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-acetoxy 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 C₉H₇O₄ and molecular weight 180.2g/mol.

Chemical Structure of Aspirin

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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 Aspirin** 6-8

<table>
<thead>
<tr>
<th>SR. No.</th>
<th>Official in</th>
<th>METHOD</th>
<th>BRIEF INTRODUCTION</th>
<th>REF. NO</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>IP-2010</td>
<td><strong>Potentiometric Titration</strong></td>
<td>Titrate: Tablet Powder Equivalent to 0.5 gm. Aspirin in 30ml of the 0.5M Sodium Hydroxide&lt;br&gt;Titrant: 0.5 M HCl&lt;br&gt;1ml of 0.5M NaOH is Equivalent to .0.04504 gm. of Aspirin</td>
<td>6</td>
</tr>
<tr>
<td>2</td>
<td>BP-2009</td>
<td><strong>Potentiometric Titration</strong></td>
<td>Titrate: 1gm Aspirin in 10ml Ethanol, Add50ml of the 0.5M Sodium Hydroxide&lt;br&gt;Titrant: 0.5 M HCl&lt;br&gt;1ml of 0.5M NaOH is Equivalent to .0.04504 gm. of Aspirin</td>
<td>7</td>
</tr>
<tr>
<td>3</td>
<td>USP30-NF25</td>
<td><strong>Liquid Chromatography</strong></td>
<td><strong>Mobile phase</strong>: Water (pH 3.4): Acetonitrile (85:15)&lt;br&gt;<strong>Column</strong>: Packing L1, (300 mm × 4.0 mm)&lt;br&gt;<strong>Flow rate</strong>: 2 ml/min&lt;br&gt;<strong>Wavelength</strong>: 285 nm.</td>
<td>8</td>
</tr>
</tbody>
</table>

**Reported Methods for Aspirin** 14-26

<table>
<thead>
<tr>
<th>SR. No.</th>
<th>DRUGS</th>
<th>METHOD</th>
<th>BRIEF INTRODUCTION</th>
<th>REF.NO</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Aspirin</td>
<td>RP-HPLC</td>
<td><strong>Mobile phase</strong>: Sodium Perchlorate Buffer, pH (2.5): Acetonitrile: Isopropyl alcohol (85:14:1)&lt;br&gt;<strong>Column</strong>: C18, (100 mm × 4.6 mm, 5µ,)&lt;br&gt;<strong>Flow rate</strong>: 1.5 ml/min&lt;br&gt;<strong>Wavelength</strong>: 275 nm.</td>
<td>9</td>
</tr>
<tr>
<td>2</td>
<td>Aspirin and Metoprolol</td>
<td>RP-HPLC</td>
<td><strong>Mobile phase</strong>: Phosphate Buffer, (pH 4.6): Methanol (20:80)&lt;br&gt;<strong>Column</strong>: Phenomenex Luna C18, (250 mm × 4.6 mm, 5µ,)&lt;br&gt;<strong>Flow rate</strong>: 0.8 ml/min&lt;br&gt;<strong>Wavelength</strong>: 230 nm.</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>Aspirin, Ramipril, Hydrochlorothiazide, Simvastatin And Atenolol</td>
<td>RP-HPLC</td>
<td><strong>Mobile phase</strong>: Methanol: Water (95:5)&lt;br&gt;<strong>Column</strong>: Hypersil Gold C18, (250 mm × 4.6 mm, 5µ,)&lt;br&gt;<strong>Flow rate</strong>: 1 ml/min&lt;br&gt;<strong>Wavelength</strong>: 230 nm.</td>
<td>11</td>
</tr>
<tr>
<td>4</td>
<td>Aspirin and</td>
<td>RP-HPLC</td>
<td><strong>Mobile phase</strong>:Acetonitrile: Acetate Buffer (75:25)</td>
<td>12</td>
</tr>
<tr>
<td>Number</td>
<td>Compounds</td>
<td>Method</td>
<td>Mobile Phase</td>
<td>Column: Kromasil-100 C18 (150 mm × 4.6 mm, 5µ,)</td>
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<td>--------</td>
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</tr>
<tr>
<td>6</td>
<td>Aspirin and Salicylic acid</td>
<td>RP-HPLC</td>
<td>Acetonitrile: Trifluoroacetic acid 0.05% (30:70)</td>
<td>6679</td>
</tr>
<tr>
<td>7</td>
<td>Amlodipine Besylate, Atenolol and Aspirin</td>
<td>RP-HPLC</td>
<td>Methanol: Phosphate Buffer (pH 7.0) (70:30)</td>
<td>6679</td>
</tr>
<tr>
<td>8</td>
<td>Aspirin And Aspirin Derivatives</td>
<td>RP-HPLC</td>
<td>Acetonitrile: Water (60:40)</td>
<td>6679</td>
</tr>
<tr>
<td>9</td>
<td>Aspirin, Caffeine and Orphenadrine citrate</td>
<td>RP-HPLC</td>
<td>Methanol: Phosphate Buffer, pH 3 (65:35)</td>
<td>6679</td>
</tr>
<tr>
<td>10</td>
<td>Aspirin and Dipyridamole</td>
<td>RP-HPLC</td>
<td>0.1 % Phosphoric acid: Acetonitrile (75:25)</td>
<td>6679</td>
</tr>
<tr>
<td>12</td>
<td>Ramipril, Aspirin and Simvastatin</td>
<td>RP-HPLC</td>
<td>Acetonitrile: Methanol: 0.5% phosphoric acid (10:70:20)</td>
<td>6679</td>
</tr>
<tr>
<td>13</td>
<td>Aspirin, Salicylic Acid, and Caffeine</td>
<td>RP-HPLC</td>
<td>Water: Methanol: Acetic acid (69:28:3)</td>
<td>6679</td>
</tr>
</tbody>
</table>
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](image)

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]

<table>
<thead>
<tr>
<th>SR. NO</th>
<th>OFFICIAL IN</th>
<th>METHOD</th>
<th>DESCRIPTION</th>
<th>REF. NO</th>
</tr>
</thead>
</table>
| 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 µm  
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]

<table>
<thead>
<tr>
<th>SR NO.</th>
<th>DRUGS</th>
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<th>BRIEF INTRODUCTION</th>
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</tr>
</thead>
</table>
| 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 µm  
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 µm  
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 |
|---|---|---|---|
| 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 |
| 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 |
| 6 | Clopidogrel | RP-HPLC | Mobile phase: Phosphate Buffer, pH 3.0: Acetonitrile (75:25)  
Column: Nova pack C18 (25 cm X 4.6 mm), 5 µm  
Flow Rate: 1.0 ml/min  
Wavelength: 247 nm |
| 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 |
| 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 |
| 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 |
| 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 |
| 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 |
| 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 |
Rosuvastatin Calcium [40]
Rosuvastatin calcium is referred as statin which is a cholesterol lowering drug. The IUPAC name of rosuvastatin is \[(E)-7-[4-(fluorophenyl)-6-isopropyl-2-[methyl-(methylsulfonyl) amino] pyrimidin-5-yl] (3R, 5S)-3, 5-dihydroxyhept-6-enioic acid\][40]. The chemical formula of rosuvastatin calcium is $C_{22}H_{27}FN_{3}O_{6}S_2$. Ca and molecular mass of rosuvastatin calcium is 1001.1 g/mol.

![Chemical structure of rosuvastatin calcium](image)

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]

<table>
<thead>
<tr>
<th>SR. NO</th>
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</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>IP 2010 (Rosuvastatin Tablet)</td>
<td>RP-HPLC</td>
<td>Mobile phase: Acetate Buffer, pH 4.0: Acetonitrile: Tetrahydrofuran (59:36:5) Column: C18 (25 cm X 4.6 mm), 5 µm Flow Rate: 1.5 ml/min Wavelength: 248 nm</td>
<td>42</td>
</tr>
<tr>
<td>SR NO.</td>
<td>DRUGS</td>
<td>METHOD</td>
<td>BRIEF INTRODUCTION</td>
<td>REF. NO.</td>
</tr>
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<td>------------------------------------------------------------------------------------</td>
<td>----------</td>
</tr>
</tbody>
</table>
| 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 Calcium           | 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-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       |
<table>
<thead>
<tr>
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<th>REF.NO.</th>
</tr>
</thead>
</table>
| 10     | Rosuvastatin and Ezetimibe                      | Stability indicating RP-HPLC | Mobile phase: Acetate Buffer, pH 6.5: Acetonitrile (55:45)  
Column: Sunfire BDS C18 (25 cm X 4.6 mm), 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 cm X 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 (25 cm 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 (25 cm X 4.6 mm), 3.5 µm  
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 (15 cm 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 OTHER 58-67**

<table>
<thead>
<tr>
<th>SR NO.</th>
<th>DRUGS</th>
<th>METHOD</th>
<th>BRIEF INTRODUCTION</th>
<th>REF.NO.</th>
</tr>
</thead>
</table>
| 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      |
### 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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