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# DEVELOPMENT AND VALIDATION OF HPTLC METHOD FOR SIMULTANEOUS ESTIMATION OF ROSUVASTATIN CALCIUM AND ASPIRIN IN CAPSULE DOSAGE FORM

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## ABSTRACT

This paper described validated high performance thin layer liquid chromatographic (HPTLC) method for estimation of Rosuvastatin Calcium (ROSU) and Aspirin (ASP) in capsule dosage form. The method involved separation of components by TLC on a precoated silica gel 60 $F_{254}$ using a mixture of n-Hexane: Acetone: Ethyl acetate: Formic acid (6:3:1:0.2 v/v) as a mobile phase. Detection of spots was carried out at 240 nm for Rosuvastatin Calcium and Aspirin both. The mean retardation factor for Rosuvastatin Calcium and Aspirin were found to be 0.31±0.02 and 0.60±0.02, respectively. The linear regression data for the calibration plots showed good linear relationship with $r^2$ value 0.995 and 0.995 in the concentration range of 500-1000 ng/spot and 3750-7500 ng/spot for Rosuvastatin calcium and Aspirin, respectively. The developed method was validated as per ICH Guidelines.

**INTRODUCTION:** Rosuvastatin calcium is chemically (3*R*, 5S, 6E)-7-[4-(4-fluorophenyl)-2-(*N*-methyl methane sulfonamido)-6-(propan-2-yl) pyrimidin-5-yl]-3, 5-dihydroxyhept-6-enoicacid. It is a competitive inhibitor of the enzyme HMG-CoA reductase, the rate limiting enzyme that converts 3-hydroxy -3-methylglutaryl mevalonate, precursor for cholesterol. It is a cholesterol lowering agent. Its approximate elimination half life is 19 hours and it's time to peak plasma concentration are reached within 3–5 hours following oral administration.

Aspirin (ASP) chemically known as acetyl salicylic acid and is used as non-steroidal anti-inflammatory and analgesic drug [1-6]. In recent years HPTLC method was reported for the quantification of Rosuvastatin calcium [7]. There was HPTLC method developed for Atorvastatin calcium and Aspirin [8]. There were also HPTLC methods reported for Aspirin and Atorvastatin calcium with combination of other drugs [9-14].

But referring to the literature survey, there is no any published HPTLC method for Rosuvastatin calcium and Aspirin combined capsule form.

The present paper describes a simple, accurate and precise method for simultaneous estimation of Rosuvastatin Calcium and Aspirin in combined capsule dosage form.

The proposed method is optimized and validated as per the International Conference on Harmonization (ICH) guidelines [15].

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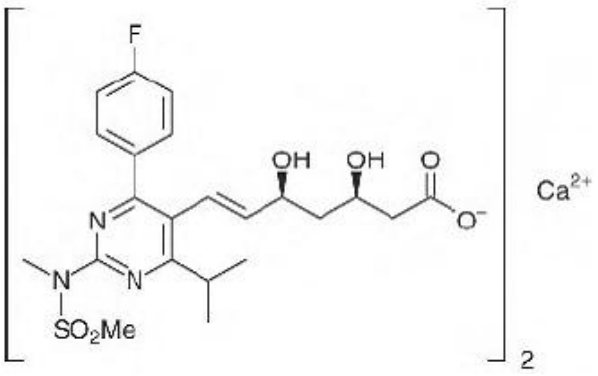
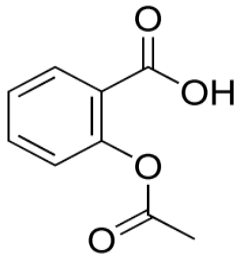


**ROSUVASTATIN CALCIUM**



**ASPIRIN**

**MATERIALS AND METHODS:**

**Chemicals and reagents:** Rosuvastatin calcium and Aspirin working standards were procured from Zydus cadila, and the tested pharmaceutical formulations (ROSU (10mg) and ASP (75mg) capsules) were procured from commercial pharmacy. Hexane, ethyl acetate, methanol, acetone, formic acid were of suitable analytical grade.

**Chromatographic Conditions:** The instrument used for the estimation was Camag Linomat V semi automatic sample applicator, Camag TLC scanner 3, CATS software for interpretation of the data, Hamilton syringe and Camag twin trough chamber. The Mobile phase was n-Hexane: Ethyl acetate: Acetone: Formic acid (6: 3: 1: 0.4). Chamber saturation time was 20min. Detection was performed at 240nm.

**Preparation of Standard Solution:** 10 mg of Rosuvastatin calcium (ROSU) and 75 mg Aspirin (ASP) were dissolved and diluted with methanol upto 100mL (100 µg/mL of ROSU and 750 µg/mL of ASP).

**Preparation of Sample Solution:** 20 capsules (UNISTAR*) were weighed with and without cap and capsule content was powdered. Powder equivalent to 10 mg of ROSU and 75 mg of ASP transferred into 100mL volumetric flask. Methanol was added to adjust level up to mark and sonicated for 30 min. The solution was filtered through whatman filter paper no. 42. First few mL of filtrate was discarded. The sample solution was used for estimation of ROSU and ASP. 6, 7 and 8 µL of sample solution was used for estimation purpose.

**RESULTS AND DISCUSSION:** Literature survey revealed that there was no any reported HPTLC method for simultaneous estimation of Rosuvastatin calcium and Aspirin. The present study was aimed at development of speedy and cost effective HPTLC technique for determination of ROSU and ASP in capsulated dosage forms.

Various blends of solvent systems in varying proportions were tried as mobile phase. However, mobile phase consisting n-Hexane: Ethyl acetate: Acetone: Formic acid in the ratio of (6: 3: 1: 0.4 v/v/v) was found to be more suitable with $R_f$ values of 0.31±0.02 and 0.60±0.02 for ROSU and ASP, respectively with saturation time of 20 minutes. The selection of wave length was based on maximum absorbance for optimum sensitivity. The drugs showed good linearity in the range of 500-1000 ng/spot for ROSU and 3750-7500 ng/spot for ASP with coefficient of correlation value 0.995 and 0.995, respectively. From the recovery studies, the accuracy results were 99.88% for ROSU and 100.04% for ASP and were found to be highly accurate.

**Validation of the Method:**

1. **Linearity and Range:** Linearity was found in the range of 500-1000 ng/spot for ROSU and 3750-7500 ng/spot for ASP. The drug peak area was calculated for each concentration level and a graph was plotted of drug concentration against the peak area. Calibration parameters are given in **table 1**.

**TABLE 1: CALIBRATION PARAMETERS**

| Parameter | Rosuvastatin calcium | Aspirin |
|---|---|---|
| Linearity range (ng/spot) | 500-1000 | 3750-7500 |
| Linearity equation | y = 5.013x + 2673 | y = 1.277x + 5727 |
| Co-relation coefficient | 0.995 | 0.995 |
| Slope | 5.013 | 1.277 |
| Intercept | 2673 | 5727 |

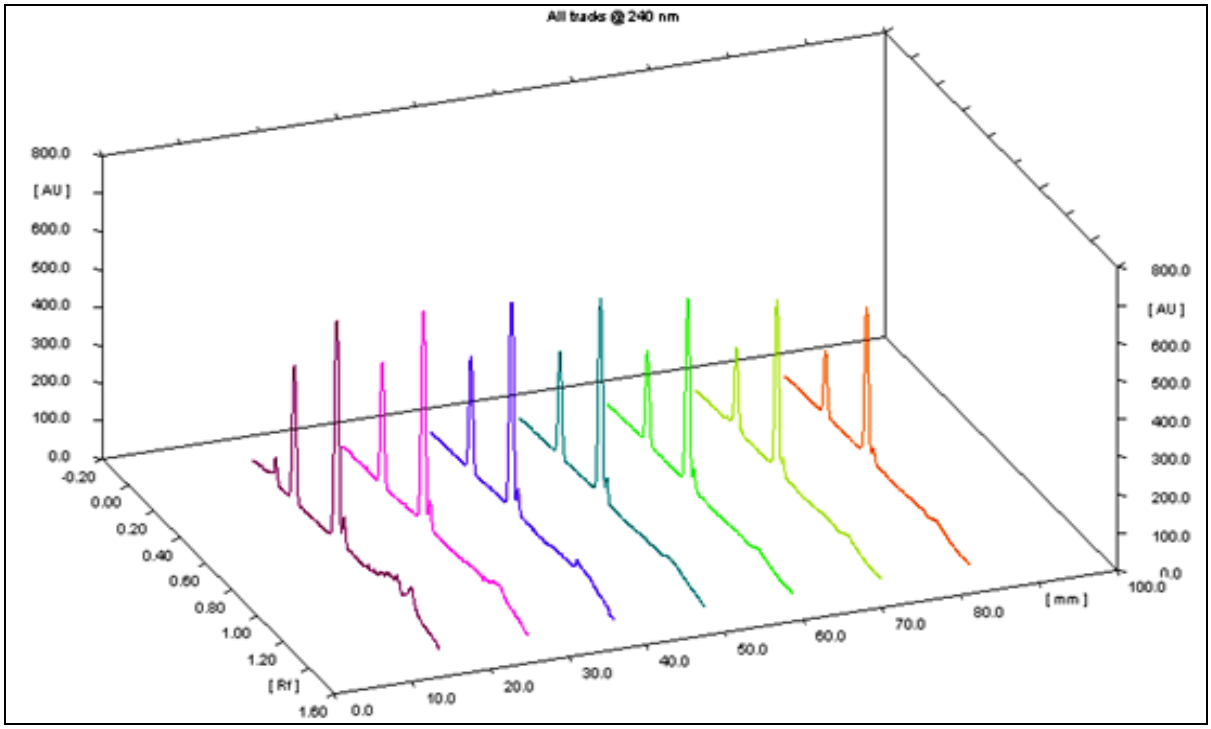



**FIGURE 1: 3D VIEW OF ALL TRACKS OF LINEARITY FOR ROSUVASTATIN CALCIUM AND ASPIRIN**

2. **Precision:** The precision expressed as standard deviation or relative standard deviation.

3. **Intraday precision:** Combined dosage form was analyzed at three levels of concentration of the assay for three times in a day. Peak Area of the solutions was measured. The % RSD for ROSU and ASP was found to be 0.882% and 0.659%, respectively.

4. **Interday precision:** Combined dosage form was analyzed at three levels of concentration of the assay for three consecutive days. Peak Area of the solutions was measured. The % RSD for ROSU and ASP was found to be 1.27% and 1.10%, respectively.

5. **Specificity:** Specificity is carried out by taking peak purity of standard and sample of each drug and standard and sample peak spectra were overlain to check specificity of each individual drug peak (**table 2**).

**TABLE 2: SPECIFICITY DATA**

| Drug | Co-relation r (s,m) | Co-relation r (m,e) | Peak purity |
|---|---|---|---|
| ROSU | 0.999 | 0.998 | Pass |
| ASP | 0.999 | 0.998 | Pass |

6. **Accuracy (Recovery study):** The accuracy of the method was established using recovery technique i.e external standard addition method. The known amount of standard was added at three different levels to preanalysed sample. Each determination was performed in triplicate. The result of recovery study is presented in **table 3**.

**TABLE 3: ACCURACY RESULTS**

| Assay level (n=3) | Capsule content taken equivalent to mgs | | Standard added mgs | | Total drug recovred mgs | | % Recovery of standard added | |
|---|---|---|---|---|---|---|---|---|
| | ROSU | ASP | ROSU | ASP | ROSU | ASP | ROSU | ASP |
| blank | 6 | 45 | 0 | 0 | 5.96 | 44.99 | 99.33 | 99.97 |
| 80% | 6 | 45 | 2 | 15 | 7.95 | 60.13 | 99.50 | 100.93 |
| 100% | 6 | 45 | 4 | 30 | 9.94 | 75.32 | 99.50 | 101.10 |
| 120% | 6 | 45 | 6 | 45 | 11.96 | 90.03 | 100.00 | 100.08 |
| | | | **Average** | | | | 99.88 | 100.04 |
| | | | **SD** | | | | 0.590 | 0.646 |
| | | | **% RSD** | | | | **0.591** | **0.646** |
| | | | **% SE** | | | | **±0.12** | **±0.04** |

**TABLE 4: ASSAY RESULT OF MARKETED FORMULATION BY HPTLC METHOD**

| Brand name | Formulation | ROSU Content (ng/spot) | ASP Content (ng/spot) | Assay (% lable claim) (n=3) | |
|---|---|---|---|---|---|
| UNISTAR* | Capsule | 100 | 750 | 100.39 | 99.71 |

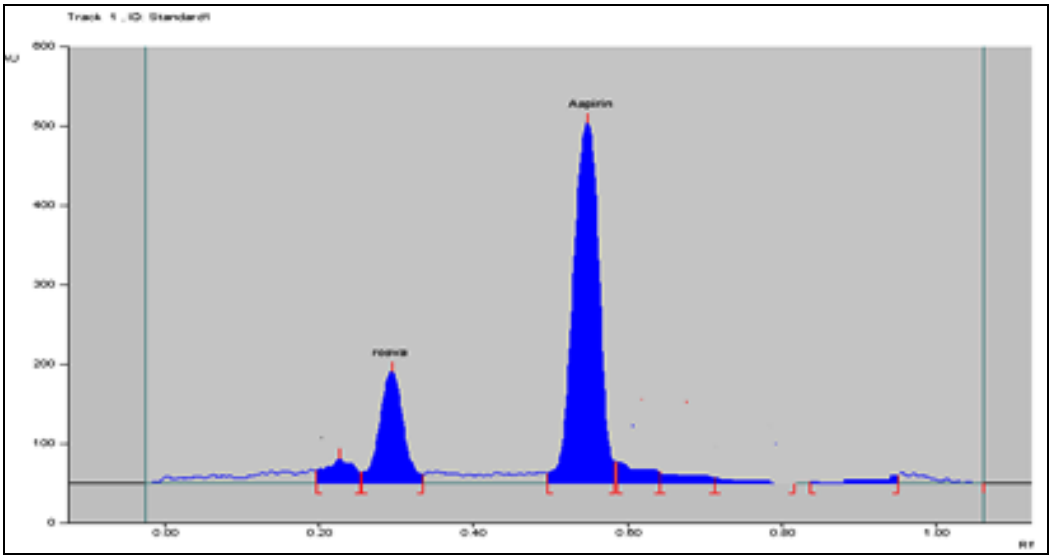



**FIGURE 2: HPTLC CHROMATOGRAM OF MARKETED FORMULATION**

**CONCLUSION:** The validated HPTLC method proved to be simple, less expensive, fast, accurate, and precise and thus can be used for routine analysis of Rosuvastatin Calcium and Aspirin in bulk and pharmaceutical dosage forms.

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