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REVIEW ON RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF ASPIRIN, ROSUVASTATIN CALCIUM, CLOPIDOGREL BISULPHATE IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

They are necessary in the treatment of a number of cardiovascular diseases. Rosuvastatin is a medicine that lowers cholesterol and other lipids in the blood to help prevent heart, brain and blood vessel problems. Clopidogrel and aspirin are medicines that prevent blood clots. These drugs are available in single and combination form in the market. Rosuvastatin is a lipoprotein-lowering agent used in the treatment of various cardiovascular, cerebrovascular, and peripheral vascular disorders. These drugs are now widely marketed both as single entities and in combination dosage forms. There are a number of well-established analytical methods for estimation of these drugs in individual and combination dosage forms.

A simple, precise, fast, and accurate RP-HPLC method was developed and validated for the simultaneous detection of aspirin, rosuvastatin, and clopidogrel in pharmaceutical dosage forms. Chromatographic separation was achieved using a reverse-phase C18 column with the appropriate mobile phase under optimal circumstances. The developed method showed good resolution, low retention times, and strong peak symmetry for all three medications. .

KEYWORD -analytical, antilipemic, medication, cerebrovascular, rp-hplc.

INTRODUCTION

Potentiometers, HPLC, and aqueous and non-aqueous titrations are only a few of the analytical methods used in the discipline. Aqueous and non-aqueous titrations are also used in the analytical field. Chromatography uses adsorption as a mass transfer technique. The basis

for separation in both the normal phase mode and the reverse phase mode is adsorption, in which the substances move or separate according to their individual affinities. HPLC is essential for separating various compounds from combinations of substances in the field of pharmaceutical studies today [3]. Reverse phase high performance liquid chromatography is one of the most popular analytical techniques for the simultaneous quantification of pharmaceutical formulations with multiple components (RP-HPLC). Aspirin and Clopidogrel bisulphate are used to treat cardiovascular disorders such myocardial infarction, hyperlipidemia, and stroke prevention.

These drugs must be estimated concurrently in combination dose forms for quality assurance and regulatory compliance. RP-HPLC is the recommended analytical technique because to its high sensitivity, specificity, repeatability, and capacity to separate compounds with different polarity. The pharmaceutical sector uses the technique extensively for impurity profiling, assay determination, stability analysis, and dissolution research.

Principle of RP-HPLC

The partition chromatography principle, which RP-HPLC employs, calls for a non-polar stationary phase and a moderately polar mobile phase. Compounds are separated through hydrophobic interactions with the stationary phase. C18 columns are widely used in pharmaceutical analysis due to their outstanding retention qualities and separation efficiency. Aqueous buffers that have been pH-adjusted and blends of organic solvents, including acetonitrile or methanol, are frequently used as the mobile phase. Due to the high UV absorbance of aspirin, clopidogrel, and rosuvastatin, UV detectors are commonly used for detection. [5]

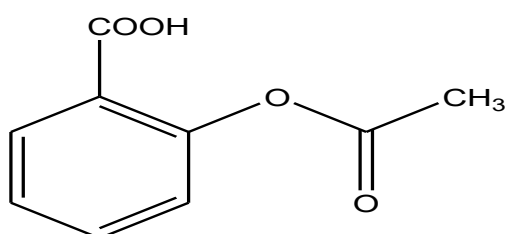
Need for RP-HPLC Method Development-



Introduction to Drug Profile



1-Aspirin –Aspirin is a common painkiller that you may be familiar with. This drug aids in lowering pain, fever, and inflammation. It's interesting to note that by preventing blood clots, it's frequently taken in small doses to help reduce the risk of heart attacks and strokes. Aspirin, chemically known as acetylsalicylic acid, is frequently taken in conjunction with other drugs such as rosuvastatin and clopidogrel since it is crucial for heart health. When combined, this help prevent heart attacks, strokes, and associated blood clot problems. Aspirin is thoroughly inspected to guarantee its identification, purity, and stability in pharmaceutical products throughout the creation of novel drug testing techniques.



Pharmacological action – By permanently blocking the cyclooxygenase-1 (COX-1) enzyme, aspirin lowers the synthesis of thromboxane A₂, which prevents platelet aggregation and blood clot formation. Because of its antiplatelet activity, aspirin is mostly used in cardiovascular conditions to prevent myocardial infarction (heart attack), ischemic stroke, and other coronary artery diseases. Prostaglandins and thromboxane A₂ are consequently produced less frequently. . Consequently, it exhibits analgesic (pain-relieving), antipyretic (fever-reducing), antiplatelet, and anti-inflammatory properties.

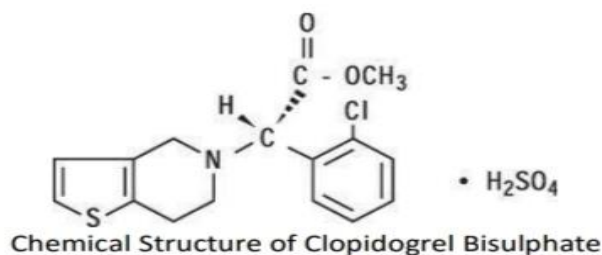
Therapeutic use

- Stopping myocardial infarction Analgesic
- anti-inflammatory qualities that prevent strokes

Reported Method of Aspirin

SR.No.	. DRUGS	METHOD	BRIEF INTRODUCTION	REF.NO
1	Aspirin and clopidogrel bisulphate	RP-HPLC	Mobile phase- Phosphate Buffer: Acetonitrile (60:40 v/v) Flow rate- 1.0 mL/min, Wavelength- 240 nm Column- C18 Column	[9]
2	Aspirin and Rosuvastatin	RP-HPLC	Mobile phase -Methanol: Water (70:30 v/v) Flow rate-- 1.0 mL/min Wavelength- 230 nm Column- C18 Column	[10]
3	Aspirin and Atorvastatin	RP-HPLC	Mobile phase Buffer; Acetonitrile (65:35v/v) Flow rate- 1.2 mL/min Wavelength- 245 nm Column- C18 Column	[11]
4	Aspirin and Prasugrel	RP-HPLC	Mobile phase -Methanol; Phosphate Buffer: (75:25 v/v) Flow rate- 1.0 mL/min Wavelength- 254 nm Column- C18 Column	[12]
5	Aspirin and Metoprolol	RP-HPLC	Mobile phase - Methanol; Buffer: (68;58 v/v) Flow rate- 1.0 mL/min Wavelength- 225 nm Column-- C18 Column	[13]
6	Aspirin and Ramipril	RP-HPLC	Mobile phase - Phosphate Buffer: Acetonitrile (60:40 v/v) Flow rate- 1.0 mL/min Wavelength- 235 nm Column- C18 Column	[14]
7	Aspirin and Simvastatin	RP-HPLC	Mobile phase - Methanol;Water;ACN(60;20;20) Flow rate- 1.0 mL/min Wavelength- 238 nm Column- C18 Column	[16]
8	Aspirin and Amlodipine	RP-HPLC	Mobile phase -- Phosphate Buffer: Acetonitrile (70:30 v/v) Flow rate- 1.0 mL/min Wavelength- 239nm Column-- C18 Column	[17]

Clopidogrel Bisulphate -One common antiplatelet medication used to prevent thrombotic events such myocardial infarction, stroke, and peripheral arterial disorders is clopidogrel bisulphate. It works by specifically preventing platelet aggregation caused by adenosine diphosphate (ADP) and is a member of the thienopyridine class of medications. In order to prevent cardiovascular problems, clopidogrel and aspirin are frequently administered as dual antiplatelet treatment.



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Pharmacological Action – By specifically preventing adenosine diphosphate (ADP) from binding to platelet receptors, clopidogrel bisulphate inhibits platelet activation and aggregation. The formation of hazardous blood clots inside blood vessels is aided by this antiplatelet activity. Because of this mechanism, the medication is primarily used to reduce the risk of ischemic stroke, heart attack, and other thrombosis-related cardiovascular problems.

Therapeutic use-

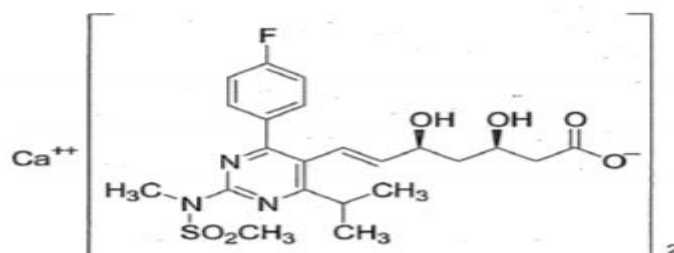
- Thrombosis prevention
- Acute coronary syndrome treatment

Reported Method of Clopidogrel Bisulphate.

SR NO.	DRUGS	METHOD	BRIEF INTRODUCTION	REF.NO.
1	Clopidogrel and Aspirin	RP-HPLC	Mobile phase -ACN: Buffer (60:40 v/v) Flow rate- 1.0 mL/min Wavelength-240 nm Column- C18 Column	21
2	Clopidogrel and Rosuvastatin	RP-HPLC	Mobile phase -Methanol: Buffer (70:30 v/v) Flow rate-1.0 mL/min Wavelength-238 nm Column- C18 Column	22

3	Clopidogrel and Atorvastatin	RP-HPLC	Mobile phase -ACN:Water (65:35 v/v) Flow rate-1.0 mL/min Wavelength-235 nm Column-- C18 Column	23
4	Clopidogrel and Atorvastatin, Aspirin	RP-HPLC	Mobile phase -Methanol: Buffer (72:28 v/v) Flow rate-1.0 mL/min Wavelength-242 nm Column- C18 Column	24
5	Clopidogrel and Metoprolol	RP-HPLC	Mobile phase - ACN: Buffer (58:42 v/v) Flow rate-1.0 mL/min Wavelength-230 nm Column- C18 Column	26
6	Clopidogrel and Telmisartan	RP-HPLC	Mobile phase - Methanol: Buffer (70:30 v/v) Flow rate-1.0 mL/min Wavelength-232 nm Column C18 Column -	27
7	Clopidogrel and Olmesartan	RP-HPLC	Mobile phase - Methanol: Buffer (65:35v/v) Wavelength-234 nm Column- C18 Column Flow rate-1.0 mL/min	28

Rosuvastatin Calcium - One member of the statin drug class that lowers cholesterol is rosuvastatin calcium. It is frequently recommended for heart disease-related lipid problems, particularly high cholesterol. This medication is frequently used either alone or in combination with other drugs like aspirin and clopidogrel to treat coronary artery disease. To ensure that rosuvastatin calcium formulations are precise, pure, and stable, researchers in the pharmaceutical industry commonly employ a method known as RP-HPLC.



Chemical structure of rosuvastatin calcium

Pharmacological action -By inhibiting a certain liver enzyme that is crucial for the production of cholesterol, rosuvastatin calcium lowers cholesterol levels. This lowers triglycerides and bad cholesterol (LDL) while raising HDL (good cholesterol). This drug effectively supports improved cardiovascular health by avoiding the accumulation of cholesterol in blood arteries, which can cause major health problems like heart attacks and strokes.

Therapeutic use

- High cholesterol
- Low cholesterol
- Avoiding heart-related illnesses

Reported Method of Rosuvastatin Calcium

SR NO	DRUGS	METHOD	BRIEF INTRODUCTION	REF. NO.
1	Rosuvastatin and Metformin	RP-HPLC	Mobile phase - ACN:Water (67:33 v/v) Flow rate-1.1 mL/min Wavelength-243 nm Column- C18 Column	30
2	Rosuvastatin and Ramipril	RP-HPLC	Mobile phase - Methanol: Buffer (70:30v/v) Flow rate-1.0 mL/min Wavelength-240 nm Column- C18 Column	31
3	Rosuvastatin and Fenofibrate	RP-HPLC	Mobile phase - ACN: Water (68:32v/v) Flow rate-1.2 mL/min Wavelength-250 nm Column- C18 Column	32
4	Rosuvastatin and Ezetimibe	RP-HPLC	Mobile phase - Methanol: Buffer (72:28v/v) Flow rate-1.0 mL/min Wavelength-242 nm Column- C18 Column	33
5	Rosuvastatin and Olmesartan	RP-HPLC	Mobile phase - ACN: Water (67:33v/v) Flow rate-1.1 mL/min	34

			Wavelength-243 nm Column- C18 Column	
6	Rosuvastatin and Pioglitazone	RP-HPLC	Mobile phase - Methanol: Buffer (70:30v/v) Flow rate-1.0 mL/min Wavelength-247 nm Column- C18 Column	35
7	Rosuvastatin and Clopidogrel	RP-HPLC	Mobile phase -ACN :Buffer(65 :35 v/v) Flow rate-1.0 mL/min Wavelength-238 nm Column- C18 Column	36
8	Rosuvastatin and Aspirin	RP-HPLC	Mobile phase - Methanol: Water (70:30v/v) Flow rate-1.0 mL/min Wavelength-230 nm Column- C18 Column	37
9	Rosuvastatin and Atorvastatin	RP-HPLC	Mobile phase - Methanol: Buffer (60:40v/v) Flow rate-1.0 mL/min Wavelength-240 nm Column- C18 Column	38

Reported Method Of Aspirin, Rosuvastatin Calcium, Clopidogrel Bisulphate With Each Other -

SR NO.	DRUGS	METHOD	BRIEF INTRODUCTION	REF.NO
1	Rosuvastatin and Atorvastatin	RP-HPLC	Mobile phase - Methanol: Buffer (60:40v/v) Flow rate-1.0 mL/min Wavelength-240 nm Column-- C18 Column	39
2	Aspirin and clopidogrel	RP-HPLC	Mobile phase - ACN: Buffer (60:40 v/v) Flow rate-1.0 mL/min Wavelength-240 nm Column--C18 Column	40
3	Rosuvastatin and Aspirin	RP-HPLC	Mobile phase - Methanol: Water (70:30v/v) Flow rate-1.0 mL/min Wavelength-230 nm	41

			Column- C18 Column	
4	Clopidogrel, Atorvastatin and Aspirin	RP-HPLC	Mobile phase - Methanol: Buffer (60:40v/v) Flow rate-1.0 mL/min Wavelength-243 nm Column- C18 Column	42
5	Rosuvastatin, Atorvastatin and Aspirin	RP-HPLC	Mobile phase - ACN: Water (68:32v/v) Flow rate-1.0 mL/min Wavelength-230 nm Column- C18 Column	43
6	Clopidogrel and Rosuvastatin	RP-HPLC	Mobile phase - ACN: Buffer (65:30v/v) Flow rate-1.0 mL/min Wavelength-238 nm Column- C18 Column	44
7	Aspirin and Rosuvastatin	RP-HPLC	Mobile phase - Methanol: Water (68:32v/v) Flow rate-1.0 mL/min Wavelength-237 nm Column- C18 Column	45

Method development

1-Selection of Column

The most often cited techniques:

Hypersil BDS C18

Water C18

BISCOF C18

ODS C18 Inertsil

Typical measurements:

- 250 mm × 4.6 mm
- Particle size: 5 µm

C18 columns provide: Enhanced symmetry at the top

- High retention
- Outstanding resolution
- A reduction in peak tailing [47]

2-Selection of Mobile Phase

Optimizing the mobile phase is essential to

- Sharp peaks • Sufficient retention time;
 - Better resolution;
 - A reduction in tailing
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 - Sufficient retention time;
 - Better resolution;
 - A reduction in tailing

3-Detection Wavelength-

Most described RP-HPLC methods used UV detection at 230 nm, 235 nm, 240 nm, and 242 nm.

Most described RP-HPLC methods used UV detection at

- 230 nm,
- 235 nm,
- 240 nm,
- 242 nm.

4-Flow Rate -

The most efficient methods used were

- 1.0 millilitres per minute,
- 1.2 millilitres per minute
- 1.5 millilitres per minute.

At a flow rate of 1.0 mL/min, better resolution and a controllable run time were achieved.

5-Retention Time- Typical retention times reported were

Drug	Retention Time
Aspirin	2–4 min
Clopidogrel	4–13 min
Rosuvastatin	4–6 min

Most methods achieved complete separation within 15 minutes

Validation According to ICH Guidelines

1-Linearity

Linearity determines the proportionate relationship between concentration and detector response.

Most studies showed: • Excellent linearity across the operating concentration range.

2-Accuracy

Recovery studies were used to assess accuracy at:

- 80%
- 100%

This verified the developed approaches' correctness.

3-Precision

The precision studies included:

- The capacity for repetition;
- Accuracy during the day;
- Accuracy throughout the day Most of the ways that are described:
- Outstanding accuracy and repeatability are indicated by percent RSD < 2%.

4-Specificity

Research on specificity confirmed: No excipient interference

Peak separation of analytes that is suitable

Accurate calculation in the formulation.[48]

4-Robustness

To assess robustness, small, deliberate changes were made to:

1. Mobile phase composition
2. pH
3. The flow rate
4. Detection wavelength

5-LOD and LOQ

LOD and LOQ studies demonstrated the exceptional sensitivity of RP-HPLC methods.

Decreased LOQ and LOD values indicate: [49].

1. Increased sensitivity
2. Suitable for examination at low concentrations

Applications of RP-HPLC

RP-HPLC techniques are widely employed for

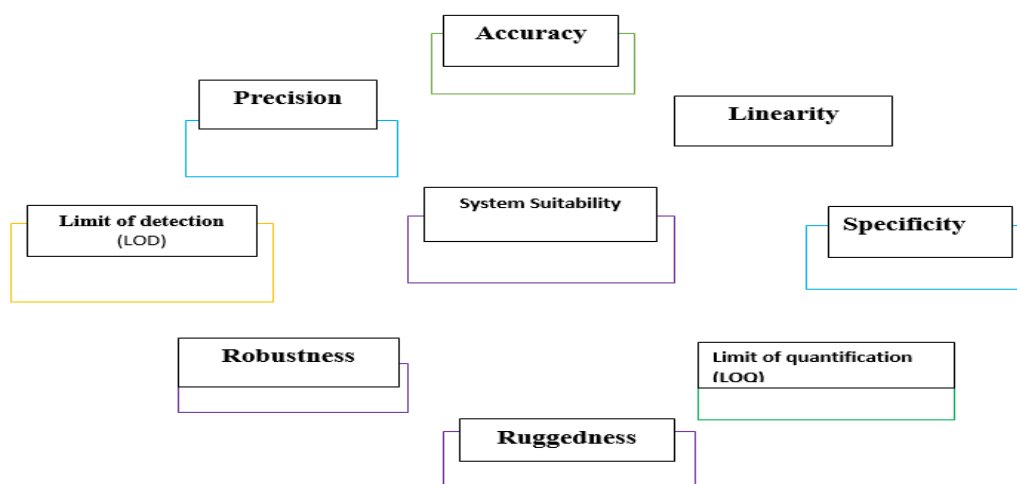
- Assay analysis;
- quality control;
- dissolving research;
- stability research; and
- bulk drug analysis
- Examination of medication dosage forms
- Profiling of contaminants.

Advantage of RP-HPLC

- Superior precision and accuracy
- The three drugs are all estimated at the same time.
- Easy and rapid analysis
- Outstanding resolution and separation
- Excellent sensitivity to low drug concentrations
- Less solvent usage • Affordable for routine quality control
- Repeatable and consistent results
- Complies with ICH validation requirements

METHOD

Validation



CONCLUSION

Aspirin, rosuvastatin calcium, and clopidogrel bisulphate are important in numerous disorders, including cardiovascular diseases. There are numerous formulations of these medications with varying dosages on the market. Numerous techniques for estimating these medications have been documented, however as of right now, no technique has been documented for simultaneously estimating these medications in their combined dosage form. For their simultaneous estimation in a combined dosage form, a suitable, accurate, and verified method must be developed.[50]

REFERENCES

1. Solanki C, Patel N. RP-HPLC technology development and validation for simultaneous aspirin and rosuvastatin calcium dose estimate in capsule form. *Int J Pharm Bio Sci.* 3(3): 577-585, 2012.
2. Masud, Begum I. Development and validation of an RP HPLC method for measuring aspirin and clopidogrel in combination tablet dosage form at the same time. 7(11): 4443-4448; *Int J Pharm Sci Res*, 2016.
3. Journal of Drug Delivery and Therapeutics, Analytical Method Development and Validation for the Simultaneous Estimation of Aspirin, Clopidogrel, and Rosuvastatin in Pharmaceutical Dosage Form, Tiwari PK, Jain A, Dubey BK, Pandey GK, Dhakad S. 2019; 9(4-s):432-438
3. Journal of Drug Delivery and Therapeutics, Analytical Method Development and Validation for the Simultaneous Estimation of Aspirin, Clopidogrel, and Rosuvastatin in Pharmaceutical Dosage Form, Tiwari PK, Jain A, Dubey BK, Pandey GK, Dhakad S. 2019; 9(4-s):432-438
4. Pisal P, Nigade G, Kale A, and Pawar S. Development and validation of a stability-indicating RP-HPLC technique for the simultaneous measurement of aspirin, rosuvastatin, and clopidogrel in pharmaceutical dosage form and bulk. Clopidogrel and aspirin in pharmaceutical dose form, *Int J Pharm Pharma Sci* 2012; 10(10): 51-56. 2016; *J Pharm Sci Bioscientific Res.* 6(2):197 206
5. Singh RD, Yadav H, Hinge M, and Patel A. Development and validation of analytical methods for measuring aspirin, clopidogrel, and rosuvastatin in pharmaceutical dosage form simultaneously. 2016; *J Pharm Sci Bioscientific Res.* 6(2):197 206
5. Singh RD, Yadav H, Hinge M, and Patel A. Development and validation of analytical methods for measuring aspirin, clopidogrel, and rosuvastatin in pharmaceutical dosage form simultaneously. 2016; *J Pharm Sci Bioscientific Res.* 6(2):197 206
5. Singh RD, Yadav

- H, Hinge M, and Patel A. Development and validation of analytical methods for measuring aspirin, clopidogrel, and rosuvastatin in pharmaceutical dosage form simultaneously. 2016; J Pharm Sci Bioscientific Res. 6(2):197 206 6-
6. Mishra A, Sharma S, Sharma A. Stability demonstrates concurrent validation of rosuvastatin, aspirin, and clopidogrel with forced degradation behavior investigation by RP-HPLC in pharmaceutical dose form. Asian Pacific Journal of Health Science, 4(3), 2017, 306-319 .
 7. Croitoru O, Spiridon AM, Belu I, Neamtu J, "Development and Validation of an HPLC Method for Simultaneous Quantification of Clopidogrel Bisulfate, Its Carboxylic Acid Metabolite, and Atorvastatin in Human Plasma: Application to a Pharmacokinetic Study," Hindawi Pub. Corp., 2015
 8. Ravichandran S, Valliapan K, Ramanathan M, "Validated RP-HPLC Method for Concurrent Determination of Phenytoin Sodium and Clopidogrel Bisulphate in Tablet Dosage Form," J. Pharm. Sci. And res., 2015, 7(11), 934 937 9-Kumar VP, Sunandama Y, "Simultaneous Determination of Clopidogrel and Pioglitazone by High Performance Liquid Chromatography in Bulk Drug and Dosage Forms," Int. J. Pharm. and res. Sci., 2(1), 1-9
 9. Ambole SR, Shirota PJ, Kondawar MS. Aspirin and rosuvastatin calcium can be simultaneously estimated from capsule dosage forms using a first order derivative spectroscopic technique. 2012; Int J Chem Tech Res., 4(3):966-970.
 10. Jain DK, Jain N, Verma J. RP-HPLC method for concurrent binary combination estimation of aspirin and prasugrel. Int J Pharm Sci Drug Res 4(3): 218-221 (2012).