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Instruction to Authors

QUANTITATIVE ESTIMATION OF PRAVASTATIN SODIUM AND ASPIRIN BY SIMULTANEOUS EQUATION METHOD

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ABSTRACT

In the present study a simple, rapid, economical, precise and accurate method for simultaneous estimation of aspirin and pravastatin sodium in combined dosage form has been developed. The method developed was simultaneous equation method and the solvent used is 0.1M Sodium hydroxide. The wavelength 297 nm and 238.40 nm were selected for aspirin and provastatin sodium respectively. Beer's range was obeyed in the concentration range of 5-45 µg/mL for aspirin and 2-18 µg/mL for pravastatin sodium. Recovery was found in the range of 98.8-101.3% for aspirin and pravastatin sodium in the physical formulation. The results of analysis have been validated statistically and recovery studies confirmed the accuracy and reproducibility of the proposed method as per ICH guidelines.

Keywords: Pravastatin Sodium, Aspirin, Spectroscopic method, Absorption, Simultaneous estimation, Sodium hydroxide

Introduction

Pravastatin (Fig 1) is chemically Sodium(3R,5R)-3,5-dihydroxy-7-[(1S,2S,6S,8S,8R)-6-hydroxy-2-methyl-8-[[(2S)-2-methylbutanoyl]oxy]-1,2,7,8,8aR,hexahydro naphthalen-1-yl]-heptanoic acid, its molecular formula is $C_{23}H_{35}O_7Na$ having molecular weight of 446.51 g/mol. It appears as a white to yellowish crystalline powder, soluble in methanol and water[1].

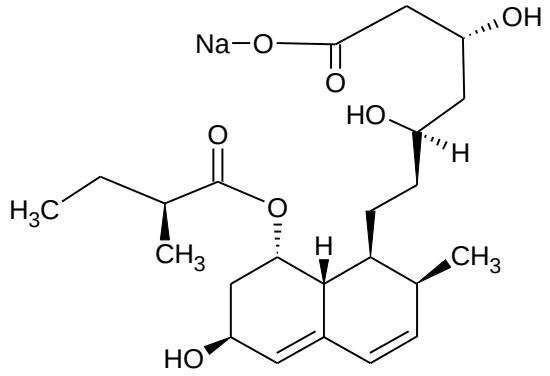

Fig 1: Structure of Pravastatin

Pravastatin act as a lipoprotein lowering drug through two pathways. Pravastatin inhibit the function of hydroxymethylglutaryl-CoA (HMG-CoA) reductase, as a reversible competitive inhibitor, Pravastatin sterically hinder the action of HMG-CoA reductase by occupying the active site of enzyme. Pravastatin also inhibits the synthesis of very-low-density-lipoproteins (VLDL) and low-density-lipoproteins (LDL). These reduction increases the cellular LDL receptors, thus LDL uptake increases, removing it from the bloodstream[2].

Aspirin (Fig 2) is chemically 2-acetyloxybenzoic acid or acetylsalicylic acid, its molecular formula is $C_9H_8O_4$ having molecular weight. It appears as colorless crystals or a white crystalline powder. It is soluble in water but partially soluble in alcohol[3].

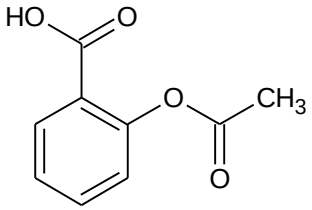

Fig 2: Structure of Aspirin

Aspirin is used as analgesic, anti-inflammatory drug. Aspirin also has an antiplatelet effect by inhibiting the production of thromboxane, which under normal circumstances binds platelet molecule together to create a patch over damaged walls of blood vessels. Because the platelet patch can become too large and also block blood flow, locally and downstream[4]. Aspirin is also used long-term at low doses, to help preventing heart attacks, strokes, and blood coagulation formation in people [5, 6]. The more wide spread and appropriate use of both provastatin sodium and aspirin in secondary prevention of cardiovascular diseases will avoid large number of pre mature death. On literature survey it was found that many analytical methods including UV methods are developed for both provastatin sodium [7-10] and aspirin[11-12] individually. But there is no method for the simultaneous estimation of aspirin and provastatin sodium in combination. The exhaustive literature survey revealed that none of the most recognized pharmacopoeias and any major journals include these drugs in combination for simultaneous estimation of provastatin sodium and aspirin by UV-visible spectroscopy. Hence, there is a need for the development of newer, rapid, accurate and reproducible method for the simultaneous estimation of provastatin sodium and aspirin in pharmaceutical dosage forms. Keeping this in mind in the present study an attempt has been made to simultaneously estimate provastatin sodium and aspirin by simultaneous equation method .

Materials and Methods

Materials

All the chemicals and reagents used for the development of proposed method to estimate pravastatin sodium and aspirin are of spectroscopic grade. The instrument UV-visible spectrophotometer (shimadzu-1800) was used for the analytical method development and validation of pravastatin sodium and aspirin. The present work was carried out at department of pharmaceutical chemistry, government college of pharmacy, Bengaluru. A pure sample of pravastatin sodium and aspirin for the current study were procured from reliable sources. Pravastatin sodium was procured from Biocon pvt Ltd., and aspirin was procured from Microlabs as gift sample, sodium hydroxide (AR grade) from Himedia, Hydrochloric acid(AR grade) from Fisher Scientific. Double distilled Milli pore water was used for the analysis and the same was collected from Milli pore Direct Q3.

Selection of Solvent for analysis

The selection of solvents for analysis was carried out by the effect of different solvents on the pure drug and tablet powder. In methanol and isopropyl alcohol the drugs were soluble. While in water the aspirin solubility proportion is less. And long storage of drugs in 1M HCl and 1M NaOH turns dark in colour. At the end of these studies, 0.1M NaOH was chosen for preparation of solutions for analysis.

Selection of analytical wavelengths

Standard stock solutions having concentration 10 µg/mL of each drug was prepared separately and they were scanned in the wavelength range of 200-400 nm and the maximum (λ_{max}) absorbance of both the drugs were found to be 229 nm and 297 nm for aspirin (Fig 3) and 238.40nm for pravastatin sodium (Fig 4)

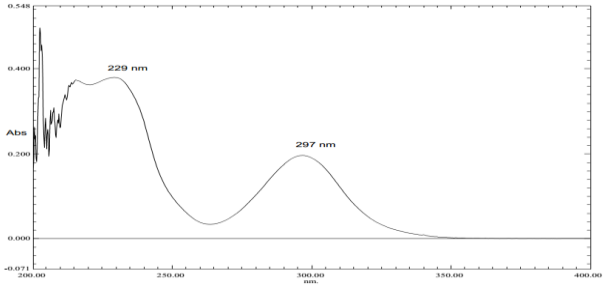

Fig 3: UV spectrum of aspirin (10µg/mL)

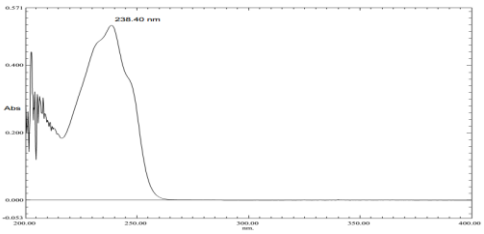

Fig 4: UV spectrum of pravastatin sodium (10µg/mL)

Preparation of Standard stock solution of aspirin and pravastatin sodium

Stock solution A was prepared by dissolving 50 mg of accurately weighed aspirin and pravastatin sodium into 100 mL volumetric flask and the final volume was adjusted to 100 ml with 0.1M NaOH to give the stock solution 500 µg/mL concentration. From the resulting solution 50 mL of aspirin and 10 mL of pravastatin sodium were placed in 100 mL volumetric flask and volume adjusted with 0.1M NaOH to give solution of 250 µg/mL of aspirin

solution and 50 µg/mL of pravastatin sodium (stock B). From stock solution B 0.5-5.0 mL of aspirin and 1-10 mL of pravastatin sodium were pipetted in to 25 mL volumetric flasks and the volume was made up with 0.1M NaOH to get concentration of 5-50 µg/mL of aspirin and 2-20 µg/mL of pravastatin sodium. The absorbance of resulting solution was measured against 297 nm and 238.40 nm. (Table 1, Fig 5&6)

Table 1: Calibration data of aspirin (5-45 µg/mL) and pravastatin sodium

(2- 18 µg/mL)

S. No	Aspirin		Pravastastin sodium	
	Concentration in µg/mL	Absorbance 297 nm	Concentration of µg/mL	Absorbance 238.40 nm
1	05	0.076	02	0.084
2	10	0.154	04	0.150
3	15	0.230	06	0.226
4	20	0.295	08	0.304
5	25	0.374	10	0.370
6	30	0.435	12	0.449
7	35	0.513	14	0.522
8	40	0.591	16	0.595
9	45	0.669	18	0.668

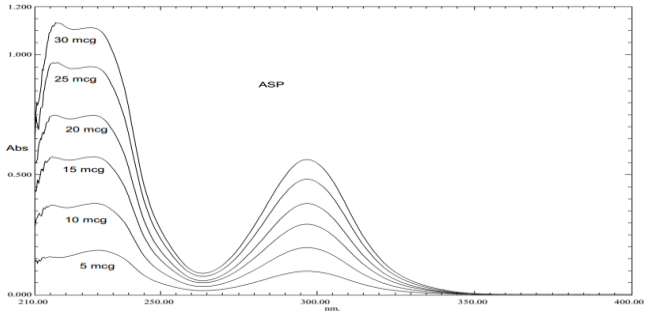

Fig 5: Calibration curves for Aspirin (5-30 µg/mL)

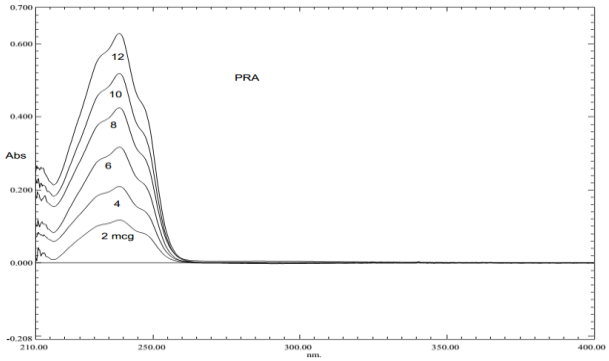

Fig 6: Calibration curves for pravastatin sodium

(2-12 µg/mL)

Formula: The following two sets of simultaneous equations are used to determine the concentrations of aspirin and provastatin sodium

$C_X = A_2a_{y1} - A_1a_{y2} / a_{x2}a_{y1} - a_{x1}a_{y2}$ or $C_X = A_1a_{y2} - A_2a_{y1} / a_{x1}a_{y2} - a_{x2}a_{y1}$

$C_Y = A_1a_{x2} - A_2a_{x1} / a_{x2}a_{y1} - a_{x1}a_{y2}$ or $C_Y = A_2a_{x1} - A_1a_{x2} / a_{y2}a_{x1} - a_{y1}a_{x2}$

Where,

Cx= concentration of aspirin in mixture,

Cy= Concentration of pravastatin sodium in mixture,

ax1 and ax2 are absorptivity of aspirin at its λ_{max} and pravastatin sodium λ_{max}

ay1 and ax2 are absorptivity of pravastatin sodium at λmax of aspirin and λmax of pravastastin sodium.

For mixture 1: A1=0.149, A2= 0.0096 (from Table 4)

A1= Absorbance of mixture at λ 238.40 nm & A2= absorbance of mixture at 297 nm

ax1= 150, ax2= 192.31, ay1=382.50, ay2=0.00 (By Table 2&3)

A1=0.149, A2= 0.0096

ax1= 150
ax2= 192.31
ay1=382.50
ay2=0.00

C_{ASP}= (0.0096*382.50 – 0.149*0.000)/ (192.31*382.50-150*0.00)

= 36.72/73558.575

= 0.0004991 mg/ml or C_{ASP}= 4.99 µg/mL

C_{PRA}= (0.149*192.31-0.096*150) / ((192.31*382.50-150*0.00)

= 28.65419/ 773558.575

= 0.0001937 mg/ml or C_{PRA} = 1.937 µg/mL

For other mixtures the calculations were done similarly as mentioned above.

Table 2: Absorbance of Aspirin at 238.40 nm and 297 nm for simultaneous estimation method

S.NO	Concentration of aspirin (µg/mL)	Absorbance		$E^{1\%}1cm$	
		238.40nm	297nm	238.40nm	297nm
1	5	0.076	0.0970	152	194
2	10	0.154	0.1960	154	196
3	15	0.230	0.2930	153	195
4	20	0.295	0.3790	147	189
5	25	0.374	0.4810	149	192
6	30	0.435	0.5630	145	187
7	35	0.513	0.6562	146	187
8	40	0.591	0.7494	147	187
9	45	0.669	0.8426	148	187
			Average	150	192

Here ay_1= 150, ay_2= 192.3

Table 3: Absorbance of pravastatin sodium at 238.40 nm and 297 nm

S.NO	Concentration of Pravastatin sodium (µg/ml)	Absorbance	$E^{1\%}1cm$		
		238.40 nm	297 nm	238.40 nm	297 nm
1	2	0.084	0	420	0
2	4	0.150	0	375	0
3	6	0.226	0	376	0
4	8	0.304	0	380	0
5	10	0.370	0	370	0
6	12	0.449	0	374	0
7	14	0.522	0	371	0
8	16	0.595	0	372	0
9	18	0.668	0	371	0
			Average	382.50	0.00

Here ax_1= 382.50, ax_2= 0.00

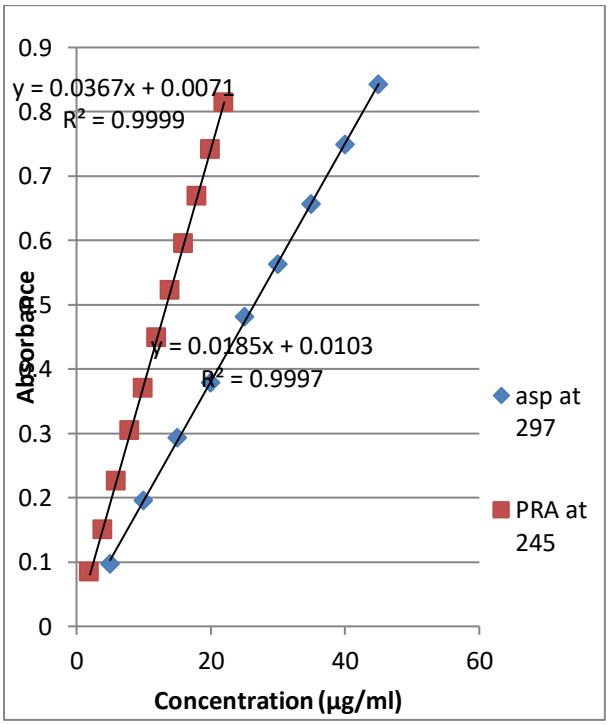

Fig 7: Calibration graph for Aspirin (5-45 µg/mL) and Pravastatin sodium (2-18 µg/mL)

Table 4: Absorbance of mix (Aspirin and pravastatin sodium) by simultaneous estimation method

S. No.	Concentration of Pravastatin sodium and aspirin (mixture in µg/mL)		Absorbance		Concentration obtained (µg/mL)		% Error	
	Pravastatin sodium	Aspirin	238.40 nm	297 nm	Provastatin sodium	Aspirin	Pravastatin sodium	Aspirin
1	2	5	0.149	0.096	1.937	4.99	-3.15	-0.200
2	4	10	0.315	0.205	4.050	10.65	1.25	6.500
3	6	15	0.484	0.334	5.800	17.36	-3.33	15.73
4	8	20	0.595	0.387	7.880	20.123	-4.17	0.615
5	10	25	0.745	0.482	9.640	25.06	-3.52	-0.252
6	12	30	0.839	0.574	11.99	29.84	-0.30	-0.553
7	14	35	1.028	0.678	13.09	35.15	-6.50	0.428

Determination of aspirin and pravastatin sodium in physical mixture

A physical mixture of aspirin and pravastatin sodium was prepared by blending appropriate quantities of each drug, powder equivalent to 10 mg of aspirin and pravastatin sodium was weighed and dissolved in 10 mL of 0.1M NaOH with the aid of ultrasonicator for 10 min. Solution was filtered through whatmann paper (No. 41) into a 100 mL volumetric flask and volume was made up to mark with 0.1M NaOH to give solutions of 100 µg/mL. Then these solutions are further diluted to 100 mL to get 250 µg/mL of aspirin and 50 µg/mL of pravastatin sodium. Various aliquots were prepared and suitably diluted with 0.1M NaOH to give final concentration of 2, 4, 6, 5, 10, 15 µg/mL in different volumetric flasks of 10 mL capacity. The absorbance of prepared aliquots mixture of aspirin and pravastatin sodium was measured against 297 nm and 238.40 nm. By substituting the values of A_1 and A_2 the values of C_x and C_y can be calculated by solving the two equations simultaneously(Table 5)

Table 5: Absorbance of assay mixtures in tablet dosage form

S.No	Aspirin (µg/mL)		Pravastatin sodium (µg/mL)		Absorbance		% Error	
	Conc. Tkn.	Conc. Obt.	Conc. Tkn.	Conc. Obt.	238.40nm (A_1)	297nm (A_2)	Aspirin	Pravastatin sodium
1	5	4.99	2	1.97	0.149	0.040	-2	-1.5
2	10	10.35	4	4.05	0.315	0.099	3.5	1.25
3	15	15.36	6	5.96	0.484	0.136	2.4	-0.66
4	5	5.1	4	4.1	0.308	0.550	2	2.5
5	5	5.2	6	6.15	0.309	0.800	4	2.5
6	10	10.08	2	2.07	0.570	0.290	0.8	3.5

Where, Tkn= Taken, Obt= Obtain, Conc.= Concentration

Method validation

The developed method was validated according to their analytical procedures as per ICH guidelines for validation of analytical procedures in order to determine linearity, precision, LOD, LOQ, and accuracy for the analyte.

Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results that are directly proportional to the concentration of analyte in the sample solution.(Table 6 & 7)

Table 6: Linearity of aspirin in 0.1M NaOH

S. No	Aspirin		
	Conc(µg/mL)	Absorbance	$E^{1\%}$ 1cm
1	5	0.097	194.0
2	10	0.196	196.0
3	15	0.293	195.0
4	20	0.379	189.5
5	25	0.481	192.4
6	30	0.563	187.0
7	35	0.645	181.7
8	40	0.727	207.0

Table 7: Linearity of Pravastatin sodium in 0.1M NaOH

S. No	Pravastatin sodium		
	Conc (µg/mL)	Absorbance	$E^{1\%}$ 1cm
1	2	0.084	420
2	4	0.150	375
3	6	0.226	376
4	8	0.304	380
5	10	0.370	370
6	12	0.449	374
7	14	0.528	379
8	16	0.607	377.1

Precision

Precision studies are carried out to ascertain the reproducibility of the proposed methods. Repeatability was determined by preparing six replicates of same concentration of the sample and the absorbance was measured(Table 8, 9,10 & 11)

Replicates	Absorbance	Simultaneous equation method	
		Absorbance	Concentration
1	1	0.574	15.00
2	2	0.575	15.00
3	3	0.576	15.01
Mean		0.581	15.00
Standard Deviation		0.008	0.040
%RSD		1.390	0.270

Table 8: Intraday Precision data for Aspirin

Table 9: Intraday Precision data for Pravastatin sodium

Replicates	Absorbance	Simultaneous equation method	
		Absorbance	Concentration
1	1	0.3160	6.00
2	2	0.3170	6.10
3	3	0.3164	6.03
Mean		0.3170	6.043
Standard Deviation		0.0005	0.051
%RSD		0.1600	0.850

Replicates	Day interval	Simultaneous equation method	
		Absorbance	Concentration
1	Day 1	0.574	15.00
2	Day 2	0.580	15.05
3	Day 3	0.590	15.08
Mean		0.581	15.00
Standard Deviation		0.008	0.040
%RSD		1.390	0.270

Table 10: Interday Precision data for Aspirin

Table 11: Interday precision data for pravastatin sodium

Replicates	Day interval	Simultaneous equation method	
		Absorbance	Concentration
1	**Day 1**	0.3160	6.00
2	**Day 2**	0.3180	6.03
3	**Day 3**	0.3190	6.1
Mean		0.3170	6.043
Standard Deviation		0.0015	0.005
%RSD		1.4800	1.850

Accuracy (% Recovery)

The accuracy of an analytical procedure expresses the closeness of agreement between the value that is accepted either as a conventional true value or as an accepted reference value and the value found. Accuracy of proposed method was determined using recovery studies. The recovery studies were carried out by adding different amount (80%,100% and 120%) of pure drug to the preanalysed formulation.(Table 12).

Ruggedness

Intermediate precision express the variation with in laboratories like Different Days, different analyst, different equipment etc. the intermediate precision was performed for aspirin and pravastatin sodium by different analyst on different day. The mixtures of samples are subjected for the UV analysis by different analysts and the obtained absorbance was recorded and the % RSD of replicates was calculated. The results obtained were presented in Table 13 & 14.

Table 13: Ruggedness data for Aspirin

	Concentration (µg/mL)		Absorbance Simultaneous equation method	
	Aspirin	**Pravastatin sodium**	**Aspirin**	**Pravastatin sodium**
ANALYST -01	15	6	0.574	0.316
	15	6	0.573	0.317
	15	6	0.573	0.315
	15	6	0.574	0.316
	15	6	0.575	0.318
		Mean	0.5736	0.316
		SD	0.0008	0.0008
		% RSD	0.15	0.15

Table 14: Ruggedness data for Pravastatin sodium

	Concentration (µg/mL)		Absorbance Simultaneous equation method	
	Aspirin	**Pravastatin sodium**	**Aspirin**	**Pravastatin sodium**
ANALYST -02	15	6	0.574	0.313
	15	6	0.573	0.318
	15	6	0.573	0.315
	15	6	0.575	0.316
	15	6	0.575	0.318
		Mean	0.574	0.316
		SD	0.001	0.0021
		% RSD	0.17	0.67

Table 12: % Recovery study data for aspirin and pravastatin sodium by simultaneous estimation method

Levels	Aspirin (µg/mL)		Pravastatin sodium (µg/mL)		Total conc. taken (µg/mL)		Absorbance		Total concentration obtained		Amt. of std. recovered (µg/ml)		% Recovery	
	Std. soln	Sample mix soln	Std. soln	Sample mix soln	ASP	PRA	ASP	PRA	ASP	PRA	ASP	PRA	ASP	PRA
80%	10	08	8	6.4	18	14.4	0.2580	0.5396	17.82	14.28	7.820	6.28	97.75	98.12
80%	10	08	8	6.4	18	14.4	0.2580	0.5434	17.81	14.30	7.810	6.30	97.60	98.43
80%	10	08	8	6.4	18	14.4	0..2580	0.5426	17.81	14.28	7.830	4.28	97.87	98.12
100%	10	10	8	8.0	20	16.0	0.2878	0.6042	19.81	15.90	9.810	7.90	98.10	98.75
100%	10	10	8	8.0	20	16.0	0.2882	0.5772	19.93	15.52	9.930	7.92	99.30	99.00
100%	10	10	8	8.0	20	16.0	0.2827	0.6064	19.8	15.96	9.960	7.966	98.00	99.57
120%	10	12	8	9.2	22	17.2	0.3175	0.644	21.90	16.90	11.90	8.90	99.16	96.73
120%	10	12	8	9.2	22	17.2	0.3161	0.6498	21.80	17.10	11.80	9.10	98.33	98.91
120%	10	12	8	9.2	22	17.2	0.3163	0.6598	21.82	17.36	11.82	9.36	98.50	101.70

Where ASP = Aspirin, PRA= Pravastatin sodium

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provide an indication of its reliability during normal usage. The evaluation of robustness should be considered during the development phase and depends on the type of procedure under study. It should show the reliability of an analysis with respect to deliberate variations in method parameters like temperature (Table 15 & 16)

Table 15: Robustness data for Aspirin

	Concentration		Absorbance	
	(µg/mL)		Simultaneous equation method	
	Aspirin	ravastatin sodium	Aspirin	Pravastatin sodium
At 18 °C	15	6	0.574	0.316
	15	6	0.563	0.317
	15	6	0.573	0.312
	15	6	0.554	0.32
	15	6	0.575	0.32
		Mean	0.5678	0.317
		SD	0.0090	0.003
		%RSD	1.60	1.05

Table 16: Robustness data for Pravastatin sodium

	Concentration		Absorbance	
	(µg/mL)		Simultaneous equation method	
	Aspirin	ravastatin sodium	spirin	Pravastatin sodium
At Room Temperature	15	6	0.574	0.313
	15	6	0.573	0.318
	15	6	0.563	0.315
	15	6	0.575	0.316
	15	6	0.575	0.318
		Mean	0.574	0.316
		SD	0.001	0.0021
		%RSD	0.89	0.67

Limit of detection and limit of quantification

Limit of detection (LOD) and limit of quantification (LOQ) were determined based on the standard deviation of response and the slope and were calculated by using the equation

$$LOD=3\times s/S \text{ and } LOQ= 10\times s/S$$

Where s is standard deviation of intercept and S is the slope of the line

Results

Aspirin and Pravastatin sodium were individually analysed by UV spectrophotometric method using the solvent 0.1M NaOH. Optical characteristics such as λ_{max}, $E^{1\%}_{1cm}$, slope intercept, correlation coefficient, linearity and range, LOD and LOQ were observed as in Table 17 & 18.

Table 17: Calibration Data graph for Aspirin

Parameter	Simultaneous equation method
λmax (nm)	297
$E^{1\%}$ 1cm	192
Slope*	0.0367
Intercept*	0.0071
Correlation coefficient	0.999
Linearity and range	5-45
LOD (µg/ml)	0.79
LOQ (µg/ml)	1.17

Table 18: Calibration Data graph for Pravastatin sodium

Parameter	Simultaneous equation method
λ_{max} (nm)	238.40
$E^{1\%}$ 1cm	382.20
Slope*	0.0185
Intercept*	0.0103
Correlation coefficient	0.9997
Linearity and range	2-18
LOD (µg/ml)	0.55
LOQ (µg/ml)	1.695

The mixture of aspirin and pravastatin sodium was analysed by UV spectroscopic method using simultaneous equation method. The method developed was validated according to the ICH guidelines. The observed results of the developed and validated method in the present study suggests that the method can be adopted for routine analysis of these drugs simultaneously.

Conclusion

A physical mixture was prepared in the laboratories of Government College of Pharmacy as no combined dosage forms of aspirin and pravastatin sodium are available in the market. As spectroscopic methods are highly powerful and convenient methods of analysis, in the present study simultaneous equation method was developed and validated for routine analysis of aspirin and pravastatin sodium in their bulk and physical mixtures. In simultaneous equation method different wavelengths are selected to calculate their concentrations in both bulk and in combination. This developed method is economical, accurate and precise. From the observations of the method developed and validated it can be concluded that, it may be used for routine analysis of aspirin and pravastatin sodium simultaneously at industrial level in their dosage forms.

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