Research Paper

Development and Validation of a Novel UV Spectrophotometric Method for Simultaneous Analysis of Amlodipine, Indapamide and Perindopril

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A simple, accurate, precise and economical ultraviolet spectrophotometric method has been developed for the simultaneous estimation of amlodipine, perindopril and indapamide. The developed method was based on determination of the three drugs using ultraviolet absorbance correction method. The three wavelengths chosen were 365 nm for amlodipine (as absorbances due to other two drugs were nil at this wavelength), 245 nm for indapamide (corrected for absorbance due to amlodipine) and 204 nm for perindopril (corrected for absorbances due to amlodipine and indapamide) with water as diluent. The Beer Lambert's range for the three drugs was 10-60 µg/ml, 5-20 µg/ml and 10-100 µg/ml for amlodipine, indapamide and perindopril respectively, with correlation coefficient (r^2) of ≥0.999. The developed method was validated as per International Conference on Harmonisation guidelines and was found to be accurate, precise, sensitive and robust. The percentage assay results were within acceptable limits. Hence the developed method can be successfully used for the routine analysis of amlodipine, perindopril and indapamide in bulk and in combination.

Key words: Absorbance correction method, amlodipine besylate, indapamide, perindopril tert-butyl amine, International Conference on Harmonisation (ICH) guidelines

Amlodipine (AMD), an antihypertensive drug belongs to the group of drugs called dihydropyridine calcium channel blockers. It is commonly used in the treatment of high blood pressure and angina. It also has antioxidant properties and ability to enhance the production of nitric oxide (NO), an important vasodilator that decreases blood pressure. Perindopril (PD) is a nonsulfhydryl prodrug belonging to the angiotensin-converting enzyme (ACE) inhibitor class of medication. It is rapidly metabolized in the liver to its active metabolite perindoprilat, following oral administration. It acts by inhibiting ACE and therefore prevents the conversion of angiotensin I to angiotensin II; consequently, angiotensin II mediated vasoconstriction and angiotensin II mediated aldosterone secretion from the adrenal cortex are inhibited thus resulting in decreased blood pressure[1]. Indapamide (IND), a thiazide-like diuretic results in an overall decrease in blood pressure through its diuretic action (increase in urine output) and vasodilatory properties (inhibiting influx of calcium or other ions)[2].

Literature survey revealed the availability of various chromatographic methods such as Thin-layer chromatography (TLC)[3], Reversed Phase-High Performance Liquid Chromatography (RP-HPLC) [4-7], stability indicating High Performance Liquid Chromatography (HPLC)[8] and Ultraviolet (UV) spectroscopic methods[9-14] like simultaneous equations method, derivative spectroscopy and absorbance correction method for analysis of only two drugs in combination. UV spectroscopic methods have the advantage of being simple, rapid and cost effective. Hence it would be worthwhile to develop a novel UV spectroscopic method for the simultaneous estimation of amlodipine besylate, indapamide and perindopril tert-butyl amine in combination[15,16].

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MATERIALS AND METHODS

AMD was supplied by Alkem Laboratories Ltd. (Mumbai, India), PD was supplied by Glenmark Pharmaceuticals (Goa, India) and IND was supplied by Adock Ingram Ltd. (Bangalore, India). Synthetic mixture containing 10 mg each of AMD and PD and 2.5 mg of IND was prepared inhouse. Methanol used was of spectroscopic grade. The analysis was carried out on Shimadzu 1800 double beam UV spectrophotometer. The software used was UV Probe.

Preparation of standard stock solution (1000 µg/ml):

About 25 mg each of AMD, IND and PD standards were weighed and transferred separately into three 25 ml volumetric flask. Volume was made up to 25 ml with methanol to obtain stock solutions of each drug (1000 µg/ml).

Preparation of working standard solutions:

Stock solutions of AMD, IND and PD were diluted with distilled water to obtain working standard solutions in the concentration ranges of 10-100 µg/ml for AMD and PD and 5-100 µg/ml for IND.

Preparation of mixed standard solution of drugs:

About 1 ml stock solution of each drug was diluted to 10 ml with distilled water to obtain a solution of 100 µg/ml concentration. From this, a mixed drug standard solution was prepared by taking 1 ml each of AMD and PD and 0.25 ml of IND into a 10 ml volumetric flask. Volume was adjusted to the mark with distilled water to obtain a concentration of 10 µg/ml for AMD, PD and 2.5 µg/ml for IND.

Selection of analytical wavelength:

Working standard solutions of AMD, IND and PD (10 µg/ml each) were scanned in the range of 200-400 nm against the blank and the spectra were recorded. The choice of the analytical wavelengths for analysis was made using the concept of absorbance corrected for interference. Accordingly, three wavelengths were selected: λ_1 for AMD as absorbances due to other two drugs were nil at this wavelength, λ_2 of absorbance of IND and AMD (corrected for absorbance due to AMD) and λ_3 of absorbance of PD, IND and AMD (corrected for absorbances due to AMD and IND).

Determination of Absorptivity:

The absorbance of the working standard solutions was recorded at their predetermined wavelengths. Calibration curves were plotted and absorptivity values for the drugs were calculated.

Method Validation:

Linearity

To determine linearity range for the drugs, a series of working standard solutions were prepared from the respective stock solutions of drugs. A volume of 0.125 ml, 0.25 ml, 0.5 ml, 0.75 ml, 1 ml, 1.25 ml, 1.5 ml, 1.75 ml, 2 ml, 2.25 ml and 2.5 ml of stock solution of each drug was pipetted into 25 ml volumetric flasks and volume was made up with distilled water to obtain solutions in concentration range of 10-100 µg/ml for AMD and PD and 5-100 µg/ml for IND. Absorbance of the solutions were recorded at predetermined wavelengths and calibration curves of absorbance vs. concentration were plotted. The linear regression equations and correlation coefficients (r^2) were determined.

Precision

Precision analysis was carried out by performing intra-day and inter-day precision studies. About 0.5 ml of mixed standard solution of drugs was pipetted into three 25 ml volumetric flasks and volume was made up to the mark with distilled water. Absorbance of the solutions was recorded against blank at predetermined wavelengths. Concentration of each drug was determined using the equations with their respective absorptivity values. Intra-day precision was performed by repeating the procedure thrice in a day while inter-day precision studies were performed on three consecutive days in triplicate.

Accuracy

The accuracy of the developed method was verified by performing percentage recovery studies at 80 %, 100 % and 120 %. About 2 mg of placebo powder was weighed and transferred into nine volumetric flasks of 25 ml capacity. To the three sets of flasks (consisting of three flask each), the mixed standard solution of drugs in a volume of 0.4 ml (80 % level), 0.5 ml (100 % level) and 0.6 ml (120 % level) was added. About 20 ml of distilled water was added to all the three sets and sonicated for about 5 min. After sonication volume was made up to 25 ml with the diluent and filtered through a Whatman filter paper. Absorbance of the filtrate was recorded at predetermined wavelengths

against blank. Sample was prepared in a similar manner omitting the drug.

Limit of detection (LOD)

LOD was calculated using slope and standard deviation response of calibration curves of drugs at the particular wavelength. DL=3.3σ/S, where, σ- standard deviation of response (y intercept) S- slope of calibration curve.

Limit of quantitation (LOQ)

LOQ was calculated using slope and standard deviation response of calibration curves of drugs at the particular wavelength.

QL=10σ/S, where, σ-standard deviation of response (y intercept), S-slope of calibration curve

Robustness

The robustness of the method was verified by performing the assay of the mixed standard solution. Deliberate minor changes were introduced in experimental conditions such as use of different UV spectrophotometer; change of analyst and by altering the composition of the diluent. The percentage assay values were determined.

Assay of synthetic mixture of drugs:

A quantity of synthetic mixture equivalent to 12.5 mg of AMD was accurately weighed and transferred to a 25 ml volumetric flask. About 20 ml of methanol was added and dissolved by shaking for about 5 min. The volume was made up to 25 ml with methanol. The solution was filtered through a Whatman filter paper no. 1. From the filtrate, 0.5 ml was withdrawn and transferred to a 25 ml volumetric flask in triplicate and volume was made up to the mark with distilled water. Absorbance of these solutions were recorded at predetermined wavelengths i.e. λ_1, λ_2 and λ_3 and respective absorbance values were noted as A_1, A_2 and A_3.

Concentration of each drug was determined using the following equations:

$A_1=ax_1Cx, A_2=ax_2Cx+ay_2Cy, \quad A_3=ax_3Cx+ay_3Cy+az_3Cz$

where Cx= Concentration of AMD in g/1000 ml, Cy= Concentration of IND in g/1000 ml, Cz= Concentration of PD in g/1000ml, A_1= Absorbance of mixture at λ_1, A_2= Absorbance of mixture at λ_2, A_3= Absorbance of mixture at λ_3, ax_1= Absorptivity of AMD at λ_1, ax_2= Absorptivity of AMD at λ_2, ax_3= Absorptivity of AMD at λ_3, ay_2= Absorptivity of IND at λ_2, ay_3= Absorptivity of IND at λ_3 az_3= Absorptivity of PD at λ_3

Bench top stability of sample solution:

The bench top stability was determined by using the mixed standard solution. About 0.5 ml of the mixed standard solution of drugs was transferred into a 25 ml volumetric flask and the final volume was made up with distilled water. This solution was kept on the bench top at room temperature and absorbance was recorded against the blank at predetermined wavelengths for time intervals up to 24 h. From the absorbance values, the percentage purity of the drugs was calculated to determine the stability period on bench top.

RESULTS AND DISCUSSION:

The choice of the diluent was based upon the solubility of the drugs. The drugs were completely soluble in methanol and partially soluble in water. Hence, methanol was used as a solvent to prepare the stock solution and distilled water was used to prepare subsequent dilutions.

The analytical wavelengths were selected by scanning the standard solutions of AMD, IND and PD (10 µg/ml each) in the range of 200-400 nm against blank. From the overlain spectra (fig. 1) the wavelengths chosen for analysis were as follows: λ_1=365 nm: absorbance due to AMD (absorbance due to other two drugs was nil at this wavelength), λ_2=245 nm: absorbance of IND (corrected for absorbance due to AMD), λ_3=204 nm: absorbance of PD (corrected for absorbances due to AMD and IND). Absorptivity values for the drugs were calculated using the absorbance values recorded for the working standard solutions of drugs at their predetermined wavelengths. The calculated mean absorptivity (lg^{-1} cm^{-1}) values for the drugs at different wavelengths

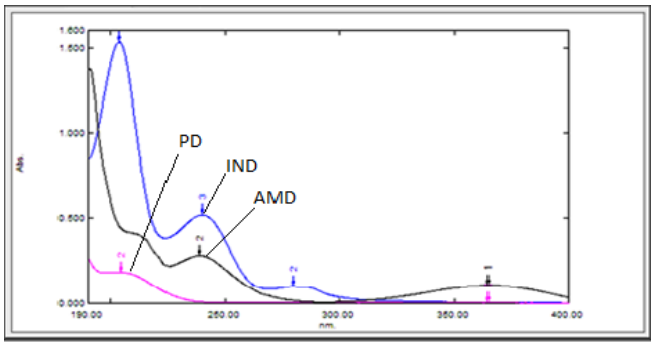

Fig. 1: Overlain UV spectra for AMD (amlodipine), IND (indapamide), PD (perindopril)

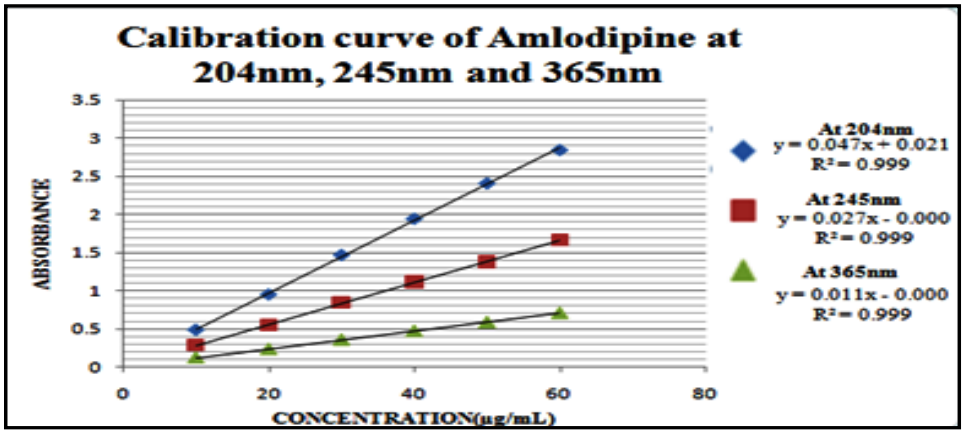

Fig. 2: Calibration curve for amlodipine at 204 nm, 245 nm and 365 nm

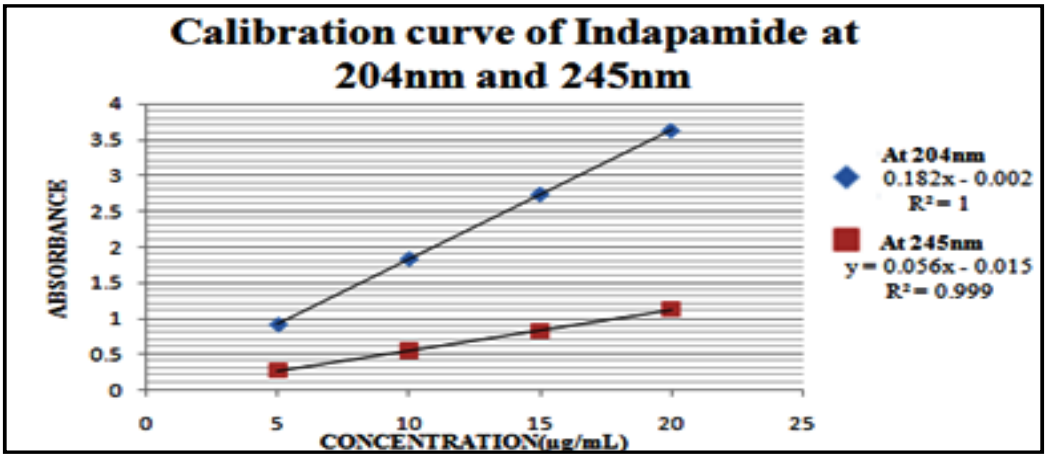

Fig. 3: Calibration curve for indapamide at 204 nm and 245 nm

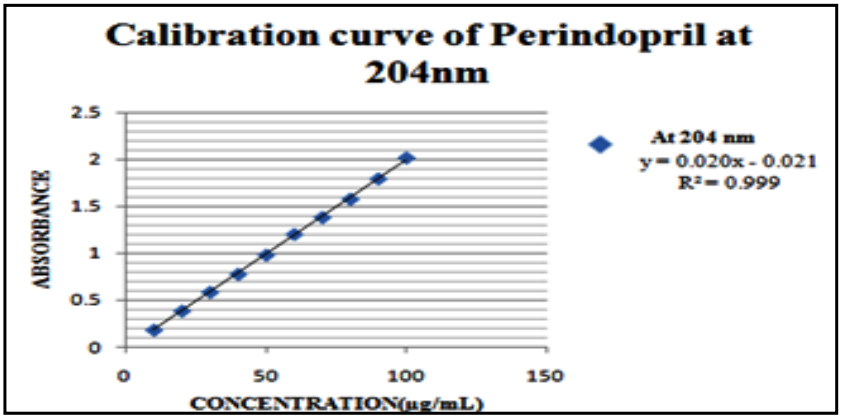

Fig. 4: Calibration curve for perindopril at 204 nm

were found to be a_{x1}=11.75, a_{x2}=27.70 and a_{x3}=48.08 for AMD, a_{y2}=54.80, a_{y3}=181.90 for IND and a_{z3}=19.60 for PD.

Linearity range for each drug was obtained by plotting calibration curves at predetermined wavelengths. The calibration plots for the drugs are displayed in fig. 2, 3 and 4. Good correlation between the absorbance and concentration of the drugs was obtained in the concentration range 10-60 µg/ml for AMD, 5-20 µg/ml for IND and 10-100 µg/ml for PD. The results of linearity for AMD, IND and PD are given in Tables 1, 2 and 3 respectively.

Precision was carried out by analysing the mixed standard solution of drugs in triplicate against the blank thrice a day for intraday studies and on three consecutive days in triplicate for interday precision analysis. The results as depicted in Table 4 and 5 gave percentage relative standard deviation (RSD) values of less than 2 %.

The accuracy of the method was determined by performing percentage recovery at three levels: 80 %, 100 % and 120 %. As seen in Table 6, 7 and 8 the mean percentage recoveries for AMD, IND and PD were found to be within the acceptance limit of 98-102 %. Hence the developed method was found to be accurate.

LOD and LOQ were calculated using slope and standard deviation response from the calibration curves of drugs at their respective wavelengths. The results as depicted in Table 9 show that the method was sensitive.

The robustness of the method was determined by introducing deliberate changes in the experimental conditions during the analysis of mixed standard solution of drugs, namely use of different instrument, change of analyst and by altering composition of diluent. The results as depicted in Table 10 show that the deliberate changes do not affect the results of analysis as percentage purity was found to be within the acceptance criteria of 90-110 %. Hence the developed UV method was found to be robust.

The synthetic mixture of drugs was analyzed at predetermined wavelengths i.e. λ_1, λ_2 and λ_3 in triplicate and the respective absorbance values were noted as A_1, A_2 and A_3. The percentage purity of AMD, IND and PD were found to be 99.70 %, 103.20 % and 97.80 % respectively, which comply with the acceptance criteria of 90-110 %. The results are depicted in Table 11.

For bench top stability, the absorbance of the mixed standard solution kept on the bench top at room temperature was recorded every hour till 6 h and then at 24 h. Stability of the sample solution was determined by calculating the percentage purity. The results as depicted in Table 12 show percentage purity in the range of 90-110 % for a period of up to 3 h. Hence the solution was found to be stable for a period of 3 h on bench top.

TABLE 1: SUMMARY OF LINEARITY DATA FOR AMLODIPINE

Parameters	At 204 nm	At 245 nm	At 365 nm
Beer's law range	10-60 µg/ml	10-60 µg/ml	10-60 µg/ml
Regression equation	y=0.047x -0.021	y=0.027x -0.000	y=0.011x -0.000
Slope (b)	0.047	0.027	0.011
Intercept (c)	-0.021	0.000	0.000
Correlation coefficient (r^2)	0.999	0.999	0.999

TABLE 2: SUMMARY OF LINEARITY DATA FOR INDAPAMIDE

Parameters	At 204 nm	At 245 nm
Beer's law range	5-20 µg/ml	5-20 µg/ml
Regression equation	y=0.182x-0.002	y=0.056x-0.015
Slope (b)	0.182	0.056
Intercept (c)	-0.002	-0.015
Correlation coefficient (r^2)	1.000	0.999

TABLE 3: SUMMARY OF LINEARITY DATA FOR PERINDOPRIL

Parameters	At 204 nm
Beer's law range	10-100 µg/ml
Regression equation	y= 0.020x-0.021
Slope (b)	0.020
Intercept (c)	-0.021
Correlation coefficient (r^2)	0.999

TABLE 4: INTRADAY PRECISION DATA

Concentration of drugs (µg/ml)	Absorbance of mixed standard			Concentration obtained (µg)		
	At 365 nm	At 245 nm	At 204 nm	AMD	IND	PD
10 µg/ml of AMD	0.119	0.420	1.140	10.10	2.54	10.20
	0.118	0.420	1.150	10.04	2.58	10.10
	0.117	0.410	1.130	10.00	2.43	10.50
2.5 µg/ml of IND	0.119	0.420	1.140	10.10	2.54	10.20
	0.118	0.420	1.160	10.04	2.58	10.60
	0.119	0.420	1.140	10.10	2.54	10.20
	0.120	0.430	1.160	10.20	2.60	10.03
10 µg/ml of PD	0.119	0.420	1.150	10.10	2.54	10.30
	0.119	0.420	1.140	10.10	2.54	10.20
Average				10.09	2.54	10.25
Percentage Purity				100.9 %	101.6 %	102.5 %
SD				0.056	0.048	0.180
Percentage RSD				0.55 %	1.88 %	1.76 %

TABLE 5: INTERDAY PRECISION DATA

	Concentration of mixed standard			Absorbance of mixed standard			Concentration obtained (µg)		
	AMD	IND	PD	At 365 nm	At 245 nm	At 204 nm	AMD	IND	PD
				0.119	0.420	1.140	10.10	2.54	10.20
Day 1				0.118	0.410	1.140	10.04	2.41	10.60
				0.119	0.420	1.150	10.10	2.54	10.32
				0.119	0.420	1.140	10.01	2.54	10.20
Day 2	10 µg/ml	2.5 µg/ml	10 µg/ml	0.119	0.420	1.150	10.10	2.54	10.30
				0.117	0.420	1.150	10.00	2.60	10.05
				0.117	0.420	1.150	10.00	2.60	10.02
Day 3				0.118	0.420	1.160	10.04	2.58	10.60
				0.120	0.430	1.160	10.20	2.60	10.03
Average							10.02	2.56	10.30
Percentage Purity							100.2 %	102.4 %	103.0 %
SD							0.06	0.03	0.22
Percentage RSD							0.60 %	1.17 %	1.94 %

TABLE 6: ACCURACY DATA FOR AMLODIPINE

Volume of mixed standard added	Spiking level	Amount present (mg)	Absorbance at 365 nm	Amount recovered (mg)	Percentage recovery	Mean percentage recovery
0.4 ml	80 %	10	0.092	9.79	97.9	98.20 %
			0.093	9.89	98.9	
			0.092	9.79	97.9	
0.5 ml	100 %	12.5	0.118	12.5	100	99.84 %
			0.118	12.5	100	
			0.117	12.44	99.52	
0.6 ml	120 %	15	0.145	15.42	102.3	102 %
			0.144	15.31	102.1	
			0.145	15.42	102.3	

TABLE 7: ACCURACY DATA FOR INDAPAMIDE

Volume of mixed standard added	Spiking level	Amount present (mg)	Absorbance at 245 nm	Amount recovered (mg)	Percentage recovery	Mean Percentage recovery
			0.330	2.55	102.00	
0.4 ml	80 %	2.500	0.330	2.52	100.80	101.40 %
			0.328	2.53	101.30	
			0.413	3.08	98.40	
0.5 ml	100 %	3.125	0.414	3.10	99.20	99.46 %
			0.413	3.15	100.80	
			0.510	3.84	102.30	
0.6 ml	120 %	3.750	0.500	3.66	102.30	100.86 %
			0.510	3.84	98.00	

A novel and simple UV absorbance correction method has been developed for the simultaneous analysis of AMD, IND and PD in combination. The developed UV spectroscopic method employed water as diluent after the preparation of stock solutions in methanol. The three wavelengths chosen for the estimation of drugs were 365 nm for AMD as absorbances due to other two drugs were nil at this wavelength, 245 nm for IND (corrected for absorbance due to AMD) and 204 nm for PD (corrected for absorbances due to AMD and IND). Method validation was done as per International Conference on Harmonisation (ICH) guidelines.

TABLE 8: ACCURACY DATA FOR PERINDOPRIL

Volume of mixed standard added	Spiking level	Amount present (mg)	Absorbance at 204 nm	Amount recovered (mg)	Percentage recovery	Mean Percentage recovery
			0.911	10.04	100.40	
0.4 ml	80 %	10	0.910	10.36	103.60	102.00 %
			0.910	10.36	103.60	
			1.130	12.70	101.60	
0.5 ml	100 %	12.5	1.120	12.00	96.00	98.72 %
			1.130	12.32	98.56	
			1.353	15.40	102.60	
0.6 ml	120 %	15	1.352	14.60	96.00	100.40 %
			1.353	15.40	102.60	

TABLE 9: LOD AND LOQ VALUES FOR DRUGS

	Drugs					
	Amlodipine			Indapamide		Perindopril
	At 365 nm	At 245 nm	At 204 nm	At 245 nm	At 204 nm	At 204 nm
LOD (µg/ml)	0.950	0.095	1.680	0.630	0.170	1.860
LOQ (µg/ml)	2.890	0.290	5.100	1.920	0.520	5.600

TABLE 10: ROBUSTNESS DATA

Sr. No	Concentration of mixed standard			Absorbance of mixed standard			Amount found (mg)			Percentage Purity		
	AMD	IND	PD	At 365 nm	At 245 nm	At 204 nm	AMD	IND	PD	AMD	IND	PD
1				0.118	0.410	1.130	10.04	2.41	10.60	100.4	96.4	106.0
2	10	2.5	10	0.119	0.402	1.150	10.10	2.50	10.30	101.0	101.6	103.0
3	µg/ml	µg/ml	µg/ml	0.118	0.420	1.140	10.04	2.06	10.10	100.4	103.2	101.0
Mean										100.6%	100.4%	103.3%

TABLE 11: ASSAY OF SYNTHETIC MIXTURE

Sr. No	Absorbance of mixed standard			Amount found (mg)			Percentage Purity		
	At 365 nm	At 245 nm	At 204 nm	AMD	IND	PD	AMD	IND	PD
1	0.117	0.407	1.140						
2	0.116	0.406	1.130	12.44	3.22	12.20	99.7	103.2	97.8
3	0.117	0.407	1.140						
Mean	0.117	0.407	1.140						

TABLE 12: BENCH TOP STABILITY OF MIXED STANDARD

Time interval (h)	Concentration of mixed standard			Absorbance of mixed standard			Percentage Purity		
	AMD	IND	PD	At 365 nm	At 245 nm	At 204 nm	AMD	IND	PD
1				0.122	0.427	1.142	103.0	103.2	90.1
2				0.122	0.426	1.139	103.0	102.0	91.0
3				0.122	0.426	1.138	103.0	102.0	90.4
4	10 µg/ml	2.5 µg/ml	10 µg/ml	0.122	0.425	1.134	103.0	102.0	89.3
5				0.122	0.420	1.127	103.0	102.0	87.3
6				0.118	0.420	1.127	100.4	101.8	88.5
24				0.110	0.400	1.100	97.8	101.9	81.1

The linearity range for the drugs were 10-60 µg/ml, 5-20 µg/ml and 10-100 µg/ml for AMD, IND and PD respectively. The method was found to be sensitive, accurate, precise and robust. The percentage assay of AMD, IND and PD in synthetic mixture was found to be 99.70 %, 103.20 % and 97.80 % respectively, which were within the acceptance criteria. Hence the developed method is a cost effective analytical tool for the simultaneous estimation of the three drugs in combination by UV spectroscopy.

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Conflict of Interests:

The authors declare no conflict of interest.

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