Jasco V-530 PC was used.
The spectrum was recorded over range 200-400 nm against solvent in 1 cm quarts cells.

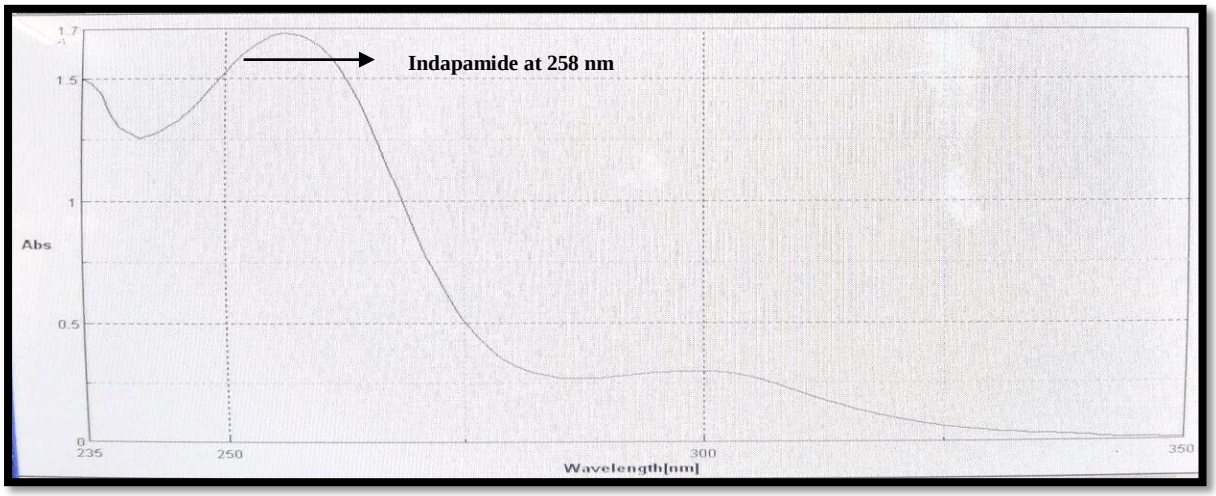
2.2 Standard solution preparations:

Accurately weighed 10 mg of Perindopril, Amlodipine and Indapamidewere transferred into volumetric flasks separately and then volume was made up to 10 ml with methanol to get a concentration of 1000 µg/ml for all three drugs. Standard stock solution (1000 µg/ml) was

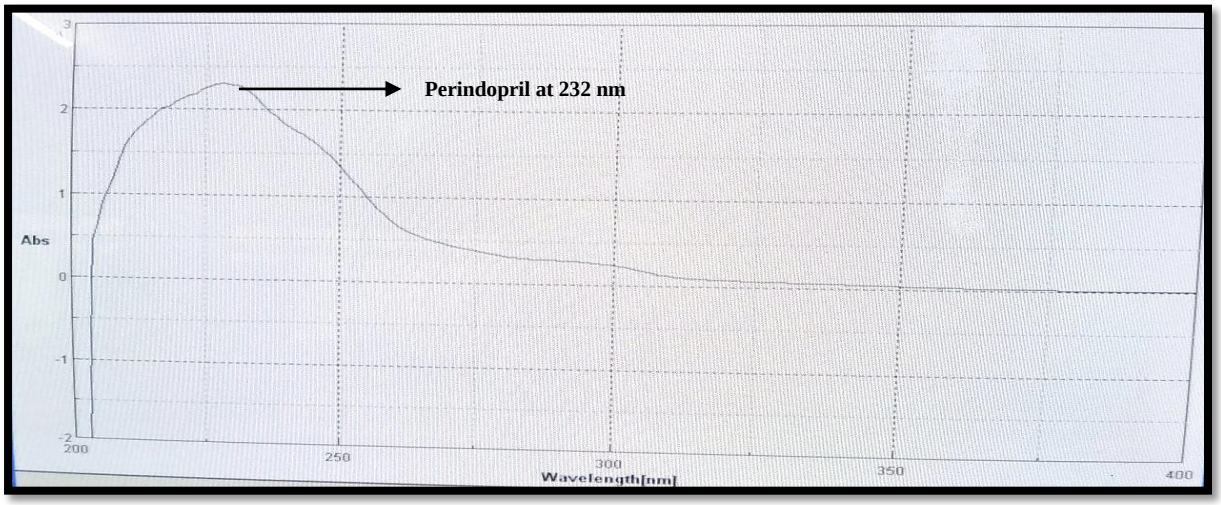
further diluted with methanol to obtain 0.4-1.2 µg/ml for perindopril, 1-3 µg/ml for amlodipine and 0.5-2.5 µg/ml for Indapamide**.**

2.3 Study of spectra and selection of wavelength:

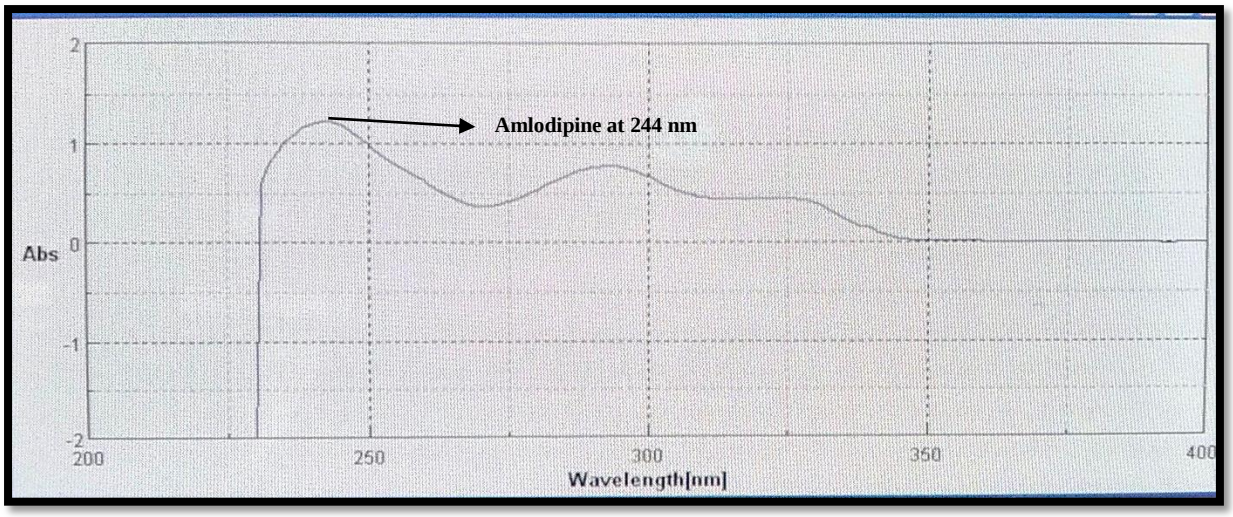
All three drugs were scanned over the range of 200-400 nm and overlay spectra was observed. While studding the overlay spectra it was observed that Indapamide shows maximum absorbance at 258 nm,Perindopril shows maximum absorbance at 232 nm and Amlodipine shows maximum absorbance at 244 nm which are given in figure 4-a, 4-b and 4-c resp. It was observed that Indapamide is interfering with Amlodipine and Perindopril at absorbance maxima but difference in absorbance maxima is sufficient and spectral characteristics are such that all three drugs can be simultaneously estimated by simultaneous equation method. Overlay spectra is given in figure 4-d.



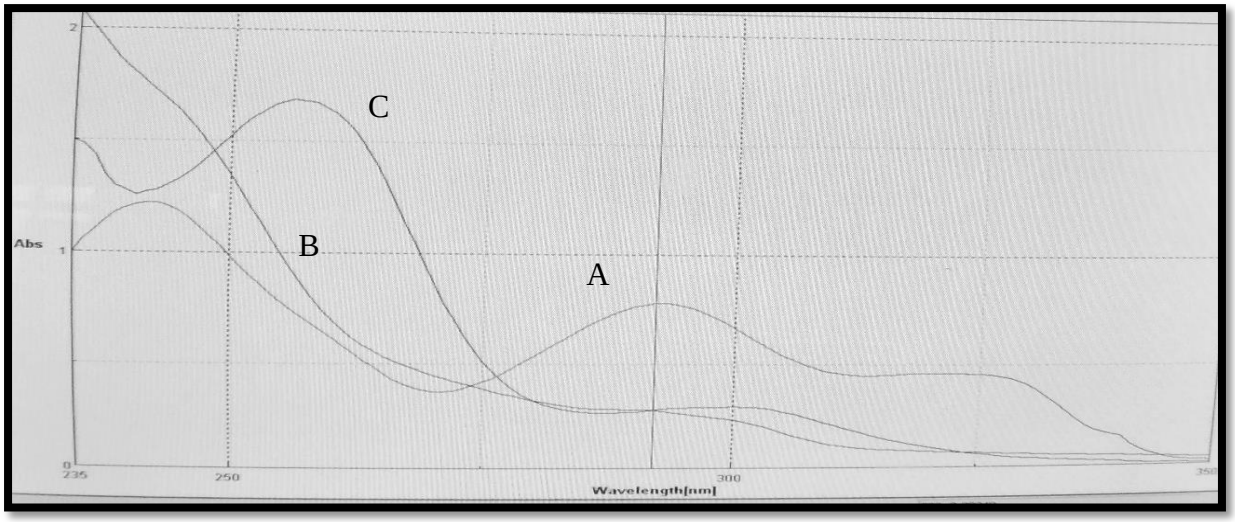
(Figure 4-a: Spectra of Indapamide at 10 µg/ml)



(Figure 4-b: Spectra of Perindopril at 10 µg/ml)



(Figure 4-c: Spectra of Amlodipine at 10 µg/ml)



(Figure 4-d: Overlay Spectra of A= Amlodipine, B= Perindopril and C= Indapamide at 10 µg/ml)

The concentration of all three drugs in mixture can be calculated by using following equations,

$$C_X = A_1 (ay_2az_3-ay_3az_2)-A_2 (ay_1az_3-ay_3az_1) +A_3 (ay_1az_2-ay_2az_1)/ax_1 (ay_2az_3)-ax_2 (ay_1az_3-ay_3az_1) +ax_3 (ay_1az_2-ay_2az_1)\ldots.. (1),$$

$$C_Y = A_1 (ax_2az_3-ax_3az_2)-A_2 (ax_1az_3-ax_3az_1) +A_3 (ax_1az_2-ax_2az_1)/ay_1 (ax_2az_3)-ay_2 (ax_1az_3ax_3az_1) +ay_3 (ax_1az_2-ax_2az_1)\ldots.. (2),$$

$$C_Z = A_1 (ax_2ay_3-ax_3ay_2)-A_2 (ax_1ay_3-ax_3ay_1) +A_3 (ax_1ay_2-ax_2ay_1)/az_1 (ax_2ay_3)-az_2 (ax_1ay_3-ax_3ay_1) +az_3 (ax_1ay_2-ax_2ay_1)\ldots.. (3),$$

Where, A_1, A_2 and A_3 are the absorbance values of mixture/ tablet solution.
ax_1, ax_2, ax_3 are absorptivities of Amlodipine at 244 nm, 232 nm and 258 nm resp.
ay_1, ay_2 and ay_3 are absorptivities of Perindopril 244 nm, 232 nm and 258 nm resp.
az_1, az_2 and az_3 are absorptivities of Indapamide 244 nm, 232 nm and 258 nm resp.

C_X, C_Y and C_Z are concentration of Amlodipine, Perindopril and Indapamide resp.
The absorptivity of all three drugs were calculated by equation (A= abc).

ax_1 = 121.99	ay_1 = 171.01	az_1 = 127.93
ax_2 = 74.402	ay_2 = 92.315	az_2 = 168.519
ax_3 = 75.89	ay_3 = 230.124	az_3 = 95.01

2.4 Preparation for analysis of tablet formulation:

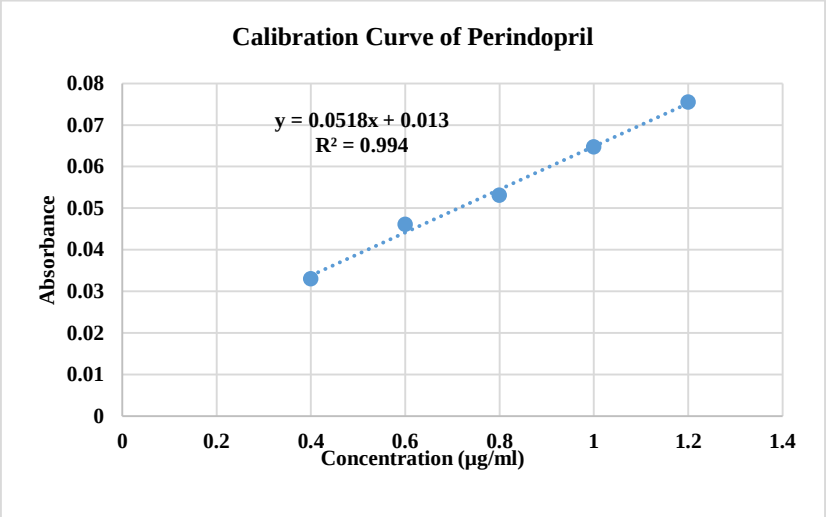
Twenty tablets were taken and their average weight was determined. They are crushed to fine powder; amount equivalent to 4 mg of Amlodipine was taken in 100 ml volumetric flask. The Perindopril and Indapamide present in this amount of tablet powder was 3.2 mg and 1 mg, the ratio of all three drugs was 4:3.2:1this was than dissolve in 50 ml of methanol by sonication for about 10 minutes. The volume is made up to the mark by methanol and filtered by Whatmann filter paper (no. 41) and the filtrate was used to prepare samples of different concentration. Now all the tablet samples was scanned in multi photometric mode and the concentration of all three drugs were obtained from the equation. Results of tablet analysis are reported in Table 1.

3. VALIDATION OF METHOD:

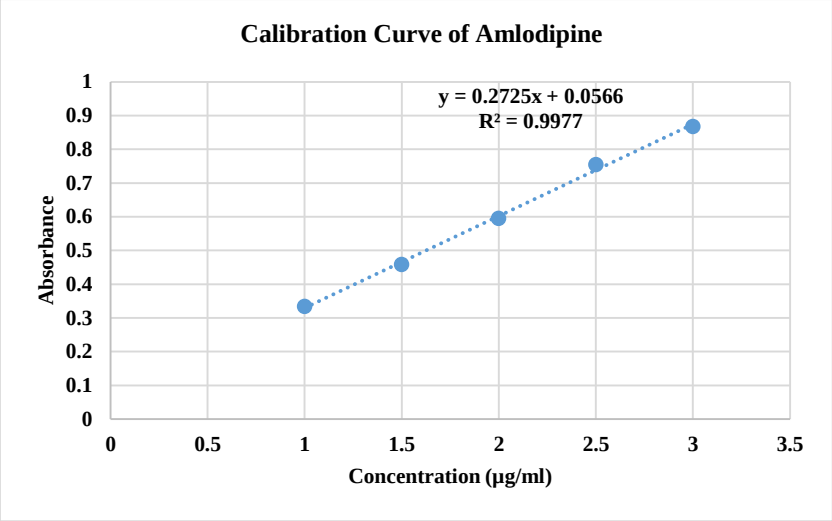
As per ICH guideline the method is validated and following parameters were evaluated [16, 48, 49].

3.1 Linearity:

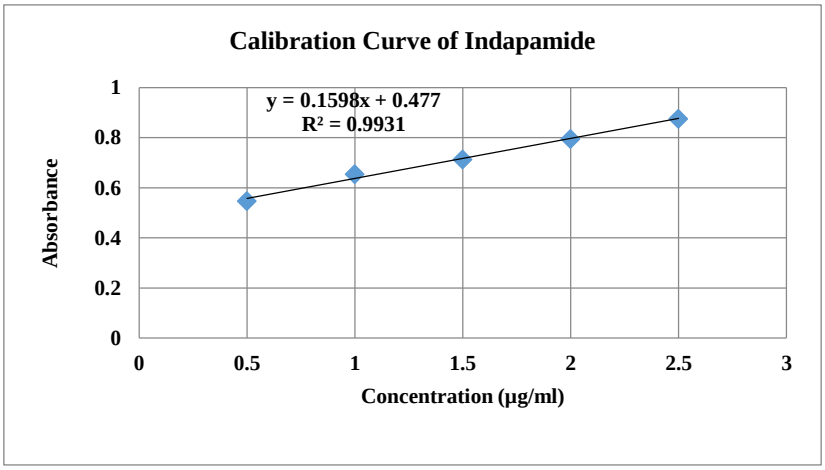
Linearity of the method was determined by diluting the stock solution to give a concentration range of 0.4-1.2 µg/ml Perindopril, 1-3 µg/ml Amlodipine and 0.5-2.5 µg/ml Indapamide respectively. The calibration curve was constructed between concentration verses absorbance. It is shown into the figure 5-a. 5-b and 5-c resp.



(Figure 5-a: Calibration Curve of Perindopril)



(Figure 5-b: Calibration Curve of Amlodipine)



(Figure 5-c: Calibration Curve of Indapamide)

3.2 Precision:

Precision was determined by repeatability, Interday precision of all three drugs. Repeatability indicates the precision under the same operating condition over short interval time. The Interday precision study is expressed within laboratory variation on different days and analyst to analyst variation by different analyst.

3.3 Limit of Detection and Limit of Quantification (LOD & LOQ):

Sensitivity of the method was determined with respect to limit of detection (LOD) and limit of quantitation. According to ICH guidelines, the limit of detection is the lowest amount of analyte in a sample that can be detected and the limit of quantitation is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy.

3.4 Accuracy (% recovery):

To a preanalysed tablet solution a definite concentration of pure drug was added (80%, 100% and 120% level) and then recovery was studied. A preanalysed tablet solution containing 5 µg/ml of Amlodipine 4µg/ml of Perindopril and 1.25 µg/ml of Indapamide were taken in 10 ml volumetric flasks and known concentrations of pure drug solution was added to them,

which were prepared from standard stock solution of Amlodipine, Perindopril and Indapamide. It was repeated at 3 concentrations and 3 replicate levels.

3.5 Robustness:

As per ICH norms, small, but deliberate variations by altering the wavelength or concentration of the solvent were made to check the methods capacity to remain unchanged. The change was made in the ratio of solvent and wavelength. Instead of 100%, 95% methanol was used as solvent and variation in wavelength also made.

4. RESULTSAND DISCUSSION:

The simultaneous equation method for estimation of Perindopril, Amlodipine and Indapamide in tablet dosage form was found to be simple, precise, accurate and reproducible. The solvent used was 100% methanol and do not shows any significant interference in the spectrophotometric assay of all three drugs.

4.1 Linearity:

The proposed method was found to be linear in the range of 0.4-1.2 µg/ml Perindopril, 1-3 µg/ml Amlodipine and 0.5-2.5 µg/ml for Indapamide with correlation coefficient 0.944, 0.997 and 0.999 resp.

Result of linearity study shown in Table 2.

4.2 Limit of Detection and Limit of Quantification (LOD& LOQ):

The standard deviation of y-intercept of regression line were determined and substituted in the following equation for the determination of detection of limit and quantification limits.

Detection limit= 3.3 σ/s

Quantification limit= 10 σ/s

Where, σ is the standard deviation of y-intercept of regression line and s is the slope of the calibration curve.

The limit of detection (LOD) and limit of quantification (LOQ) data are given in Table 2.

4.3 Accuracy:

The validity and reliability of proposed methods were assessed by recovery studies. The recovery of added standards (80%, 100% and 120%) was found at three concentrations level. The value of mean of recoveries was found to be in ranging from 98.79 to 102.03% for Perindopril, 99.67 to 101.93 % for Amlodipine and 94.32 to 102.02% for Indapamide. The value of SD and %RSD are less than 2 indicate the accuracy of method.

Result of recovery study shown in Table 3.

4.4 Precision:

Precision was determined by repeatability and Interday precision of all three drugs.

a) Repeatability:

The repeatability was performed for six concentrations in linearity range 0.4, 0.6, 0.8, 1, 1.2 and 1.4 µg/ml for Perindopril, 1, 1.5, 2, 2.5, 3 and 3.5 µg/ml for Amlodipine, and 0.5, 1, 1.5, 2, 2.5,3 µg/ml for Indapamide indicates the precision under the same operating condition over short interval time.

b) Interday precision:

Interday precision was also performed within laboratory variation on different days for all three drugs simultaneously in three replicate at three concentrations.

Result of precision shown in Table 4.

4.5 Robustness:

Standard stock solution of 1000 µg/ml of Perindopril, Amlodipine and Indapamide were prepared using methanol as a solvent. From standard stock solution, sub stock solution of 100µg/ml of Perindopril, Amlodipine and Indapamide were prepared separately. From these standard stock solutions of drugs, appropriate dilutions was prepared to get mixed standard solutions of all three drugs in 4:5:1.25 ratio (Perindopril, Amlodipine and Indapamide).

Result of robustness shown in Table 5.

Table 1: Result of Tablet formulation.

Sr. No.	Drug Name	Labelled Amount (mg)	S.D.	% COV
1	Perindopril	4	0.079	94.49%
2	Amlodipine	5	0.035	96.36%
3	Indapamide	1.25	0.023	94.93%

(Where, S.D. = Standard Deviation, %COV= % Recovery)

Table 2: Linear regression parameters for Perindopril, Amlodipine and Indapamide by both proposed methods.

Sr. No.	Parameter	Perindopril	Amlodipine	Indapamide
1	Wavelength (nm)	232	244	258
2	Calibration range (µg/ml)	0.4-1.2	01-03	0.5-2.5
3	Correlation coefficient (R^2)	0.944	0.9977	0.9931
4	Slope (m)	0.0518	0.2725	0.1598
5	Intercept (c)	0.013	0.0566	0.477
6	Limit of detection (µg/ml)	22. 82	38.26	23.57
7	Limit of Quantitation (µg/ml)	63. 21	115.96	68. 45

Table 3: Recovery study at three concentration levels for Perindopril, Amlodipine and Indapamide by both proposed methods.

Sr. No.	Drug	Concentration of standard added	S.D.	% RSD	% COV
1	Perindopril	80%	0.0029	0.89	98.79
		100%	0.0082	0.73	101.37
		120%	0.0024	0.56	102.03
2	Amlodipine	80%	0.0027	0.29	99.67
		100%	0.0109	1.26	101.34
		120%	0.4654	0.99	101.93
3	Indapamide	80%	0.0041	0.36	94.32
		100%	0.0012	0.17	101.78
		120%	0.0127	1.46	102.02

Table 4: Precision study at three concentration levels for Perindopril, Amlodipine and Indapamide by both proposed methods.

Sr. No.	Parameter	S.D.			% RSD		
1	Drug	PERI	AMLO	INDA	PERI	AMLO	INDA
2	Repeatability	0.0041	0.0124	0.0100	0.24	0.60	0.61
3	Interday Precision	0.0004	0.0112	0.0046	0.68	0.83	0.47

(Where, PERI = Perindopril, AMLO = Amlodipine, INDA = Indapamide)

Table 5: Robustness study at three concentration levels for Perindopril, Amlodipine and Indapamide by both proposed methods.

Sr. No.	Drug	Wavelength (nm)	S.D.	% RSD
1	Perindopril	230	0.0123	1.23
		234	0.0077	0.73
2	Amlodipine	242	0.0085	0.79
		246	0.0044	0.41
3	Indapamide	256	0.0153	1.20
		260	0.0124	0.95

5. CONCLUSION:

This work formulates a new approach to the simultaneous analysis of ternary mixtures of Perindopril, Amlodipine and Indapamide which have overlapping spectra. This method does not require any derivatization step or complex algorithms. It needs only some simple mathematical calculation. This method has advantages of novel and smart spectrophotometric method development. It is more time & money saving than other spectrophotometric methods. Hence, they can be easily applied in quality control laboratory tests in the dosage form.

6. ACKNOWLEDGEMENTS:

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7. CONFLICTS OF INTEREST:

The authors declare that there is no conflict of interest.

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