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Analytical Methods Development and Validation for Simultaneous Estimation of Rosuvastatin Calcium and Megestrol Acetate in Synthetic Mixture

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ABSTRACT:
Rosuvastatin is a statin used to lower cholesterol by inhibiting HMG-CoA reductase, reducing cardiovascular risk. Megestrol acetate is a hormonal drug used in breast cancer treatment by controlling abnormal cell growth. Their combination was evaluated in Phase III clinical trials for prevention of atypical endometrial hyperplasia, where patients received Megestrol acetate (160 mg) and Rosuvastatin (10 mg) daily, showing a 36.1% complete response rate at 16 weeks, though higher BMI reduced efficacy. Literature review revealed no validated method for simultaneous estimation of both drugs in synthetic mixture; hence this study aimed to develop and validate UV spectrophotometric and RP-HPLC methods as per ICH Q2 (R2) guideline. For UV method, two techniques were done as Simultaneous Equation (Vierordt's) and first order derivative UV method. In the Vierordt's method, detection was performed at 243 nm and 287 nm. Linearity was observed in the range of 1-5 µg/ml for Rosuvastatin calcium and 16-80 µg/ml for Megestrol acetate with correlation coefficients of 0.9997 and 0.9996. Precision was within %RSD < 2. Accuracy showed recovery of 99.00%-99.38% and 99.74%-99.91%. LOD/LOQ values were 0.04-0.08 µg/ml and 0.13-0.25 µg/ml. Assay results were 99.00% and 99.90%. In the First Order Derivative method, zero-crossing points were 293 nm and 227 nm. Linearity was 1-5 µg/ml and 16-80 µg/ml with correlation coefficients of 0.9993 and 0.9982. Recovery ranged from 99.33%-99.60% and 99.77%-99.92%. LOD/LOQ values were 0.07-0.64 µg/ml and 0.20-1.95 µg/ml. Assay values were 99.5% and 99.96%. The RP-HPLC was developed using Acetonitrile: Phosphate Buffer (pH 3.6 adjusts with 10% Ortho phosphoric acid) 58:42 %v/v at 253 nm with retention time of 2.3 min and 4.5 min. Linearity was excellent with correlation coefficient of 0.9962 and 0.9986. Accuracy ranged from 99.93%-99.99%, with LOD/LOQ values of 0.02-0.58 µg/ml and 0.06-1.75 µg/ml. All methods were accurate, precise, reproducible, economical, and suitable for routine quality control.

1. INTRODUCTION:

Rosuvastatin belongs to a group of medicines called statins. It is used to lower cholesterol and to reduce the risk of heart disease. Cholesterol is a fatty substance that builds up in your blood vessels and causes narrowing, which may lead to a heart attack or stroke. Rosuvastatin works by blocking an enzyme in the liver called HMG-CoA reductase, which leads to the liver making less cholesterol[1]. Megestrol acetate is a hormonal therapy drug. It is used to treat breast cancer. Breast cancer is a disease in which abnormal breast cells grow out of control and form tumors. If left unchecked, the tumors can spread throughout the body and become fatal[2]. The combination effect of Rosuvastatin calcium and Megestrol acetate was studied in clinical trial phase III[3].The combination effect of Rosuvastatin

calcium and Megestrol acetate was found to be in prevention of atypical endometrial hyperplasia The effect of rosuvastatin combined with oral megestrol acetate on fertility-preserving treatment in patients with atypical endometrial hyperplasia[4-6].A comprehensive literature survey revealed several reported analytical methods for the estimation of Rosuvastatin calcium either alone or in combination with other drugs, including RP-HPLC methods[7-12], UV spectrophotometric methods[13-15], stability-indicating liquid chromatographic method[16-17], HPLC/UV[18-19], HPTLC[20], liquid chromatography-tandem mass spectrometry method in human plasma[21-22] and LC-MS/MS[23]. Despite the availability of these sophisticated and well-established analytical techniques, all reported methods focus on the individual estimation of these drugs or their determination in biological matrices. To the best of our knowledge, no validated spectrophotometric and chromatographic method has been reported for the simultaneous quantification of Rosuvastatin calcium and Megestrol Acetate in a synthetic mixture. The absence of a unified, cost-effective, and time-efficient analytical approach for their concurrent estimation highlights a significant analytical gap in the literature. Therefore, the present study was undertaken to develop and validate novel, accurate, precise, and robust RP-HPLC and simultaneous equation UV spectrophotometric methods for the concurrent estimation of both drugs, in accordance with ICH Q2 (R2)[24] guideline, thereby providing a reliable analytical tool suitable for routine quality control analysis.

2. EXPERIMENTAL MATERIALS AND INSTRUMENTATION:

2.1 Chemicals and reagents

Rosuvastatin calcium was obtained as a gift sample from Cadila Pharmaceuticals, Ahmedabad, Gujarat, India. Megestrol acetate was procured from Jigs Chemical Limited, Ahmedabad, Gujarat, India. HPLC-grade methanol and water were purchased from Finar Chemicals Pvt. Ltd., Ahmedabad, Gujarat, India. All other chemicals and reagents used in the study were of analytical reagent (AR) grade or HPLC grade and were used without further purification.

2.2 Instrumentation

UV spectroscopic analysis was performed using a Shimadzu UV-1900 UV-Visible spectrophotometer (Shimadzu Corporation, Kyoto, Japan) equipped with UV Probe 2.7 software, a spectral bandwidth of 1 nm, and 1.0 cm matched quartz cuvettes over the wavelength range of 200-400 nm.

Chromatographic analysis was carried out using a Systronics RP-HPLC system (Model SYS-LC-138, Systronics, India) coupled with a UV detector. The pH of the buffer solutions was measured using a Systronics pH meter (Systronics, Naroda, Ahmedabad). An analytical balance (Scale-Tec, India) was used for accurate weighing of samples. The mobile phase was degassed by sonication using a Digital Pro+ sonicator (Model PS-10A, Broleo, India) prior to use.

2.3 Preparation of Solutions

2.3.1 Preparation of Stock Solution

Precisely weighed quantities of 10 mg each of Rosuvastatin calcium and Megestrol acetate were quantitatively transferred into a 100 ml volumetric flask and subsequently diluted to volume with methanol to achieve a final concentration of 100 µg/mL. The solutions were sonicated for 5 mins to ensure complete dissolution.

2.3.2 Preparation of calibration curve

The calibration standards in the concentration range of 1-5 µg/mL for rosuvastatin calcium and 16-80 µg/mL for megestrol acetate, appropriate aliquots of the respective stock solutions were transferred into a series of 10 mL volumetric flasks. For Rosuvastatin calcium, aliquots of 0.1, 0.2, 0.3, 0.4, and 0.5 mL were diluted to volume with methanol to yield final concentrations of 1, 2, 3, 4, and 5 µg/mL, respectively. Similarly, for Megestrol acetate, aliquots of 1.6, 3.2, 4.8, 6.4, and 8.0 mL were diluted to volume with methanol to obtain concentrations of 16, 32, 48, 64, and 80 µg/mL, respectively. The prepared solutions were analyzed under optimized spectrophotometric conditions using a 1 cm matched quartz cuvette. For chromatographic analysis, 20 µL of each working standard solution was injected into the RP-HPLC system under optimized chromatographic conditions.

3. METHODOLOGY

3.1 Method I: UV SEPCTROPHOTOMETRIC METHOD DEVELOPMENT

Pipetted out 0.2 ml solution of Rosuvastatin calcium (100 µg/ml) and 3.2 ml standard stock solution of Megestrol acetate (100 µg/ml) into different 10 ml volumetric flask and diluted up to mark with Methanol to get the 2 µg/ml of Rosuvastatin calcium and 32 µg/ml of Megestrol acetate. Each solution was scanned between 200 to 400 nm.

3.1.1 Simultaneous equation as Vierordt's method

Solutions of Rosuvastatin calcium (2 µg/ml) and megestrol acetate (32 µg/ml) prepared in methanol were subjected to a spectral scan from 200 to 400 nm at a medium speed, utilizing pure methanol as the reagent blank. For the analytical determination, the absorption maxima (λmax) were established at 243 nm for Rosuvastatin calcium and 287 nm for Megestrol acetate. This procedure applies the Simultaneous Equation technique based on Vierodt's principle, where the precise concentration of each drug within the sample is calculated according to the following mathematical expressions.

Standard Stock solutions of Rosuvastatin calcium and Megestrol acetate in the concentration range 1-5 µg/mL and 16-80 µg/ml were made in the methanol and absorbance of these solutions was measured at 243 nm and 287 nm. Calibration curves were plotted to confirm the Beer's law and the absorptivity values calculated at the respective wavelengths for both the drugs. Two simultaneous equations as below were formed using these absorptivity values A (1%, 1 cm).

$$\text{At } \lambda 1 \; A1 = ax1bCx + ay1bCy \ldots\ldots\ldots\ldots\ldots (1)$$

$$\text{At } \lambda 2 A2 = ax2bCx + ay2\, bCy \ldots\ldots\ldots\ldots\ldots\ldots (2)$$

For measurements in 1 cm cells b=1,

Rearrange eq. (2),

$$Cy = A2 - ax2Cx/ay2$$

Substituting for Cy in eq (1) and rearranging

$$Cx = A2ay1 - A1\, ay2/ax2\, ay1 - ax1\, ay2 \ldots\ldots\ldots\ldots\ldots\ldots (3)$$

$$Cy = A1ax2 - A2\, ax1/ax2\, ay1 - ax1\, ay2 \ldots\ldots\ldots\ldots\ldots\ldots (4)$$

Where C_x and C_y are the concentration of Rosuvastatin calcium and megestrol acetate, respectively, A_1 and A_2 are absorbance at 243 nm and 287 nm, respectively, ax_1 and ax_2 are absorptivity of Rosuvastatin calcium at 243 nm and 287 nm, respectively; ay_1 and ay_2 are absorptivity of megestrol acetate at 287 nm and 243 nm, respectively. By solving the two simultaneous equations, the concentrations of Rosuvastatin calcium & megestrol acetate in sample solutions were obtained.

3.1.2 Simultaneous equation as First Order Derivative Method

Solutions of Rosuvastatin calcium (2 µg/ml) and megestrol acetate (32 µg/ml) prepared in methanol were subjected to a spectral scan from 200 to 400 nm at a medium speed, utilizing pure methanol as the reagent blank. For the analytical determination, Each solution was scanned in the range of 200-400 nm. All Zero-order Spectrum (D0) were converted to First Derivative Spectrum (D1) using delta lambda 2.0 and scaling factor 8. The overlain first derivative spectrum of Rosuvastatin calcium and Rosuvastatin Calcium at different concentration were recorded. The Zero-crossing point (ZCP) of Rosuvastatin calcium was found to be 293 nm and ZCP of Megestrol acetate was found to be 227 nm.

Standard Stock solutions of Rosuvastatin calcium and Megestrol acetate in the concentration range 1-5 µg/mL and 16-80 µg/ml were made in the methanol and absorbance of these solutions was measured at 227 nm and 293 nm. Graph of Absorbance v/s Concentration was plotted.

3.2 Method II: Reverse Phase High Performance Liquid Chromatography Method

Chromatographic analysis was performed via isocratic elution, wherein various mobile phase configurations including Methanol: Water, ACN: Phosphate Buffer (pH 5) were evaluated in varying ratios. Optimal resolution of both analyte peaks was achieved using a mixture of Acetonitrile: Phosphate Buffer (pH 3.6 adjusts with 10% Ortho phosphoric acid) (58:42 % v/v)at a consistent flow rate of 1 mL/min. All solvents underwent filtration through a 0.45 µm membrane and were degassed via sonication for 30 minutes before use. Separation was executed on a Kromstar C_{18} (250 mm × 4.6 mm, 5 µm) stationary phase, with the eluent monitored using a UV Detector and chromatograms specifically extracted at 253 nm nm. Calibration curves were subsequently established by plotting

the measured peak areas against their respective concentrations to derive the corresponding linear regression equations.

3.3 METHOD VALIDATION

The analytical methodologies employed in this research were rigorously validated in accordance with the regulatory standards established by the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) under the ICH Q2 (R2)[35] guidelines for analytical procedure validation.

3.3.1 Specificity

Specificity denotes the capacity of an analytical procedure to accurately and distinctly quantify the target analyte despite the potential interference of co-existing substances. Within a complex sample, these extraneous components commonly encompass synthesis impurities, degradation products, or various matrix constituents that could otherwise confound the measurement.

3.3.2. Linearity and Range (n=6)

The linearity of the analytical procedure was evaluated through the preparation of five distinct concentrations of standard solutions. Rosuvastatin calcium & megestrol acetate demonstrated linear responses within the concentration ranges of 1-5 µg/mL and 16-80 µg/mL, respectively. The proportionality of both analytes was statistically assessed by calculating the slope, y-intercept, and correlation coefficient (R^2) from the resulting calibration curves.

3.3.3. Precision

The precision of both analytical methodologies was evaluated across three distinct parameters: repeatability, intraday (intermediate) precision, and interday (reproducibility) precision. To assess intraday precision, standard solutions of rosuvastatin calcium (1, 2, 3 µg/mL) and megestrol acetate (08, 16, 24 µg/mL) were analyzed in triplicate at three separate time intervals within a single day. Interday precision was similarly established by evaluating the same concentration levels over three consecutive days. Furthermore, repeatability was rigorously determined through six replicate injections of a single concentration level 2 µg/mL for Rosuvastatin calcium and 16 µg/mL for megestrol acetate. All precision data were statistically quantified and reported as the percentage relative standard deviation (%RSD) to ensure compliance with ICH Q2 (R2) guideline.

3.3.4 Limit of Detection (LOD):

Limit of detection can be calculated using following equation as per ICH guidelines.

$$\mathrm{LOD} = 3.3 * \frac{\sigma}{S}$$

Where, σ = standard deviation of the calibration curve
S = slope of the calibration curve

3.3.5 Limit of Quantification (LOQ):

Limit of quantification can be calculated using following equation using the standard deviation of the Y-intercept (σ) and the mean slope (S) of the calibration curve according to ICH Q2 (R2) guideline.

$$\mathrm{LOQ} = 10 * \frac{\sigma}{S}$$

Where, σ = standard deviation of the calibration curve
S = slope of the calibration curve

3.3.6 Accuracy (Recovery study) (n=3)

The accuracy of an analytical procedure denotes the proximity of the experimental result to the accepted reference value or conventional true value. To confirm the accuracy of the proposed method, recovery studies were conducted in accordance with ICH Q2(R2) Guidelines at three distinct concentration levels: 50%, 100%, and 150%. These evaluations targeted Rosuvastatin calcium (**2 µg/ml**) and Megestrol acetate (**16 µg/ml**). using the standard addition technique, with each level analyzed in triplicate. The methodology's accuracy was subsequently established by calculating the percentage recovery of both analytes across these fortified concentrations.

3.3.7 Assay as analysis of Synthetic Mixture

A synthetic mixture containing Rosuvastatin calcium and megestrol acetate in the ratio of 1:16 was prepared. Accurately weighed quantities of Rosuvastatin calcium (10 mg) and Megestrol acetate (160 mg) were blended with commonly used excipients, namely microcrystalline cellulose (08 mg), lactose (10 mg), magnesium stearate (3 mg), talc (4 mg), and croscarmellose sodium (5 mg), using a mortar and pestle to obtain a homogeneous mixture. An accurately weighed portion of the prepared blend equivalent to 10 mg of Rosuvastatin calcium 160 mg of megestrol acetate was transferred into a 100 mL volumetric flask. The mixture was sonicated to ensure complete dissolution of the drugs. The volume was then made up to the mark with methanol and mixed thoroughly. The resulting solution was filtered through Whatman filter paper to remove insoluble excipients. The obtained stock solution contained 100 µg/mL of Rosuvastatin calcium and 1600 µg/mL of megestrol acetate. For sample analysis, 0.2 mL of this stock solution was accurately transferred into a 10 mL volumetric flask and diluted to volume with methanol to yield final concentrations of 2 µg/mL of Rosuvastatin Calcium and 32 µg/mL of Megestrol acetate. The prepared sample solution was analyzed using the optimized RP-HPLC and UV spectrophotometric methods, and the percentage assay of both drugs was calculated.

3.3.8 Robustness

Robustness of the developed RP-HPLC and UV spectrophotometric methods was evaluated by deliberately introducing small and systematic variations in analytical conditions and assessing their effect on the assay results. For the RP-HPLC method, robustness was examined by varying the flow rate (±1 mL/min from 0.2 mL/min), mobile phase composition (±2% variation in organic phase), and detection wavelength (±2 nm from 253 nm). The effects of these changes on retention time, peak area, tailing factor, and resolution were studied. For the UV spectrophotometric method, robustness was assessed by varying the detection wavelength (±2 nm from 253 nm for rosuvastatin calcium and 276 nm for Megestrol Acetate and evaluating the effect of slight variations in solvent composition.

3.3.9 System Suitability Tests

A system suitability test is an integral part of liquid chromatography. They are used to verify that resolution and reproducibility of chromatography system are adequate for the analysis to be done. The test includes the Resolution, Column efficiency, Tailing factor and Theoretical plates (table 1).

4. RESULTS AND DISCUSSION:

4.1 Selection of wavelength

For the simultaneous equation method, standard solutions of Rosuvastatin calcium (2 µg/mL) and megestrol acetate (16 µg/mL) in methanol were subjected to spectral scanning between 200 and 400 nm at medium speed, with methanol employed as the blank solution. For the analytical determination, the absorption maxima (λmax) were established at 243 nm for Rosuvastatin calcium and 287 nm for Megestrol Acetate (Figure 1). This procedure applies the Simultaneous Equation technique based on Vierodt's principle, where the precise concentration of each drug within the sample is calculated.

To determine the wavelength for measurement, Rosuvastatin calcium (2 µg/ml) and megestrol acetate (16 µg/ml) solutions were scanned between 200-400 nm. Absorbance maximum was obtained at their λmax 243 nm and 287 nm for measurement of Rosuvastatin calcium and megestrol acetate, respectively. For first order derivative technique, Measurement and determination of Rosuvastatin calcium and megestrol acetate were observed at 227 nm and 293 nm, respectively. (Figure 2).

For RP-HPLC method, coupled with UV detection, is fundamentally dependent upon the strategic selection of an optimal detection wavelength. Both analytes exhibited significant molar absorptivity at 253 nm, leading to its selection for the simultaneous quantification of rosuvastatin calcium and megestrol acetate within the synthetic mixture. The spectral rationale for this choice of detection wavelength is showed in Figure 1.

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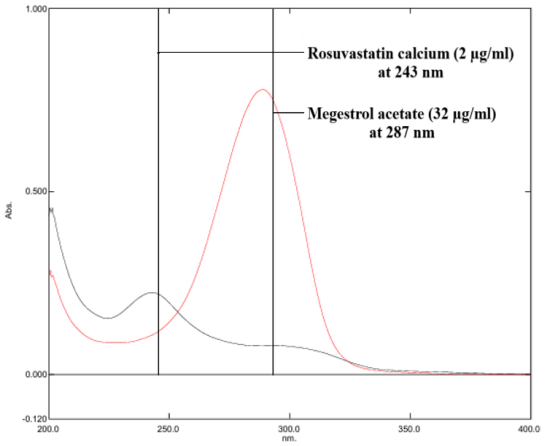

Figure 1: Overlain UV Spectra of Rosuvastatin calcium (2 µg/ml) 243 nm and Megestrol acetate (32 µg/ml)

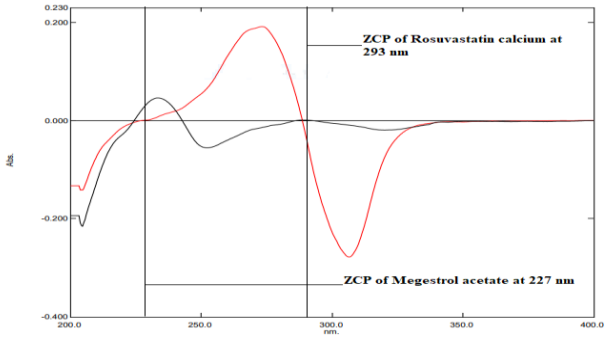

Figure 2: Overlain UV Spectra of Rosuvastatin calcium (2 µg/ml) and Megestrol acetate (32 µg/ml) in Methanol (First Order

*4.2 Simultaneous equation (***Vierordt's***) method*

For multi-component UV analysis, Vierordt's method is named after the German scientist Karl Vierordt. UV Spectra of Rosuvastatin calcium (1-5 µg/mL) and megestrol acetate16-80 µg/mL) over the linearity and range had been showed in Figure 3 and 4, respectively.

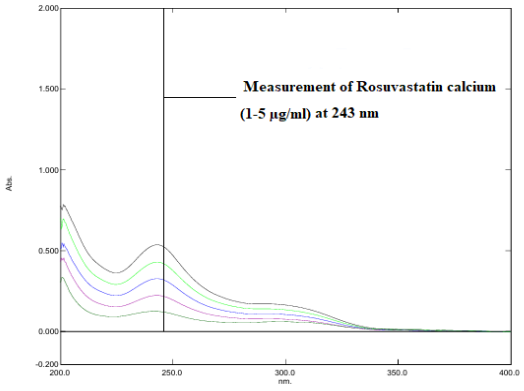

Figure 3: Overlain UV Spectra of Rosuvastatin calcium (1-5 µg/ml) at 243 nm

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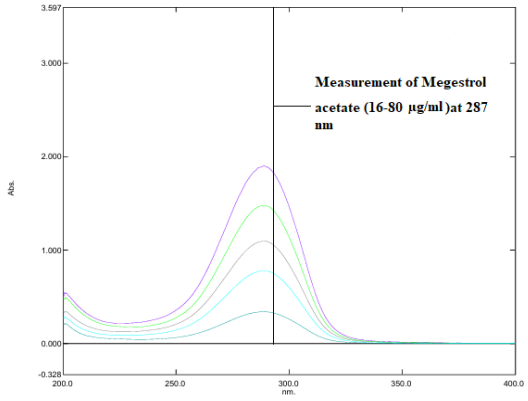

Figure 4: Overlain UV Spectra of Megestrol acetate (16-80 µg/ml) at 287 nm

4.3 Simultaneous equation as First Order Derivative Method

Overlain UV Spectra of Rosuvastatin calcium (1-5 µg/ml) and Megestrol acetate 16-80 µg/ml) In methanol (First Order) have been shown in Figure 5 and 6 at the wavelength of 227and 293.

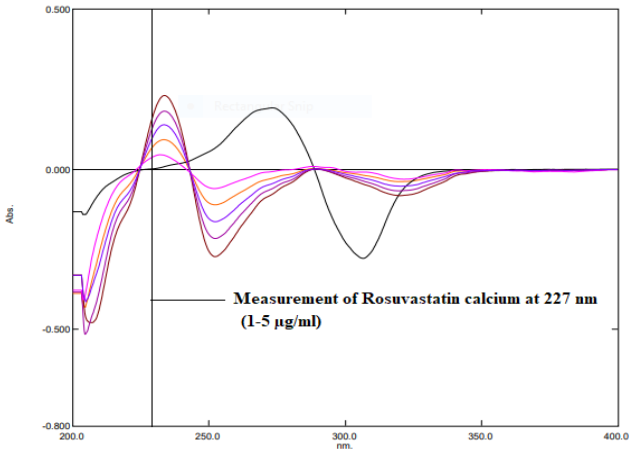

Figure 5: Overlain UV Spectra of Rosuvastatin calcium (1-5 µg/ml) at 227 nm (First Order)

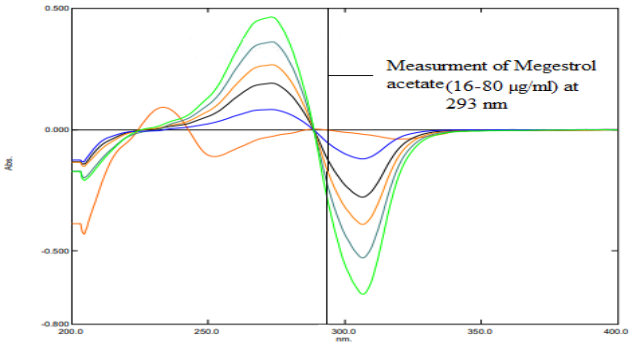

Figure 6: Overlain UV Spectra of Megestrol acetate (16-32 µg/ml) in Methanol (First Order)

4.3 RP-HPLC Method Development

An RP-HPLC method coupled with UV detection was developed for the concurrent quantification of Rosuvastatin calcium and Megestrol acetate, with the primary objective of achieving optimal peak symmetry and high theoretical plate counts within an efficient analytical runtime. Chromatographic parameters were refined through the systematic

evaluation of various stationary and mobile phase compositions. Among the reversed-phase C_8 and C_{18} columns assessed, the Kromstar C_{18} (250 × 4.6 mm, 5 µm) demonstrated superior performance, yielding highly symmetric peaks and the most favorable retention times. The optimal mobile phase was identified as a mixture Acetonitrile: Phosphate Buffer (pH 3.6 adjusts with 10% Ortho phosphoric acid) (58:42 % v/v) at 253 nm. Although alternative ratios of this buffer and solvent were investigated, they resulted in undesirable peak tailing and excessive retention for both analytes.

4.4 VALIDATION OF THE PROPOSED METHODS

4.4.1 Specificity

Specificity is defined as the ability of an analytical method to unequivocally assess the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components. The specificity of the developed RP-HPLC method was evaluated by comparing chromatograms of the mobile phase (blank), placebo (excipients), and the test preparation solution. The chromatogram of the blank showed no peaks at the retention times corresponding to Rosuvastatin calcium and Megestrol acetate. Similarly, no interfering peaks from excipients were observed at the respective retention times of the analytes in the sample chromatogram. These results demonstrate that the developed method is specific and free from interference due to mobile phase components or formulation excipients, thereby confirming its suitability for the simultaneous estimation of Rosuvastatin calcium and Megestrol acetate. Retention time was found to be 2.3 min and 4.5 min for Rosuvastatin calcium and Megestrol acetate, respectively showed in table 1.

Table 1: System suitability parameters for Rosuvastatin calcium and Megestrol acetate

Sr. No.	System suitability parameters	Rosuvastatin calcium	Megestrol acetate
1.	Retention time	2.3	4.5
2.	Theoretical Plates	7728	6846
3.	Tailing Factors	0.67	0.53
4.	Resolution	2.2	

4.4.2 Linearity and range

Simultaneous equation as Vierordt's method, UV Spectra of Rosuvastatin calcium (1-5 µg/ml) and Megestrol acetate (16-80 µg/ml) over the linearity and range had been showed in Figure 2 and 3, respectively. For UV, rosuvastatin calcium exhibited a linear response in the concentration range of 1–5 µg/mL at 243 nm and 287 nm. The correlation coefficients (r^2) were found to be 0.9997 and 0.9981 at 243 nm and 287 nm, respectively, indicating excellent linearity. The mean absorbance values (n = 6) showed low standard deviation with %RSD values below 1.5%, demonstrating good precision and repeatability. Megestrol acetate showed linearity over the concentration range of 1-5 µg/mL at 287 nm and 243 nm, with correlation coefficients (r^2) of 0.9996 and 0.9997, respectively.

For, First Order Derivative Method UV Spectra of Rosuvastatin calcium (1-5 µg/ml) and Megestrol acetate (16-80µg/ml) over the linearity and range had been showed in Figure 5 and 6, respectively. For UV, Rosuvastatin calcium exhibited a linear response in the concentration range of 1–5 µg/mL at 227 nm. The correlation coefficients (r^2) were found to be 0.9993at nm, respectively, indicating excellent linearity. The mean absorbance values (n = 6) showed low standard deviation with %RSD values below 2 %, demonstrating good precision and repeatability. Megestrol acetate showed linearity over the concentration range of µg/mL at 293 nm, with correlation coefficients (r^2) of 0.9982, respectively.

The RP-HPLC chromatogram of rosuvastatin calcium (1-5 µg/mL) and megestrol acetate (16-80 µg/mL). The Peak Area was found. Calibration graphs were plotted between concentrations and peak areas were observed. The regression equation of calibration curve was generated and

Correlation Coefficient for rosuvastatin calcium 0.9962 and for megestrol acetate 0.9986, respectively. The %RSD values were less than 2.0%, confirming acceptable precision and reproducibility of the developed method. The linearity data are summarized in Table 2.

Table 2: Linearity and sensitivity data of Rosuvastatin calcium and Megestrol acetate

Parameters	UV Spectrophotometry				First Order Derivative Method		HPLC	
	ROSU		MEGE		ROSU	MEGE	ROSU	MEGE
Wavelength (nm)	243 nm	287 nm	287 nm	243 nm	227 nm	293 nm	253 nm	
Beer's Law Limit (µg/mL)	1-5 µg/ml	1-5 µg/ml	16-80 µg/ml	16-80 µg/ml	1-5 µg/ml	16-80 µg/ml	1-5	16-18
Regression equation (y=mx+c)	Y= 0.1025x+ 0.0221	Y= 0.0296x+ 0.0232	Y= 0.0243x- 0.0656	Y=0.0035 x- 0.0032	Y= 0.0247x + 0.0095	Y = 0.0039x - 0.0132	y = 65.607x - 38.677	y = 11.494x - 24.711
Correlation Coefficient (r^2)	0.9997	0.9981	0.999	0.9992	0.9993	0.9982	0.9962	0.9986
Accuracy (% Recovery=3)	99.0 %-99.38 %		99.74 %-99.91%		99.33-99.60 %	99.79-99.92 %	99.93%-99.96%	99.97%-99.99%
LOD	0.04	0.08	2.93	0.76	0.07	0.64	0.02	0.58
LOQ	0.13	0.25	8.88	2.31	0.20	1.95	0.06	1.75
% Assay	99.00%		99.90%		99.5 %	99.96%	99.90%	99.97%

4.4.2.1 Calculation for Simultaneous Equation Method for Rosuvastatin calcium and Megestrol acetate in *Synthetic Mixture*

Rosuvastatin calcium (2 µg/ml) and Megestrol Acetate (32 µg/ml) in methanol, both the solutions were scanned over range of 200-400nm against methanol as blank, using medium scan speed. The sampling wavelength for analysis includes 243 nm for Rosuvastatin calcium and 287 nm for Megestrol Acetate The method employs Simultaneous Equation as per Vierodt's method and the concentrations of drugs in sample solution were determined by using the following formula:

Rosuvastatin calcium,

$$C_x = \frac{A2 \times ay1 - A1 \times ay2}{ax2 \times ay1 - ax1 \times ay2}$$

Where ax_1 and ax_2 represented the absorptivity of Rosuvastatin calcium at 243 nm and 287 nm, respectively; ay_1 and ay_2 denoted the absorptivity of Megestrol acetate at 287 nm and 243 nm, respectively; and A_1 and A_2 corresponded to the absorbance of the sample measured at 243 nm and 287 nm, respectively.

C (Rosuvastatin calcium) = 1.9 µg/mL; The concentration of Rosuvastatin calcium (Cx), calculated using Vierordt's simultaneous equation method, was found to be 1.9 µg/mL.

For megestrol acetate,

$$C_y = \frac{A1 \times ax2 - A2 \times ax1}{ax2 \times ay1 - ax1 \times ay2}$$

where a_{x1} and a_{x2} are the absorptivity values of *Rosuvastatin calcium* at 243 nm and 287 nm, respectively; $a_{\gamma 1}$ and $a_{\gamma 2}$ represent the absorptivity of Megestrol Acetate at 287 nm and 243 nm, respectively; and A_1 and A_2 are the absorbance values of the sample measured at 243 nm and 287 nm, respectively.

C (Megestrol acetate) = 30.80 µg/mL; The concentration of Megestrol Acetate (Cy), calculated using Vierordt's simultaneous equation method, was found to be 30.80 µg/mL.

4.4.3 Precision

Methodological precision was evaluated through intraday, inter-day, and repeatability assessments using triplicate analyses of Rosuvastatin calcium (1, 2 and 3 µg/ml) and Megestrol Acetate (16, 32 and 48 µg/ml) across three consecutive days and within a single diurnal period. Absorbance values were recorded for these concentrations to establish intermediate precision, while repeatability was specifically determined using concentrations of 2 µg/ml for Rosuvastatin calcium and 32 µg/ml for Megestrol Acetate. The outcomes, expressed as Relative Standard Deviation (% RSD) for each precision parameters were less than two.

4.4.4 LOD and LOQ

The limits of detection (LOD) and quantification (LOQ) are calculated using the standard deviation responses and slopes obtained from the calibration curves of each drug at their specific wavelengths. The results of LOD and LOQ were displayed in Table 2.

4.4.5 Accuracy

To evaluate the accuracy of the proposed methodology, recovery studies were performed using the standard addition technique, in which pre-analyzed samples were spiked with known concentrations of pure Rosuvastatin calcium and Megestrol acetate. These assessments were executed at three levels 50%, 100%, and 150% and conducted in triplicate to ensure statistical reliability. The accuracy was expressed as the percentage recovery of the added standards. For the UV spectrophotometric approach, the percentage recovery was found to be within range of 99.0 %-99.38 %for Rosuvastatin calcium and 99.74 %-99.91%for Megestrol acetate. For RP-HPLC method, the percentage recovery was found to be within the range 99.93%-99.96%for Rosuvastatin calcium and 99.97%-99.99%% for Megestrol acetate, with detailed results provided in Table 3.

Table 3: Recovery study data for UV and RP-HPLC Method

Vierordt's Method						
Name of Drug	**% Level of recovery**	**Test Amount (µg/mL)**	**Amount of drug taken (µg/mL)**	**Total Std Amt (µg/mL)**	**Total amount Recovered (µg/mL)**	**% Mean Recovery ± SD(n=3)**
Rosuvastatin calcium	50	2	1	3	2.97	99.00±0.104
	100	2	2	4	3.97	99.23±0.115
	150	2	3	5	4.97	99.38±0.163
Megestrol acetate	50	32	16	48	47.88	99.74±0.104
	100	32	32	64	63.91	99.84±0.108
	150	32	48	80	79.93	99.91±0.126
First Order Derivative Method						
Rosuvastatin calcium	50	2	1	3	2.97	99.00±0.104
	100	2	2	4	3.97	99.23±0.115
	150	2	3	5	4.97	99.38±0.163
Megestrol acetate	50	32	16	48	47.88	99.74±0.104
	100	32	32	64	63.91	99.84±0.108
	150	32	48	80	79.93	99.91±0.126
RP-HPLC Method						
Rosuvastatin calcium	50	2	1	33	2.998	99.93±0.6175
	100	2	2	44	3.998	99.95±0.7383
	150	2	3	55	4.998	99.96±0.8591
Megestrol acetate	50	32	16	45	47.99	99.97±0.2165
	100	32	32	60	63.99	99.98±0.5254
	150	32	48	75	79.992	99.99±0.7541

4.4.6 Assay as Analysis of Synthetic mixture

From assay, the concentration of Rosuvastatin Calcium 2 µg/mL and megestrol acetate 32 µg/mL were run into UV and RP-HPLC. The Percentage assay of Dabigatran etexilate and megestrol acetate were found to be 99.00%and 99.90% respectively in UV for Vierordt's Method. And The Percentage assay of Rosuvastatin Calcium and megestrol acetate were found to be 99.50% and 99.96% respectively in UV for first order derivative method. For RP-HPLC the Percentage assay of Rosuvastatin Calcium and megestrol acetate were found to be 99.90% and 99.97%, respectively. Its results showed in Table 4.

Table 4: Assay results for UV and RP-HPLC Method

Vierordt's Method				
Name of Drug	**Amount in synthetic mixture (µg/mL)**	**Mean Amount found (µg/mL)**	**% Assay ± SD (n=3)**	**%RSD**
Rosuvastatin Calcium	2	1.98	99.00± 0.047	0.047
Megestrol Acetate	32	31.97	99.90 ± 0.012	0.012
First Order Derivative Method				
Rosuvastatin Calcium	2	1.98	99.00± 0.047	0.047

Megestrol Acetate	32	31.97	99.90 ± 0.012	0.012
RP-HPLC Method				
Rosuvastatin Calcium	2	1.998	99.90±1.0146	1.02
Megestrol Acetate	32	31.991	99.97±1.1042	1.11

4.4.7 Robustness

Chromatographic analysis was used to analyses the effects of changes in analysts, and the results showed that there was no statistically significant difference in the % RSD of technique II. Additionally, small changes were performed to assess the robustness of the created HPLC procedures. The approaches' robustness was demonstrated by the% RSD, which remained constant despite minor variations in flow rate, run time, and detection. It was determined that the created approaches were essential. The results indicated that minor deliberate variations in method parameters did not produce significant changes in analytical responses. The percentage relative standard deviation (%RSD) values were found to be within acceptable limits (<2%), demonstrating that the developed methods are robust and reliable for routine analysis.

5. CONCLUSION:

The current investigation focused on the development and validation of streamlined, cost-effective, and precise analytical protocols for the concomitant quantification of Rosuvastatin calcium and Megestrol acetate, in a synthetic matrix. While previous literature documents various techniques for these analytes in isolation, a literature gap was identified regarding their simultaneous determination. Consequently, UV-Spectrophotometric and RP-HPLC methodologies were established and validated in strict accordance with ICH Q2 (R2) regulatory standards. For the UV-Spectrophotometric approach, the Vierordt's (simultaneous equation) method and First Order Derivative Method was employed employed, utilizing analytical wavelengths of 243 nm and 287 nm for Rosuvastatin calcium and Megestrol acetate, respectively for Vierordt's (simultaneous equation) method. This method exhibited robust linearity over concentration intervals of 1-5 µg/ml and 16-80 µg/ml, respectively, yielding correlation coefficients nearer to 0.9997, 0.9981, 0.9996 and 0.9997. Comprehensive validation encompassing accuracy, precision, repeatability, and sensitivity (LOD/LOQ) yielded results within established acceptance criteria. Furthermore, recovery experiments and assay data substantiated the method's reliability for estimating both components within the synthetic mixture. For First Order Derivative Method utilizing analytical wavelength 227 nm and 293 nm for Rosuvastatin calcium and Megestrol acetate, this method exhibited robust linearity over concentration intervals of 1-5 µg/ml and 16-80 µg/ml, respectively, yielding correlation coefficients nearer to 0.9973 and 0.9982. Additionally, a highly sensitive RP-HPLC method was optimized using a C_{18} stationary phase and a mobile phase comprised of Acetonitrile: Phosphate Buffer (pH 3.6 adjusts with 10% Ortho phosphoric acid) (58:42 % v/v) The system operated at a flow rate of 1 ml/min with UV detection at 253 nm, resulting in well-resolved peaks and favorable system suitability metrics. The chromatographic technique demonstrated superior linearity, precision, and robustness. The obtained assay percentages confirmed that this method is highly suitable for standardized quantitative assessments. In conclusion, both newly developed analytical platforms proved to be efficient, accurate, and reproducible. These validated methods are highly recommended for routine quality control and the simultaneous monitoring of Rosuvastatin calcium and Megestrol Acetate in synthetic mixtures.

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CONFLICT OF INTEREST:

The authors declare that there is no conflict of interest.

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