

A Review on Analytical Methods for Estimation of Aspirin, Clopidogrel Bisulphate and Rosuvastatin Calcium in Pharmaceutical Dosage Form

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ABSTRACT

This review article is intended to highlight the analytical methods of aspirin, clopidogrel and rosuvastatin in individual as well as combined pharmaceutical dosage form. Aspirin, clopidogrel and rosuvastatin play an important role in the various cardiovascular diseases. Aspirin and clopidogrel are the antiplatelet whereas Rosuvastatin is antilipemic agent which is used in the treatment of various cardiovascular diseases, cerebrovascular and peripheral vascular diseases. Now these days these drugs are easily available in the market in their individual form as well as in their combined dosage form. Aspirin, clopidogrel and rosuvastatin are official in the pharmacopoeias. Various analytical methods have been reported for the estimation of these drugs in their individual form as well as in their combined dosage form.

Keywords: Cardiovascular, RP-HPLC, Mobile Phase, Column, Wavelength, Flow rate.

INTRODUCTION TO ANALYTICAL METHOD

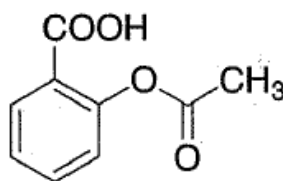
There are various analytical methods are used now these days for the estimation. Various analytical methods like potentiometer, HPLC, aqueous and non-aqueous titrations are used in the field of analysis. Aqueous and non-aqueous titrations are also used in the field of analysis. But now these days HPLC plays an important role in the field of analysis for the quantitative determination.

HPLC is referred as high pressure liquid chromatography which is a separation technique based on the solid stationary phase and liquid mobile phase^[1]. Chromatography is mass transfer process involve adsorption. The active component of the column is adsorbent which is granular material of solid particles (silica, polymers). The principle of separation in the normal phase mode and reverse phase mode is adsorption in which the substances travel /separate according to their relative affinities. Now these days HPLC plays an important role in the field of pharmaceutical analysis for the separation of various substances from the mixture of substances^[2].

Introduction to Drug Profile

Aspirin

Aspirin is known as acetylsalicylic acid which is still the most commonly used NSAID to treat pain and inflammation [3]. Aspirin is 2-acetyloxy benzoic which is COX inhibitor. Aspirin is white crystalline powder [4] which is freely soluble in chloroform and in ether, slightly soluble in water with having molecular formula $C_9H_8O_4$ and molecular weight 180.2g/mol.



Chemical Structure of Aspirin

Pharmacological action [5]

The analgesic, antipyretic, and anti-inflammatory effects of acetylsalicylic acid are due to actions by both the acetyl and the salicylate portions of the intact molecule as well as by the active salicylate metabolite. Acetylsalicylic acid directly and irreversibly inhibits the activity of both types of cyclooxygenase (COX-1 and COX-2) to decrease the formation of precursors of prostaglandins and thromboxane's from arachidonic acid. This makes acetylsalicylic acid different from other NSAIDS (such as diclofenac and ibuprofen) which are reversible inhibitors.

Summary of Analytical Methods for Aspirin**Official Methods for Aspirin⁶⁻⁸**

SR. No.	Official in	METHOD	BRIEF INTRODUCTION	REF. NO
1	IP-2010 (Aspirin tablet)	Potentiometric Titration	Titrate: Tablet Powder Equivalent to 0.5 gm. Aspirin in 30ml of the 0.5M Sodium Hydroxide Titrant: -0.5 M HCl 1ml of 0.5M NaOH is Equivalent to .04504 gm. of Aspirin	6
2	BP-2009	Potentiometric Titration	Titrate: 1gm Aspirin in 10ml Ethanol, Add50ml of the 0.5M Sodium Hydroxide Titrant: 0.5 M HCl 1ml of 0.5M NaOH is Equivalent to .04504 gm. of Aspirin	7
3	USP30-NF25 (Aspirin Tablet)	Liquid Chromatography	Mobile phase: Water (pH 3.4): Acetonitrile (85:15) Column: Packing L1, (300 mm × 4.0 mm) Flow rate: 2 ml/min Wavelength: 285 nm.	8

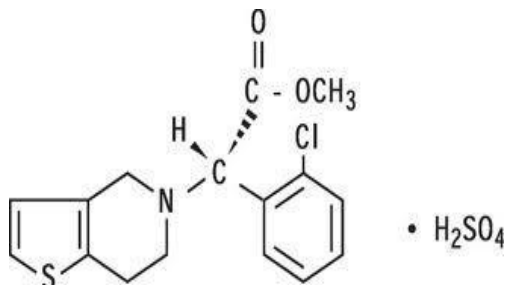
Reported Methods for Aspirin¹⁴⁻²⁶

SR.No.	DRUGS	METHOD	BRIEF INTRODUCTION	REF.NO
1	Aspirin	RP-HPLC	Mobile phase: Sodium Perchlorate Buffer, pH (2.5): Acetonitrile: Isopropyl alcohol (85:14:1) Column: C18, (100 mm × 4.6 mm, 5 μ ,) Flow rate: 1.5 ml/min Wavelength: 275 nm.	9
2	Aspirin and Metoprolol	RP-HPLC	Mobile phase: Phosphate Buffer, (pH 4.6): Methanol (20:80) Column: Phenomenex Luna C18, (250 mm × 4.6 mm, 5 μ ,) Flow rate: 0.8 ml/min Wavelength: 230 nm.	10
3	Aspirin, Ramipril, Hydrochlorothiazide, Simvastatin And Atenolol	RP-HPLC	Mobile phase: Methanol: Water (95:5) Column: Hypersil Gold C18, (250 mm × 4.6 mm, 5 μ ,) Flow rate: 1 ml/min Wavelength: 230 nm.	11
4	Aspirin and	RP-HPLC	Mobile phase: Acetonitrile: Acetate Buffer (75:25)	12

	Prasugrel		Column: Luna C18 (150 mm × 4.6 mm, 5μ, Flow rate: 0.6 ml/min Wavelength: 245 nm	
5	Aspirin and Prasugrel	Stability indicating RP-HPLC	Mobile phase: Acetonitrile: Methanol: Water pH (3) (30:10:60) Column: Kromasil-100 C18 (150 mm × 4.6 mm, 5μ, Flow rate: 1 ml/min Wavelength: 245 nm	13
6	Aspirin and Salicylic acid	RP-HPLC	Mobile phase: Acetonitrile: Trifluoroacetic acid 0.05% (30:70) Column: Waters C ₁₈ (250 mm × 4.6 mm, 5μ, Flow rate: 1.0 ml/min Wavelength: 230 nm	14
7	Amlodipine Besylate, Atenolol and Aspirin	RP-HPLC	Mobile phase: Methanol: Phosphate Buffer (pH 7.0) (70:30) Column: BDS C ₁₈ (250 mm × 4.6 mm, 5μ, Flow rate: 1.0 ml/min Wavelength: 235 nm	15
8	Aspirin And Aspirin Derivatives	RP-HPLC	Mobile phase: Acetonitrile: Water (60:40) Column: Kromasil C ₁₈ (180 mm × 4.6 mm, 5μ, Flow rate: 1.0 ml/min Wavelength: 277 nm	16
9	Aspirin, Caffeine and Orphenadrine citrate	RP-HPLC	Mobile phase: Methanol: Phosphate Buffer, pH3 (65:35) Column: Acclaim C ₁₈ (250 mm × 4.6 mm, 5μ, Flow rate: 1.0 ml/min Wavelength: 215 nm	17
10	Aspirin and Dipyridamole	RP-HPLC	Mobile phase: 0.1 % Phosphoric acid: Acetonitrile (75:25) Column: RP C ₁₈ (50 mm × 4.6 mm, 3.5μ, Flow rate: 1.0 ml/min Wavelength: 227 nm	18
11	Aspirin and Esomeprazole Magnesium	RP-HPLC	Mobile phase: Acetonitrile: Methanol: Phosphate Buffer, pH 3.0 (25:25:50) Column: ODS BP C ₁₈ (200 mm × 4.6 mm, 5μ, Flow rate: 1.0 ml/min Wavelength: 230 nm	19
12	Ramipril, Aspirin and Simvastatin	RP-HPLC	Mobile phase: Acetonitrile: Methanol: 0.5% phosphoric acid (10:70:20) Column: ODS BP C ₁₈ (200 mm × 4.6 mm, 5μ, Flow rate: 1.0 ml/min Wavelength: 226 nm	20
13	Aspirin, Salicylic Acid, and Caffeine	RP-HPLC	Mobile phase: Water: Methanol: Acetic acid (69:28:3) Column: Hypersil C ₁₈ (150 mm × 4.6 mm, 5μ, Flow rate: 1.0 ml/min Wavelength: 275 nm	21

Clopidogrel Bisulphate [22-23]

Clopidogrel Bisulphate is an antiplatelet agent which is used to inhibit the aggregation of platelets which inhibits the blood clots. The drug is Methyl (+)-(S)- α -(2-chlorophenyl)-6,7-dihydrothieno [3,2-c]pyridine-5(4H)-acetate sulfate. Clopidogrel bisulphate is insoluble in water. Clopidogrel Bisulphate is an irreversible inhibitor of P2Y₁₂. The molecular formula of clopidogrel bisulphate is C₁₆H₁₆ClNO₂S.H₂SO₄ and the molecular mass is 419.03 g/mol.



Chemical Structure of Clopidogrel Bisulphate

Pharmacological Action ^[23]

Clopidogrel is an anti-platelet agent which acts by direct inhibition of ADP. The anti-aggregating activity of the clopidogrel bisulphate is due to the biotransformation of the drug to 2-oxo-clopidogrel by enzyme P450-1A. Clopidogrel Bisulphate is mostly used in the myocardial infarction, stroke and peripheral artery disease.

Summary of Analytical Methods for Clopidogrel Bisulphate**Official Methods for Clopidogrel Bisulphate [24-25]**

SR. NO	OFFICIAL IN	METHOD	DESCRIPTION	REF. NO
1	IP 2010 (Clopidogrel Tablet)	Chiral Chromatography	Mobile phase: Phosphate Buffer: Acetonitrile (75:25) Column: Chiral Recognition Protein (15 cm X 4.6 mm), 5 μ m Flow Rate: 1.0 ml/min Wavelength: 220 nm	24
2	USP30-NF25 (Clopidogrel Tablet)	Chiral Chromatography	Mobile phase: Phosphate Buffer: Acetonitrile (75:25) Column: Packing L57 (15 cm X 4.6 mm) Flow Rate: 1.0 ml/min Wavelength: 220 nm	25

Reported Methods for Clopidogrel Bisulphate [26-39]

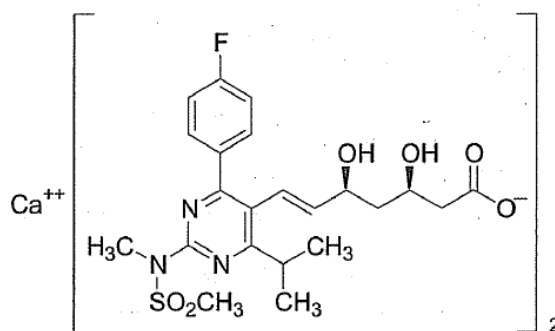
SR NO.	DRUGS	METHOD	BRIEF INTRODUCTION	REF.NO.
1	Clopidogrel Bisulphate	RP-HPLC	Mobile phase: Phosphate Buffer, pH 2.8: Acetonitrile (35:65) Column: Develosil ODS (15 cm X 4.6 mm), 5 μ m Flow Rate: 1.0 ml/min Wavelength: 225 nm	26
2	Clopidogrel Bisulphate	RP-HPLC	Mobile phase: Phosphate Buffer, pH 4.0: Acetonitrile (32:68) Column: Hypersil BDS C18 (25 cm X 4.6 mm), 5 μ m Flow Rate: 1.0 ml/min Wavelength: 220 nm	27

3	Clopidogrel Bisulphate	RP-HPLC	Mobile phase: Phosphate Buffer: Acetonitrile, Methanol (10:80:10) Column: Knauer C18 (25 cm X 4.6 mm),5 µm Flow Rate: 0.9 ml/min Wavelength: 240 nm	28
4	Clopidogrel Bisulphate	RP-HPLC	Mobile phase: Phosphate Buffer, pH 3.0: Acetonitrile (40:60) Column: C18 (15 cm X 4.6 mm),5 µm Flow Rate: 1.0 ml/min Wavelength: 224 nm	29
5	Clopidogrel Bisulphate	RP-HPLC	Mobile phase: 0.1% Trifluoroacetic acid: Acetonitrile (30:70) Column: Inertsil ODS C18 (25 cm X 4.6 mm),5 µm Flow Rate: 1.0 ml/min Wavelength: 220 nm	30
6	Clopidogrel	RP-HPLC	Mobile phase: Phosphate Buffer, pH 3.0: Acetonitrile (75:25) Column: ODS C18 (25 cm X 4.6 mm),5 µm Flow Rate: 1.0 ml/min Wavelength: - 247 nm	31
7	Clopidogrel Bisulphate	RP-HPLC	Mobile phase: Phosphate Buffer, pH 8.0: Acetonitrile (30:70) Column: Nova pack C18 (25 cm X 4.6 mm),5 µm Flow Rate: 0.8 ml/min Wavelength: 210 nm	32
8	Clopidogrel Bisulphate	Stability Indicating RP-HPLC	Mobile phase: Tetrabutyl ammonium Hydrogen Sulfate Buffer: Acetonitrile (70:30) Column: Symmetry C8 (15 cm X 3.9 mm),5 µm Flow Rate: 1.0 ml/min Wavelength: 225 nm	33
9	Clopidogrel Bisulphate	Stability Indicating RP-HPLC	Mobile phase: Phospahte Buffer, pH 4.0: Acetonitrile (80:20) Column: C18 (15 cm X 4.6 mm),5 µm Flow Rate: 0.5 ml/min Wavelength: 235 nm	34
10	Clopidogrel Bisulphate and Atorvastatin Calcium	RP-HPLC	Mobile phase: Acetonitrile: Water (65:35) Column: Sphere-100 C18 (25 cm X 4.6 mm),5 µm Flow Rate: 0.5 ml/min Wavelength: 227 nm	35
11	Clopidogrel Bisulphate and Atorvastatin Calcium	RP-HPLC	Mobile phase: Solvent A: 0.1% Trifluoro acetic acid in water Solvent B: 0.1% Trifluoro acetic acid in Acetonitrile Column: X-Bridge C18 (15 cm X 4.6 mm),5 µm Flow Rate: 1.0 ml/min Wavelength: 215 nm	36
12	Clopidogrel Bisulfate, Its Carboxylic Acid Metabolite,	RP-HPLC	Mobile phase: Phosphate Buffer, pH 2.6: Acetonitrile: Methanol Column: Hypersil BDS C18 (25 cm X 4.6 mm),5 µm Flow Rate: 1.0 ml/min	37

	and Atorvastatin		Wavelength:- 220 nm	
13	Phenytoin Sodium and Clopidogrel Bisulphate	RP-HPLC	Mobile phase: Water, pH 3.0: Acetonitrile (30:70) Column: Phenomenx Luna C18 (15 cm X 4.6 mm), 5 μ m Flow Rate: 0.5 ml/min Wavelength: 215 nm	38
14	Clopidogrel And Pioglitazone	RP-HPLC	Mobile phase: Water, pH 4.6: Acetonitrile: Methanol (10:10:80) Column: C18 (15 cm X 4.6 mm), 5 μ m Flow Rate: 1 ml/min Wavelength: 230 nm	39

Rosuvastatin Calcium [40]

Rosuvastatin calcium is referred as statin which is a cholesterol lowering drug. The IUPAC name of rosuvastatin is [(E)-7-[4-(4-fluorophenyl)-6-isopropyl-2-[methyl-(methylsulfonyl) amino] pyrimidin-5-yl] (3R, 5S)-3, 5-dihydroxyhept-6-enoic acid][40]. The chemical formula of rosuvastatin calcium is $C_{22}H_{27}FN_3O_6S_2 \cdot Ca$ and molecular mass of rosuvastatin calcium is 1001.1 g/mol.



Chemical structure of rosuvastatin calcium

Pharmacological Action [41]

Rosuvastatin is lipid lowering agent which inhibits the HMG-CoA which prevents the conversion of 3-hydroxy -3-methylglutaryl-coenzyme-A to melvonate which is precursor of cholesterol [41]. Rosuvastatin is also used in the treatment of atherosclerosis, heart attack, stroke and peripheral vascular disease.

Summary of Analytical Methods of Rosuvastatin Calcium

Official Methods of Rosuvastatin Calcium [42]

SR. NO	OFFICIAL IN	METHOD	DESCRIPTION	REF. NO
1	IP 2010 (Rosuvastatin Tablet)	RP-HPLC	Mobile phase: Acetate Buffer, pH 4.0: Acetonitrile: Tetrahydrofuran (59:36:5) Column: C18 (25 cm X 4.6 mm), 5 μ m Flow Rate: 1.5 ml/min Wavelength:- 248 nm	42

Reported Methods for Rosuvastatin Calcium [43-57]

SR NO.	DRUGS	METHOD	BRIEF INTRODUCTION	REF. NO.
1	Rosuvastatin Calcium	RP-HPLC	Mobile phase: Water, pH 3.5: Acetonitrile (60:40) Column: YMC C8 (15 cm X 4.6 mm),5 µm Flow Rate: 1.5 ml/min Wavelength: 242 nm	43
2	Rosuvastatin Calcium	RP-HPLC	Mobile phase: Phosphate Buffer, pH 3.0: Acetonitrile (50:50) Column: Thermo Hypersil C18 (10 cm X 4.6 mm),5 µm Flow Rate: 0.5 ml/min Wavelength: 243 nm	44
3	Rosuvastatin Calcium	RP-HPLC	Mobile phase: Phosphate Buffer, pH 4.5: Acetonitrile: Methanol (50:50) Column: Luna C18 (25 cm X 4.6 mm),5 µm Flow Rate: 1.0 ml/min Wavelength: 248 nm	45
4	Rosuvastatin	RP-HPLC	Mobile phase: Phosphate Buffer, pH 6.8: Acetonitrile: (60:40) Column: RP C18 (10 cm X 4.6 mm),3 µm Flow Rate: 0.6 ml/min Wavelength: 242 nm	46
5	Rosuvastatin Calcium	RP-HPLC	Mobile phase: Acetonitrile: Water (75:25) Column: Enable C18 (25 cm X 4.6 mm),5 µm Flow Rate: -0.6 ml/min Wavelength:- 252 nm	47
6	Rosuvastatin Calcium	Stability indicating RP-HPLC	Mobile phase: Solvent-A: Acetonitrile: Water: Methanol: Tetrahydrofuran (10:40:2:5) Solvent-B: Acetonitrile: Methanol: Tetrahydrofuran (50:5:0.5) Column:- Luna C18 (25 cm X 4.6 mm),5 µm Flow Rate: 2.0 ml/min Wavelength: 243 nm	48
7	Rosuvastatin Calcium and Ezetimibe	RP-HPLC	Mobile phase: Acetonitrile: Water (75:25) Column: Enable C18 (25 cm X 4.6 mm),5 µm Flow Rate: -0.6 ml/min Wavelength:- 252 nm	49
8	Rosuvastatin and Ezetimibe	RP-HPLC	Mobile phase: Phosphate Buffer: Acetonitrile: Methanol (40:15:45) Column: Zorbax C18 (15 cm X 4.6 mm),3.5 µm Flow Rate: 1.5 ml/min Wavelength: 242 nm	50
9	Rosuvastatin and Ezetimibe	RP-HPLC	Mobile phase: Phosphate Buffer, pH 8.0: Acetonitrile: Water (50:40:10) Column: Waters C18 (25 cm X 4.6 mm),3.5 µm Flow Rate: 1.0 ml/min Wavelength: 230 nm	51

10	Rosuvastatin and Ezetimibe	Stability indicating RP-HPLC	Mobile phase: Acetate Buffer, pH 6.5: Acetonitrile (55:45) Column: Sunfire BDS C18(25cm X 4.6mm), 3.5 µm Flow Rate: -0.8 ml/min Wavelength:- 230 nm	52
11	Rosuvastatin and Fenofibrate	RP-HPLC	Mobile phase: Water, pH 2.5: Acetonitrile (30:70) Column: Inertsil ODS C18 (25 cmX 4.6 mm),3.5 µm Flow Rate: 1.0 ml/min Wavelength- 248 nm	53
12	Rosuvastatin Calcium and Fenofibrate	RP-HPLC	Mobile phase: Water: Acetonitrile: Methanol (20:40:40) Column: Agilent ODS C18 (25cm X 4.6 mm),3.5 µm Flow Rate: 1.0 ml/min Wavelength: 252 nm	54
13	Rosuvastatin Calcium and Fenofibrate	RP-HPLC	Mobile phase: Phosphate Buffer, pH 5.5: Methanol (25:75) Column: Phenomenex C18 (25cmX 4.6mm),3.5µm Flow Rate: 1.0 ml/min Wavelength: 272 nm	55
14	Rosuvastatin Calcium and Niacin	RP-HPLC	Mobile phase: Phosphate Buffer: Acetonitrile (50:50) Column: Inertsil ODS C18 (15cm X 4.6 mm),3.5 µm Flow Rate- 1.0 ml/min Wavelength: 254 nm	56
15	Rosuvastatin calcium and Amlodipine besylate	RP-HPLC	Mobile phase: Acetonitrile: Tetrahydrofuran and Water, pH 3.0 (68:12:20) Column: Qualisil C8 (25 cm X 4.6 mm),3.5 µm Flow Rate: 1.0 ml/min Wavelength: 251 nm	57

REPORTED METHOD FOR ASPIRIN, CLOPIDOGREL BISULPHATE AND ROSUVASTATIN CALCIUM WITH EACH OTHER⁵⁸⁻⁶⁷

SR NO.	DRUGS	METHOD	BRIEF INTRODUCTION	REF.NO.
1	Aspirin and Clopidogrel	RP-HPLC	Mobile phase: 3% o-Phosphoric acid: Acetonitrile (65:35) Column: Phenomenex C18 (25 cm X 4.6 mm),5 µm Flow Rate: 1.0 ml/min Wavelength: 266 nm	58
2	Aspirin and Clopidogrel Bisulphate	RP-HPLC	Mobile phase: Acetonitrile: Phosphate Buffer, pH 3.0: Methanol (50:30:20) Column: C18 (25 cm X 4.6 mm),5 µm Flow Rate: 1.5 ml/min Wavelength: 240 nm	59
3	Aspirin and Clopidogrel	Stability indicating RP-HPLC	Mobile phase: Solvent A: Phosphate Buffer, pH 2.3 Solvent B: Methanol: Acetonitrile (50:50) Column: Phenyl Hexyl (25 cm X 4.6 mm),5 µm Flow Rate: 1.0 ml/min Wavelength: 220 nm	60

4	Aspirin and Clopidogrel Bisulphate	Ion pair RP-HPLC	Mobile phase: Acetonitrile: Tetrabutylammonium Hydrogen Sulphate Buffer (50:50) Column: Lichrosphere-100 (25 cm X 4.6 mm),5 µm Flow Rate: 1.0 ml/min Wavelength: 240 nm	61
5	Aspirin, Atorvastatin Calcium and Clopidogrel Bisulphate	RP-HPLC	Mobile phase: Acetonitrile: Phosphate Buffer, pH 3.0 (50:50) Column: Inertsil ODS (15 cm X 4.6 mm),5 µm Flow Rate: 1.2 ml/min Wavelength: 235 nm	62
6	Aspirin, Atorvastatin Calcium and Clopidogrel Bisulphate	RP-HPLC	Mobile phase: Acetonitrile: Water, pH 3.0:Methanol (50:40:10) Column: Hypersil BDS C18 (25 cm X 4.6 mm),5 µm Flow Rate: 1.0 ml/min Wavelength: 248 nm	63
7	Aspirin and Rosuvastatin	RP-HPLC	Mobile phase: Methanol: Buffer (45:55) Column: X-Terra C18 (15 cm X 4.6 mm),5 µm Flow Rate: 1.0 ml/min Wavelength: 215 nm	64
8	Aspirin and Rosuvastatin Calcium	RP-HPLC	Mobile phase: Water with 0.5% Triethylamine: Acetonitrile (50:50) Column: Smart C18 (25 cm X 4.6 mm),5 µm Flow Rate: 1.0 ml/min Wavelength: 243 nm	65
9	Aspirin and Rosuvastatin Calcium	RP-HPLC	Mobile phase: Phosphate Buffer, pH 3.0: Acetonitrile (45:55) Column: Hyper chrome ODS BP (20 cm X 4.6 mm),5 µm Flow Rate: 1.0 ml/min Wavelength: 241 nm	66
10	Clopidogrel Bisulphate and Rosuvastatin Calcium	RP-HPLC	Mobile phase: Perchlorate Buffer, pH 2.5: Acetonitrile (65:35) Column: Nova pack C18 (10 cm X 3.9 mm),4 µm Flow Rate: 1.0 ml/min Wavelength:- 242 nm	67

CONCLUSION

Aspirin, clopidogrel bisulphate and rosuvastatin calcium play an important role in the many cardiovascular diseases, and in various diseases. These drugs are available in the market in many formulations with their different dose. Many methods have been reported for the estimation of these drugs but currently not any method have been reported for the simultaneous estimation of these drugs in their combined dosage form. So there is need to develop a suitable, accurate and validated method for their simultaneous estimation in combined dosage form.

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