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Method Development and Validation for the Simultaneous Estimation of Amlodipine Besylate-Rosuvastatine Calcium in Bulk Drugs and Dosage form by HPLC and Spectrophotometric Techniques

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Abstract: *The present communication deals with the development of a new drug, simple, specific, sensitive, rapid and economical procedure for simultaneous estimation of Rosuvastatine calcium and amlodipine besylate in a combined dosage form. The method is based on the native ultraviolet absorbance maxima of the two chemotherapeutic agents. As both Compounds do not interact chemically in methanol; two wavelengths 246 nm for atorvastatin calcium and 360 nm for amlodipine besylate were used. Both the drugs obeyed Beer's law in the concentration range that was employed in the method.*

Keywords: Amlodipine besylate, Rosuvastatine calcium HPLC and spectrophotometric etc.

I. INTRODUCTION

AMLODIPINE

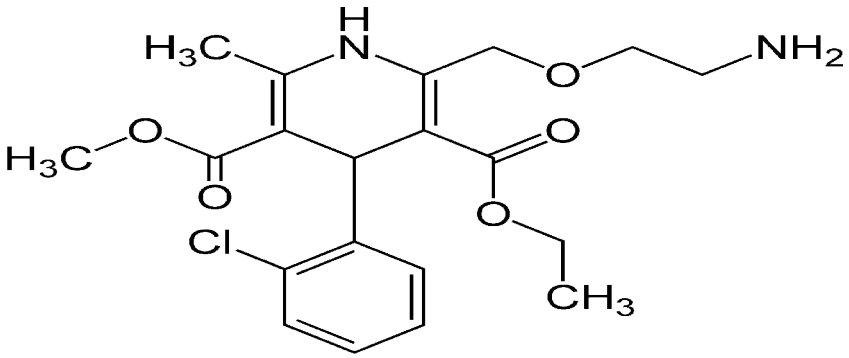

Figure: Structures of the Amlodipine Besylate

Amlodipine besylate, sold under the trademark Norvasc, is used to treat high blood pressure, heart disease and common side effects are swelling, fatigue, stomach upset and nausea. Serious side effects can include low blood pressure or a heart attack.

Amlodipine was patented in 1982 and approved for use in healthcare in 1990. This is the WHO list of the most important drugs that contain the safest and most effective drugs needed for healthcare.

1.1 Medical Purpose

Amlodipine besylate is used to treat high blood pressure and coronary heart disease in people with persistent angina pectoris (chest pain usually caused by physical or mental stress) or angina pectoris (which sometimes occurs) and without heart failure. It can be used alone or in combination with high blood pressure or heart disease. Amlodipine can be given to adults and children aged 6 to 17 years.

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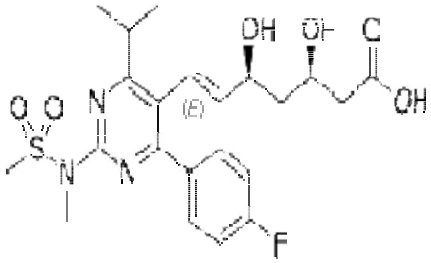
1.2 Combination Therapy Amlodipine can be given in Combination with Various Medicines

- Amlodipine because of high blood pressure and the prevention of cardiovascular disease or if a person has too much cholesterol.
- Amlodipine / aliskiren or amlodipine / aliskiren / hydrochlorothiazide should not lower blood pressure unless amlodipine alone is possible. COSCO renin is aliskiren works for primary hypertension to reduce (without known cause) the binding to Hutchinson and prevent

The renin-angiotensin (Raas) result from the trigger to increase blood pressure.
Hydrochlorothiazide is a diuretic, and it is reduced in whole blood.

II. ROSUVASTATIN CALCIUM:

2.1 Structures of the Rosuvastantine Calcium



IUPAC Name: bis [(E)-7-[4- (4-fluorophenyl)-6-is[methyl (methyl sulfonic)amino] pyramid in- 5-yl](3R,5S)-3,5-dihydroxyhept-6-enoic acid] calcium Salt

CASE No : 287714-41-4
Empirical Formula : (C22H28FN3O6S) 2Ca
Molecular Weight : 1001.14
Bioavailability : 20%
Solubility : Sparingly Soluble in Water & Methanol and Slightly Soluble in Ethanol
Mechanism : Rosuvastatine is a selective and competitive inhibitor of HMG-CoA

Reeducate, the enzyme responsible for the conversion of HMG- CoA to mevalonate, and decreases the hepatic biosynthesis of cholesterol. The drug Rosuvastatine calcium, sold under the brand name CRESTOR, which is used for the prevention of cardiovascular diseases in patients at high risk and to Lipids abnormal processing. It is recommended for use in diet changes, fitness and weight loss. It is taking normally.

Common side effects include abdominal pain, nausea, headache and muscle aches. Serious side effects can include rhabdomyolysis, liver problems and diabetes. Can be used during pregnancy is harmful to the baby. Like all statins, rosuvastatine calcium works by inhibiting HMG- Co-Areductase, an enzyme in the liver that plays a role in cholesterol production.

Rosuvastatine calcium was patented in the year 1991 and is approved for medical use in the United States in the year2003. It is available as generic. In 2017, it was the 39th most commonly prescribed drug in the United States with more than 19 million prescription drugs.

2.2 Medical uses

Rosuvastatine calcium (sold as Crestor) 10 mg tablets. The main use of rosuvastatine is for the prevention of cardiovascular disease in exposed patients and for the treatment of abnormal lipids.

2.3 Effect on Cholesterol Levels

A meta-analysis showed that rosuvastatine calcium can increase both high-density lipoprotein (HDL) cholesterol and other stain light. A 2014 Cochrane Review found good evidence of a linear decrease in non-HDL levels with rosuvastatine calcium. HDL increases 7% without dose effect.

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Side effects and contraindications:

The following side effects should be reported to the prescribing doctor if they persist or get worse:

- Constipation
- Heartburn
- Dizziness
- Sleeplessness
- Depression
- Joint pain
- Cough
- Memory loss or forgetfulness
- Confusion

III. Objectives of the Work

1. To develop method for Amlodipine besylate and Rosuvastatine Calcium.
2. To validate the developed method of Amlodipine besylate and Rosuvastatine Calcium.

3.1 Instruments and Chemicals

The instruments and chemicals which were required to perform this study are as shown in table:

S. No	Particular Equipment's	Company Name
1.	HPLC System	Labronics laboratory instruments LT-3201 self priming pump SPD-M10A Detector Accuracy <+ -1%or +-2 microliter/min. whichever is greater, Precision:0.25% from 0.1ml/min to 10ml/min at 20 degrees, Pmax: 6000 psi Dimensions: 25.5cm(W)x32.5 cm (Dx 15.6cm (H) Weight:13 lbs.(5.9 kg) ,Power Input: 85 to 264 VAC,47 to 63 Hz,60 Watts
2.	Balance	Shimadzu AX-200
3.	Ultrasonicator	Toshcon by Toshniwal
4.	pH meter	Chemiline digital meter
5.	UV Spectrophotometer	Shimadzu 1600 UV Spectrophotometer
	Chemicals	
1.	Acetonitrile	HPLC Grade – Spectro chem. Pvt ltd
2.	Methanol	HPLC Grade - Spectro chem. Pvt ltd
3.	Water	HPLC Grade - Spectro chem. Pvt ltd
4.	Hydrochloric acid	AR grade - Ranbaxy Fine Chemicals
5.	Ortho phosphoric acid	AR grade - Ranbaxy Fine Chemicals
6.	Sodium hydroxide	AR grade - Ranbaxy Fine Chemicals
	Glassware's	
1.	Volumetric flask	Qualigens A grade
2.	Pipettes	Qualigens A grade

IV. Research Methodology

In this chapter various techniques and methods is described which are used to perform the needed experiment and analysis of the work. Starting from the The Gas chromatography and then followed by various techniques and the experiment performed. Gas chromatography, High performance liquid chromatography, paper chromatography, thin layer chromatography etc.

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- **Mobile Phase Preparation:-** In the present investigation a new RP-HPLC method is developed for the simultaneousness himation of Rosuvastatine Calcium and Amlodipine besylate in tablet formulation by taking Acetonitrile : Phosphate buffer (45:55) as mobile phase and will be validated as per ICH guideline. The mobile phase was consisted of 20mM potassium dihydrogen phosphate buffer pH 3.5 Acetonitrile (55:45, v/v) for the preparation of buffer solution, 2.72 g Potassium dihydrogen phosphate was weighed and dissolves in 1000 ml water, pH was adjusted to 3.5 with the help of dilute ortho-phosphoric acid. Mobile phase was filtered through a 0.45 µm nylon filter and degassed in an ultrasonic bath.
- **Preparation Of Stock Solution:-**Stock solutions of rosuvastatine calcium (500 µg / ml) and amlodipine besylate (250 µ g / ml) were prepared by transferring 25 mg rosuvastatine calcium and 12.5 mg amlodipine basylate to a 50 ml vial and adding buffered potassium phosphate, potassium, acetone. Potassium 55:45, volume / volume) the mixture was stirred for 2 minutes to dissolve the ingredients and the solution was diluted by volume with the same solvent mixture.
- **Preparation of Standard Solution:** - Standard solution, Rosuvastatine calcium (100µ g/mL) and Amlodipine besylate (50µg/mL) was prepared by diluting 10 ml standard stock solution to 50 mL, in a volumetric flask, with the same solvent mixture.
- **Preparation of Stock and Test Solutions**:-Prepare a stock solution (500 + 250 µg / ml) for the test, weigh and mix 20 tablets. Carefully weigh the powder sample corresponding to the weight of 5 tablets and transfer it to a 100 ml bottle. Potassium dioxide phosphate buffer, pH 3.5 acetonitrile (55:45, v / v), 60 ml was added to 60 ml and the mixture was tested for 10 min, shaking normally. The contents of the flask were then returned to room temperature and diluted in volume with the same solvent mixture. This solution (20 ml) was filtered through a 0.45 µm nylon spray filter. Preparation of the test solution (100 + 50 µ g / ml) for the test Transfer 10 ml of the test mass solution to a 50 ml vial and dilute in volume with potassium phosphate buffer solution potassium dioxide, pH 3.5 acetonitrile (55:45, v /v).
- **Equipment used:-**Waters alliance equipped HPLC system with a photo diode array detector is used for the method development and force degradation studies. The HPLC system used for method validation is waters HPLC system with variable wavelength detector (VWD) and Shimadzu 2010 series LC system with UV detector. The data is monitored and processed by using LC-solution Software. The chromatographic column used is YMC pack ODS-AQ, (250 mm x 4.6 mm 5µm).

V. Results and Discussion

5.1 Method Development and Optimization

The new HPLC method is optimized with a view to develop a stability indicating method of amlodipine besylate and Rosuvastatine calcium its impurities. 3.1g of ammonium acetate in 1000 mL of water and mixed well. The pH of the solution is adjusted to 6.4±0.05 with dilute acetic acid and mixed well and used as mobile phase A. Mixture of mobile phase A, methanol and acetonitrile in the ratioof40:40:20v/vas mobile phase B for initial trail on ainertsil ODS (250x4.6) mm, 5µ Stationary phase with a 25 cm length, 4.6 mm ID and 5 µm particle size.

5.2 LOD and LOQ

The constraint of recognition and breaking point of measurement were assessed by successive
Weakening of amlodipine besylate and rosuvastatine in calcium answer for acquire a sign to-commotion proportion of 3:1 LOD and 10:1 LOQ.

5.3 Linearity

For linearity, seven focuses alignment bend were acquired in a concentration range from the 20-80 µg/ml for the Amlodipine and also the 40-160 µg/ml for Rosuvastatin.

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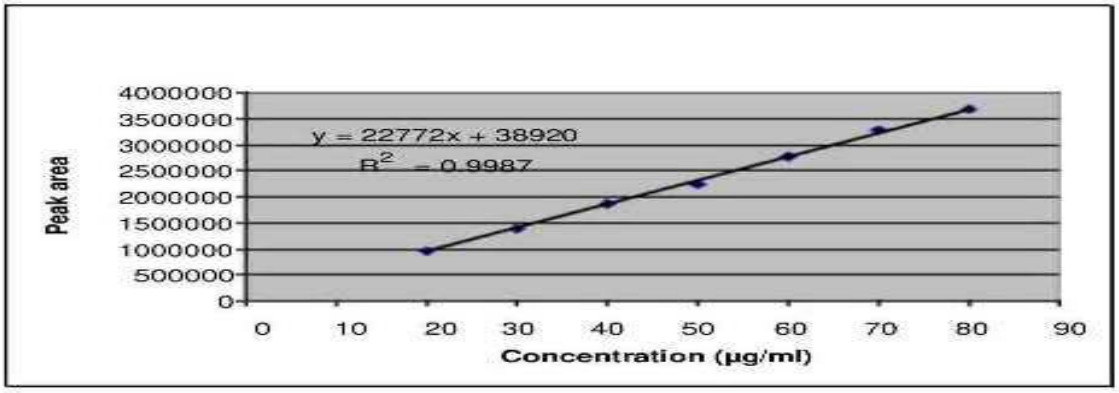
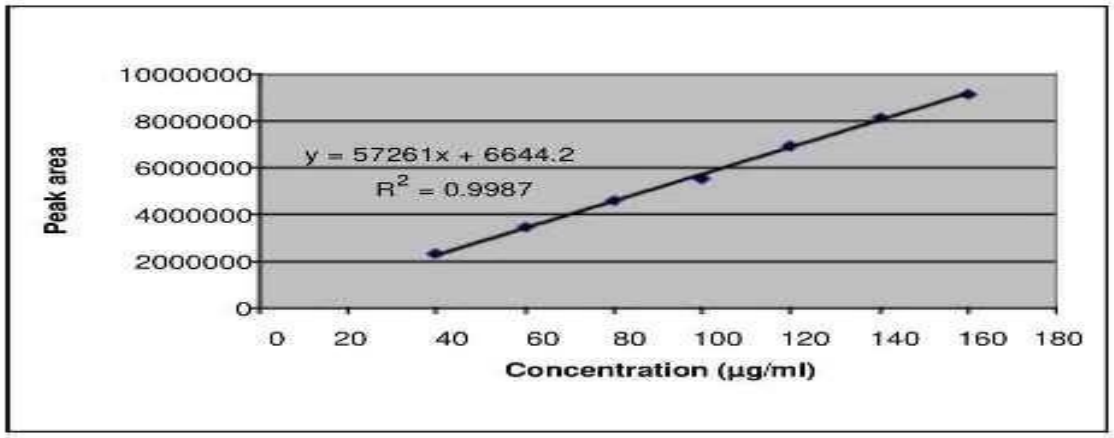


Linearity based curve for the Amlodipine besylate.



Linearity based curve for the Rosuvastatin calcium.

5.4 Accuracy

Precision was evaluated by deciding the recuperation method at three distinct focuses (comparing to the groupings of test arrangement 50, 100 and 150%) with known measures of amlodipine (25, 50 and 75 µg/ml) and rosuvastatin (50, 100 and 150 µg/ml) dissolvable and the measure of amlodipine and rosuvastatin. Three layers were made for every fixation and infused in copy. The% yield was determined and recorded for each stage as per Tables 6.2 and 6.3. The mean calcium admission of rosuvastatin went from 98.96% to 101.94% and the mean recuperation of amlodipine besylate went from 98.37% to 101.79%, which is palatable.Table for Rosuvastatin Calcium and Amlodipine besylate :

Accuracy Level	Amount Added	Amount Found	% Recovery	Mean	Std. dev.	% RSD
50%	49.92	50.8041	101.76	101.87	0.09	0.09
	50.24	51.2147	101.94			
	50.08	51.0367	101.91			
100%	100.12	99.0767	98.96	99.56	0.54	0.54
	99.88	99.8898	100.01			
	100.16	99.8678	99.71			
150%	150.04	150.071	100.02	100.47	0.42	0.41
	149.96	151.229	100.85			
	149.92	150.765	100.56			

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Accuracy Level	Amount Added	Amount Found	% Recovery	Mean	Std. dev.	% RSD
50%	24.92	24.9377	100.07	101.17 %	0.95	0.94
	25.04	25.4542	101.65			
	25.08	25.5287	101.79			
100%	49.84	49.0252	98.37	98.5%	0.11	0.11
	49.92	49.1868	98.53			
	49.96	49.2613	98.60			
150%	74.8	73.6240	98.43	98.77%	0.3	0.3
	74.88	74.1461	99.02			
	74.84	73.9902	98.86			

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