Original Article

Area under Curve Method for the Simultaneous Quantitative Estimation of Pravastatin Sodium and Aspirin

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ABSTRACT

In the present study an economic, a precise UV-spectrosphotometric method for estimating Aspirin and Pravastatin sodium in bulk and physical mixture is carried out as there is less paucity of literature on simultaneous estimation of these drugs by area under curve method. 0.1M sodium hydroxide is used as a solvent in the estimation. The method determines Pravastatin sodium and Aspirin at the wavelength range of 292-302 nm and 233-243 nm respectively. Beer's range was observed at 5-45 μg/mL for Aspirin, 2-18 μg/mL for Pravastatin sodium. 97.91-99.0% for Aspirin and 92.3-99.0% for Pravastatin sodium in the physical mixture are the percentage recoveries. The results observed were statistically validated and ICH guidelines were followed to check the reproducibility of the proposed method via recovery studies. The method may be used for routine analysis of Aspirin and Pravastatin sodium simultaneously at the industrial level and research laboratories in bulk and in their dosage forms.

Keywords: Pravastatin sodium, Aspirin, Area under the curve, 0.1M Sodium hydroxide, Physical mixture, Recovery studies.

INTRODUCTION

(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 is the IUPAC name of Pravastatin Sodium (Figure 1), $C_{23}H_{35}O_7Na$ having molecular weight of 446.51 g/mol is its molecular formula. Pravaststin sodium appears in the form of a white, yellowish crystalline powder and soluble in methanol, water.[1]

Pravastatin sodium is a reversible competitive Inhibitor of hydroxymethylglutaryl-CoA (HMG-CoA) reductase. It sterically hinders the function of the enzyme HMG-CoA reductase by interacting with its active site. Also very low density lipoproteins and low-density-lipoproteins synthesis are inhibited by Pravaststin. As a result the cellular Low density lipoprotein receptors are increased, inturn increases the uptake of low density lipoproteins.[2]

Aspirin (Figure 2) is acetylsalicylic acid. Having the molecular formula $C_9H_8O_4$ and a molecular weight of 180.16 g/mol. It occurs in the form of colorless crystals or as a white crystalline powder. It is soluble in alcohol and partially soluble in alcohol.[3]

Acetyl salicylic acid is an analgesic and anti-inflammatory agent. Aggregation of Platelets is inhibited by Aspirin as it inhibit the production of thromboxane. Thromboxane binds to platelet molecules and forms a patch over the damaged blood vessel walls.[4] Acetyl salicylic acid at a lower dose is used to prevent stroke, heart attack and coagulation of blood.[5-6]

A deep survey revealed the fact that UV, HPLC, RP-HPLC methods were developed for the estimation of Pravastatin sodium and Aspirin individually.[7-12] There is no methods are found for the simultaneous estimation of Aspirin and Pravastatin sodium in combination. This encouraged

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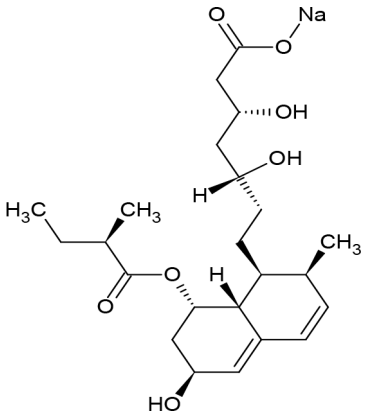

Figure 1: Structure of Pravastatin sodium.

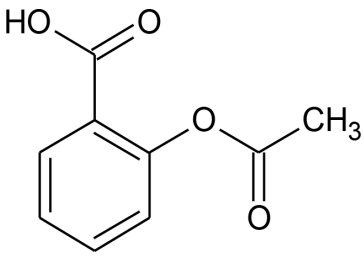

Figure 2: Structure of Aspirin.

us to develop and validate a new analytical method to estimate simultaneously of Pravastatin sodium and Aspirin in pharmaceutical dosage forms, which must be reproducible, rapid and sensitive. Hence the earlier work on the estimation of Aspirin and Pravastatin sodium is continued.[13]

MATERIALS AND METHODS

Materials

Pravastatin sodium and Aspirin are estimated by using spectroscopic grades of chemicals and reagents. The 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 the Department of Pharmaceutical Chemistry, Government College of Pharmacy, Bengaluru. Pravastatin sodium was procured from Biocon Pvt Ltd. Aspirin from Microlabs as a gift sample, Sodium hydroxide (AR grade) from Himedia, Hydrochloric acid (AR grade) from Fisher Scientific. Millipore water collected from Millipore Direct Q3 was used.

Solvent

Drugs were soluble in methanol and isopropyl alcohol but in water the aspirin is not completely soluble. A dark colour was produced when drugs were stored for a longer period of time in 1M hydrochloric acid and 1M Sodium hydroxide. Hence 0.1M Sodium hydroxide was chosen as a solvent for the preparation of solution for analysis.

Analytical Wavelengths

Pravastatin and Aspirin standard stock concentration of 10µg/mL was prepared. The absorbance was measured at wavelength 200-400 nm. λ_{max} of both the drugs were 229 nm and 297 nm for Aspirin (Figure 3) and 238.40 nm for Pravastatin sodium (Figure 4).

The Area under the curve was measured at ± 10 nm of both the λ_{max}, i.e., at λ 292-302 nm and 233-243 nm respectively for Aspirin (Figure 5) and Pravastatin sodium (Figure 6).

Pravastatin sodium and Aspirin standard stock solution

50 mg of Aspirin and Pravastatin sodium was weighed into a 100 mL standard volumetric flask, dissolved in few ml 0.1 M Sodium hydroxide and then made up the volume to 100 ml. It is stock solution A of concentration 500 µg/mL. From this 50 mL of Aspirin and 10mL of Pravastatin sodium were placed in a 100 mL standard volumetric flask diluted with 0.1M Sodium hydroxide to produce 250 µg/mL of Aspirin and 50 µg/mL of Pravastatin sodium (stock B). From stock solution B, 0.5- 4.5 mL of Aspirin and 1-9 mL of Pravastatin sodium placed in a 25 mL standard volumetric flasks, diuted with 0.1M sodium hydroxide to get 5-45 µg/mL

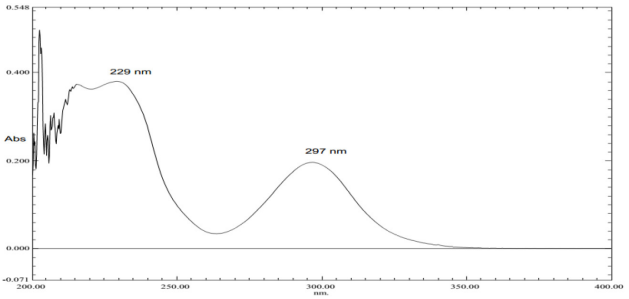

Figure 3: UV spectrum of aspirin (10µg/mL).

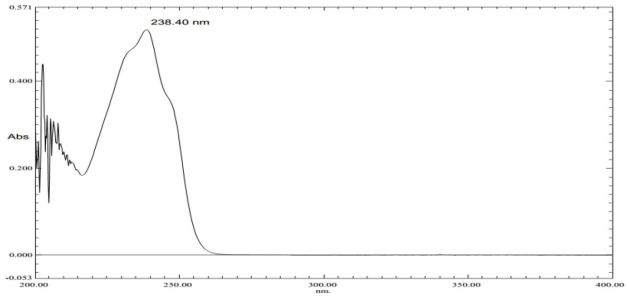

Figure 4: UV spectrum of Pravastatin sodium (10µg/mL).

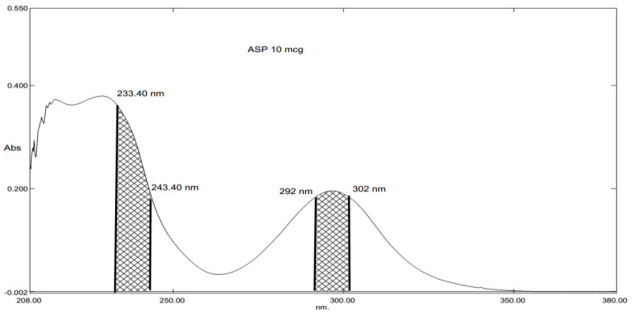

Figure 5: Area under curve of Aspirin (10µg/mL).

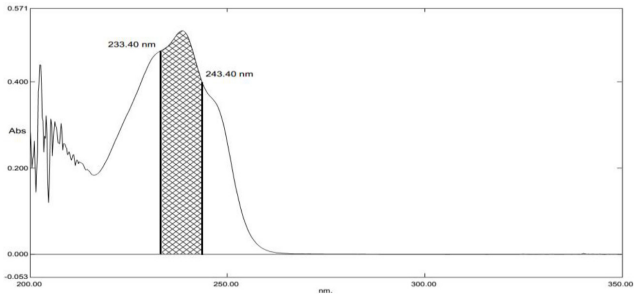

Figure 6: Area under curve of Pravastatin sodium (10µg/mL).

Table 1: Calibration data of AUC of Aspirin (5-45 µg/mL) and Pravastatin sodium (2- 18 µg/mL).

Sl. No	Aspirin		Pravastastin sodium	
	Concentration in µg/mL	AUC at 292-302 nm	Concentration of µg/mL	AUC at 234-244 nm
1	05	0.950	02	1.094
2	10	1.913	04	1.954
3	15	2.870	06	2.958
4	20	3.710	08	3.971
5	25	4.704	10	4.845
6	30	5.505	12	5.851
7	35	6.455	14	6.945
8	40	7.405	16	8.025
9	45	8.355	18	9.119

AUC= Area under curve

of Aspirin and 2-18 µg/mL of Pravastatin sodium. The Area under the curve was determined in 292-302 nm and 233 -243 nm. (Table 1, Figure 7).

Formula: The following two sets of simultaneous equations are used to determine the concentrations of Aspirin and Pravastatin sodium.

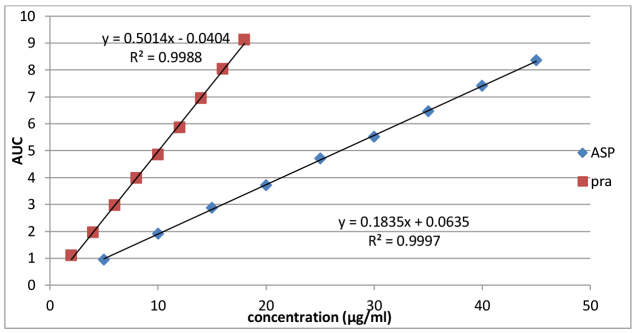

Figure 7: Calibration graph for Aspirin (5-45 µg/mL) and Pravastatin sodium (2- 18 µg/mL).

CX = A2ay1- A1ay2/ ax2ay1- ax1ay2 or
CX= A1ay2-A2ay1/ax1ay2-ax2ay1

CY = A1ax2- A2ax1/ax2ay1- ax1ay2 or
CY = A2ax1-A1ax2/ay2ax1-ay1ax2

Where, A1 is the AUC of mixture at 292-302 nm and A2 is the AUC of mixture at 233-243 nm. ax1 is the absorptivity of aspirin at 292-302 nm. ay1 is for the absorptivity of Pravastatin sodium. Similarly ax2 is aspirin absorptivity and ay2 is of Pravastatin sodium at 233-243 nm.

Absorptivity ($E^{1\%}$ 1cm) = (Area under curve / concentration in µg/ml) × 10000

For Mixture 1:

A1=0.936, A2= 2.937 (From Table 2)

ax1= 1882.6, ax2= 2866.6, ay1= 0.000, ay2= 4990 (From Table 3 and 4)

CAspirin= (2.937*0.00-0.936*4994) / 2866*0.00-1882*4990
= -4674.384/-9391180
= 0.0004977
CAspirin=4.977µg/ml

CPravastatin = (0.936*2866.6-2.937*1882.6) / (2866*0.00-1882.6*4990)
= (2683.1376-4512.5922)/-9394174
= -1829.45174/-9394174
= 0.0001947 mg/ml
CPravastatin =1.947µg/ml

For other mixtures, similar calculations were done as mentioned above.

Table 2: Area under curve of Aspirin at 292-302 nm and 234-244 nm.

Sl. No	Concentration of Aspirin (µg/ml)	Area under curve (AUC)		$E^{1\%}1cm$	
		292-302nm	234-244nm	292-302nm	234-244nm
1	5	0.950	1.44	1900	2880
2	10	1.913	2.918	1913	2918
3	15	2.870	4.375	1913	2916
4	20	3.710	5.66	1855	2830
5	25	4.704	7.174	1881	2869
6	30	5.505	8.368	1835	2789
			Mean	1882.6	2866

Here ax1=1882.6, ax2= 2866.

Table 3: Area under curve of Pravastatin sodium at 292-302nm and 234-244nm.

Sl. No	Concentration of Pravastatin sodium (µg/ml)	Area under curve (AUC)		$E^{1\%}1cm$	
		292-302nm	234-244nm	292-302nm	234-244nm
1	2	0.0000	1.094	0.000	5470
2	4		1.954		4885
3	6		2.958		4930
4	8		3.971		4963
5	10		4.845		4845
6	12		5.851		4875
7	14		6.945		4960
8	16		8.025		5015
9	18		9.119		5066
	20		**Mean**	**0.000**	**4990**

Here ay1= 0.000, ay2= 4990

Table 4: Area under curve of mixture of Aspirin and Pravastatin sodium.

Sl. No.	Concentration of PRA and ASP (mix in µg/ml)		AUC		Concentration obtained		% Error	
	ASP	PRA	291-301	234-244	PRA	ASP	PRA	ASP
1	05	02	0.936	2.397	5.6	1.9	12.0	-5.00
2	10	04	2.004	4.961	10.6	3.8	6.00	-5.00
3	15	06	3.265	7.907	15.40	5.78	2.66	-3.66
4	20	08	3.787	9.642	20.11	7.7	0.55	-3.75
5	25	10	4.718	12.067	25.06	9.78	0.20	-2.20
6	30	12	5.620	14.310	29.85	11.56	0.50	-3.66
7	35	14	6.608	16.631	35.01	13.24	0.028	-5.14

Where, ASP=Aspirin, PRA=Pravastatin.

Table 5: Absorbance of assay mixtures.

Sl. No.	Aspirin (µg/mL)		Pravastatin sodium (µg/mL)		AUC		% Error	
	Conc. Tkn.	Conc. Obt.	Conc. Tkn.	Conc. Obt.	291-301nm (A_1)	233-243nm(A_2)	ASP	PRA
1	5	4.3	2	1.90	0.900	2.09	-2.0	-1.50
2	10	10.1	4	4.10	2.00	5.00	3.5	1.25
3	15	15.89	6	5.96	3.28	7.90	2.4	1.66
4	5	5.2	4	4.12	3.8	9.80	2.0	2.50
5	5	5.1	6	6.22	3.82	12.0	4.0	2.50
6	10	10.10	2	2.21	2.04	13.0	0.8	3.50

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

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, powdered mixture of 10 mg of Aspirin and Pravastatin sodium was dissolved in 10 mL of 0.1M Sodium hydroxide under sonication, filtered in No. 4 Whatman paper, diluted to get 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 suitably diluted to give final concentration 2, 4, 6, 5, 10, 15 µg/mL. Area under the curve of prepared aliquots mixture of Aspirin and Pravastatin sodium was measured against 292-302 nm

and 233-243 nm. Absorbance of assay mixtures is depicted (Table 5).

Method Validation

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

Linearity

Linearity of Aspirin and Pravastatin sodium are obtained as given below (Table 6, 7).

Table 6: Linearity of Aspirin in 0.1M Sodium hydroxide.

Sl. 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 Sodium hydroxide.

Sl. 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

Table 8: Intraday precision data for Aspirin.

Replicates	Absorbance	Area under curve 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
SD		0.008	0.040
%RSD		1.390	0.270

Table 9: Intraday precision data for Pravastatin sodium.

Replicates	Absorbance	Area under curve 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
SD		0.0005	0.051
%RSD		0.1600	0.850

Table 10: Interday precision data for Aspirin.

Replicates	Day interval	Area under curve 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
SD		0.008	0.040
%RSD		1.390	0.270

Table 11: Interday precision data for Pravastatin sodium.

Replicates	Day interval	Area under curve 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
SD		0.0015	0.005
%RSD		1.4800	1.850

Precision

The results of Precision studies carried out are depicted (Table 8, 9, 10, 11).

Accuracy (% Recovery)

Results of recovery studies carried out are tabulated (Table 12).

Ruggedness

The mixtures of samples are subjected to UV analysis by different analysts. Percentage relative standard deviation of replicates are calculated and reported (Table 13, 14).

Table 12: % Recovery study data for Aspirin and Pravastatin sodium by AUC method.

Levels	Aspirin (µg/mL)		Pravastatin (µg/mL)		Total conc. taken (µg/mL)		AUC		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
80%	10	08	8	6.4	18	14.4	3.339	7.14	7.89	5.94	99.0	99.0
80%	10	08	8	6.4	18	14.4	3.336	7.14	7.87	5.93	98.83	98.8
80%	10	08	8	6.4	18	14.4	3.339	7.11	7.89	5.94	98.62	99.0
100%	10	10	8	8.0	20	16.0	3.710	8.09	9.90	7.39	99.0	92.3
100%	10	10	8	8.0	20	16.0	3.700	8.09	9.90	7.39	99.0	92.3
100%	10	10	8	8.0	20	16.0	3.720	8.10	9.90	7.39	99.0	92.3
120%	10	12	8	9.2	22	17.2	4.070	8.83	11.8	9.56	98.33	95.6
120%	10	12	8	9.2	22	17.2	4.010	8.82	11.7	9.56	98.1	95.6
120%	10	12	8	9.2	22	17.2	4.080	8.80	11.7	9.58	97.91	95.8

Table 13: Ruggedness data for Aspirin.

	Concentration (µg/mL)		Absorbance AUC 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 AUC 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 15: Robustness data for Aspirin.

	Concentration (µg/mL)		Absorbance AUC method	
	Aspirin	Pravastatin sodium	Aspirin	Pravastatin sodium
At 18 ºC	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 (µg/mL)		Absorbance AUC method	
	Aspirin	Pravastatin sodium	Aspirin	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

Table 17: Calibration Data for Aspirin.	
Parameter	**Area under curve method**
λ_{max} (nm)	292-302
$E^{1\%}$ 1cm	1882.6
Slope*	0.5014
Intercept*	0.0404
Correlation coefficient	0.9999
Linearity and range	5-45
LOD (µg/mL)	1.4
LOQ (µg/mL)	4.3

Table 18: Calibration Data for Pravastatin sodium.	
Parameter	**Area under curve method**
λ_{max} (nm)	233-243
$E^{1\%}$ 1cm	4990
Slope*	0.01835
Intercept*	0.0635
Correlation coefficient	0.9997
Linearity and range	2-18
LOD (µg/mL)	0.58
LOQ (µg/mL)	0.98

Robustness

At two different temperature, robustness of the procedure adopted for analysis are reported (Table 15, 16) and it is the indication of reliability of the analysis carried out.

LOD and LOQ

LOD and LOQ were calculated and reported.

RESULTS

Aspirin, Pravastatin sodium were individually analyzed by UV spectrophotometric method using the solvent 0.1M Sodium hydroxide. Optical characteristics such as λ_{max}, E1%1cm, slope intercept, correlation coefficient, linearity and range, LOD, and LOQ were observed as in Table 17, 18.

Mixture of Aspirin and Pravastatin sodium was analyzed by UV spectroscopic method using Area under curve method. The observed results in the developed and validated method of the present study suggest the fact that simultaneously Aspirin and Pravastatin sodium can be estimated on routine basis.

CONCLUSION

A physical mixture was prepared in the laboratories of the Government College of Pharmacy, Bengaluru as the availability of formulations of Aspirin and Pravastatin sodium is not marketed. As spectroscopic methods are potent and convenient method of analysis, in the present study, area under curve method was developed. It is validated for routine analysis of the drugs of the present study. In this method, different ranges of wavelengths are selected to calculate their concentrations in both bulk and in physical mixture. This developed method is economical, accurate, and precise. From observations of the method developed and validated, it is clear that the method may be used for routine analysis of Aspirin and Pravastatin sodium simultaneously at the industrial level and research laboratories in bulk and in their dosage forms.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

ASP: Aspirin; **PRA:** Pravastatin sodium; **LOD:** Limit of detection; **LOQ:** Limit of Quantitation; **AUC:** Area under the curve; **ICH:** International Conference on Harmonization; **mL:** Milli Litre; **nm:** nanometer; **cm:** Centi meter; **UV:** Ultra violet; µg: Micro gram; **Std. soln:** Standard solution; **Tkn:** Taken; **Obt:** Obtained; **Conc.:** Concentration; **SD:** Standard Deviation; **%RSD:** Percentage Relative Standard Deviation.

REFERENCES

1. CAS number 81131-70-6 and 1554206 USP.
2. Tripathi K. Essentials of medical pharmacology, Jaypee publication edition. 7th ed.
3. The Merck. Index. 9th ed. Vol. 874. Merck and CO, INC. p. 114.
4. Hennekens CH, Sacks FM, Tonkin A, Jukema JW, Byington RP, Pitt B, *et al*. Additive benefits of pravastatin and Aspirin to decrease risks of cardiovascular disease: Randomized and observational comparisons of secondary prevention trials and their meta-analyses. Arch Intern Med. 2004;164(1):40-4. doi: 10.1001/archinte.164.1.40, PMID 14718320.
5. Available from: http://en.wikipedia.org/wiki/Pravastatin; 20/03/2016. Wikipedia.
6. The Indian Pharmacopoeia Commission. Ghaziabad. Vol. 2014;III:2532-4.
7. Balaji S, Katteboina S. Development of spectrophotometric method for determination of pravastatin sodium in bulk and tablet formulation. Int J Pharm Tech Res. 2009;1(4):1017-9.

8. Doshi D, Bhatt P. Development and validation of UV spectrophotometric methods for the estimation of pravastatin sodium in bulk and tablet dosage form. Res J Pharm Technol. 2010;3(3):869-79.
9. Trivedi G, Hasumati R. A Review of analytical methods for determination of pravastatin in pharmaceutical dosage forms and biological Fluids; Pharma Tutor. 2014;2(11):59-65.
10. Kadikar HK, Shah R. Simultaneous UV spectrophotometric estimation of pravastatin and Co-enzyme Q10 in their formulated combined dosage forms and synthetic mixture. Int. J Pharm Res Biosci. 2012;1(4):112-27.
11. Ambadekar SR, Barabde GR. Comparative study of estimation of Aspirin form commercial sample by UV-Visible Spectrophotometer and HPLC method. IOSR JAC. 2014;7(9):57-61. doi: 10.9790/5736-7915761.
12. Bhattacharyya I, Bhattacharyya SP, Talukdar S, Medya S. Development of difference spectroscopic method for the estimation of Aspirin in formulation using hydrotropy. Int Res Pharm Sci. 2011;2(1):84-7.
13. Chaluvaraju KC, Ramgopal MD, Naveen Kumar KL, Niranjan MS. Quantitative Estimation of pravastatin sodium and Aspirin By Simultaneous estimation method. J Pharm Chem. 2020;14(3):13-22.

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