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Simultaneous determination of Aspirin and Rosuvastatin Calcium in capsules by using RP-HPLC coupled with photo diode array detection

Ankita Panchal, Gaurav Sanghvi, Ashish Vachhani, Navin Sheth, Devendra Vaishnav*

Department of Pharmaceutical Sciences, Saurashtra University,
Rajkot - 360 005, Gujarat, India
Telefax: +91 281 2585083

*E-mail address: devvaishnav@gmail.com

ABSTRACT

A simple, sensitive, specific, and cost effective method for simultaneous determination of Aspirin and Rosuvastatin calcium was developed and validated in single dosage formulation. The sample solution of ASP and RSTC was prepared using methanol as a solvent. Separation of ASP and RSTC was achieved with a mobile phase consisting of 20 mM KH_2PO_4 : Methanol (30:70 v/v) at a flow rate of 1.0 ml/min. Separations were performed on Merck hibar 250-4.6 RP18 (5 μm) column (150 mm X 3.0 mm), using a Shimadzu Prominence HPLC system equipped with a Shimadzu SPD-20A detector, Rhenodyne 7725i injector with 20 μL loop, LC-20 AD pump, CBM-20 Alite controller and LC Solution software. Retention times of ASP and RSTC were 3.747 and 5.969 minutes respectively. Absolute recovery of ASP and RSTC was 100.3 and 100.03 % respectively. The lower limit of quantification (LLOQ) of ASP and RSTC was 0.3097 and 0.1063 ppm and lower limit of detection (LLOD) of ASP and RSTC was 0.01535 and 0.01358 ppm respectively. Linearity was established for the range of concentrations 15.00-90.0 μg/ml and 2.0-12.0 μg/ml for ASP and RSTC respectively with the coefficient of determination (R^2) of 0.994 and 0.999 for both the compounds. The inter- and intra-day precision in the measurement of ASP quality control (QC) sample 75 μg/ml, were in the range 0.1-0.2 % relative standard deviation (R.S.D.) and 0.2-0.3 % R.S.D., respectively. The inter- and intra-day precision in the measurement of RST quality control (QC) sample 10 μg/ml, were in the range 0.1-0.2 % R.S.D., and 0.0-0.3 % R.S.D., respectively. The developed method would be applicable for routine quality control of ASP And RSTC in bulk as well as in pharmaceutical formulations.

***Keywords*:** Aspirin; Rosuvastatin calcium; RP-HPLC; Capsule; Validation

1. INTRODUCTION

Aspirin (ASP) 2-acetyloxybenzoic acid (Fig. 1a) is an analgesic and anti-platelet agent acting by irreversibly acetylating a serine residue in platelet prostaglandin G/H synthase, an

enzyme colloquially known as cyclooxygenase [1,2]. Rosuvastatin calcium (RSTC) (e)-(3r, 5s)-7-3, 5-dihydroxyhepten-6-oic acid calcium (Fig. 1b) is an anti-lipidemic agent act by inhibiting HMG-CoA reductase enzyme [3,4].

The physico-chemical properties of both ASP and RSTC are given in Table 1. The numerals of methods are published for the determination of ASP and RSTC alone or in combination with other drugs in bulk and dosage forms or in biological fluids, including UV Spectroscopy [5-7], Second Kind Electrode detection [8], HPLC [9-14], HPTLC [15-18], Ultra HPLC [19], LC-MS/MS [20-22] and HPLC/Q-TOF-MS [23]. High-performance liquid chromatography coupled with diode array detector (HPLC-DAD) has proved to be one of the most powerful tools for the analysis of drugs alone or in combination with other drugs [24].

However the comprehensive literature study revealed that none of the pharmacopoeias or any journals includes these drugs in combination for the simultaneous quantification of ASP and RSTC. With this regards, the present investigation was carried out to develop a reverse phase high performance liquid chromatography (RP-HPLC) procedure which will serve a reliable, accurate, sensitive and fast method for the simultaneous determination of ASP and RSTC.

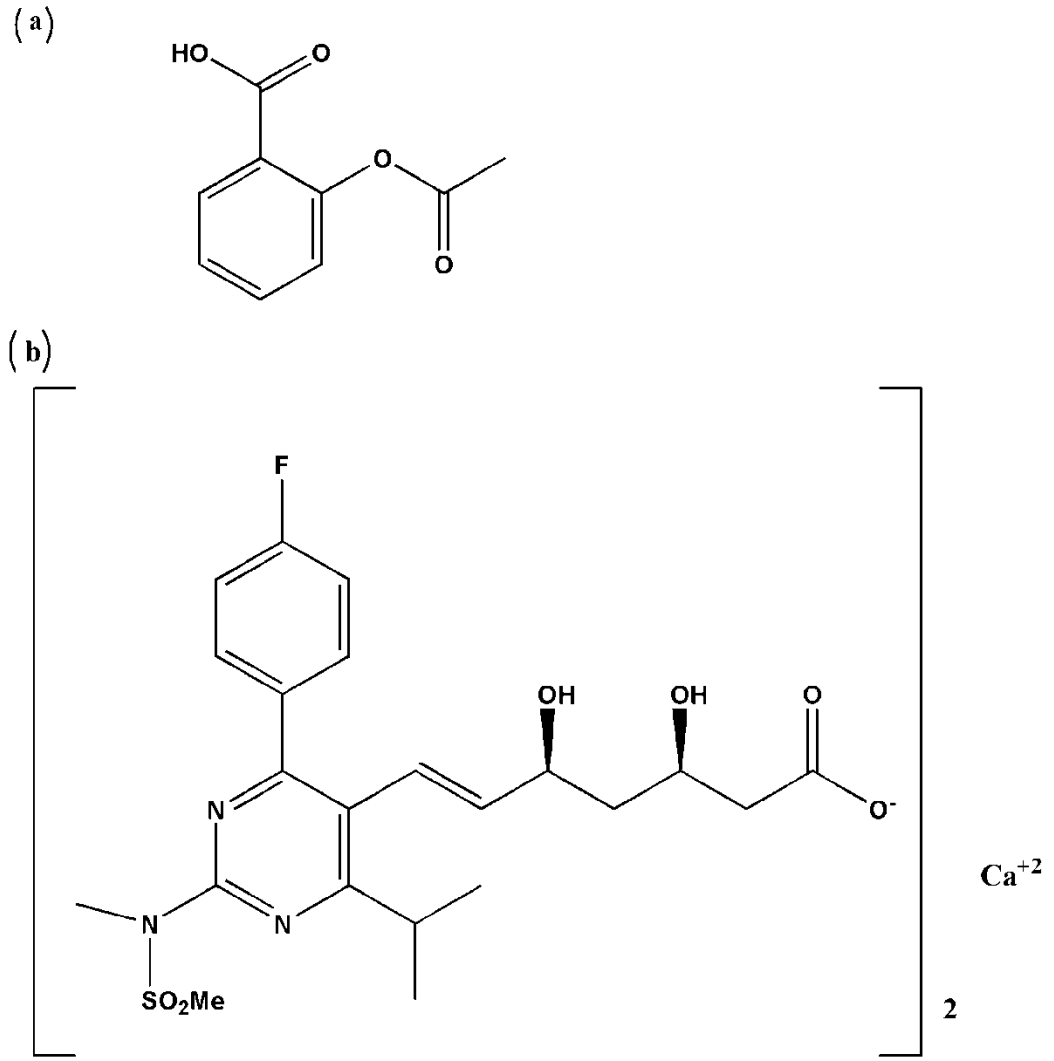

Figure 1. Chemical structure of a) Aspirin b) Rosuvastatin Calcium.

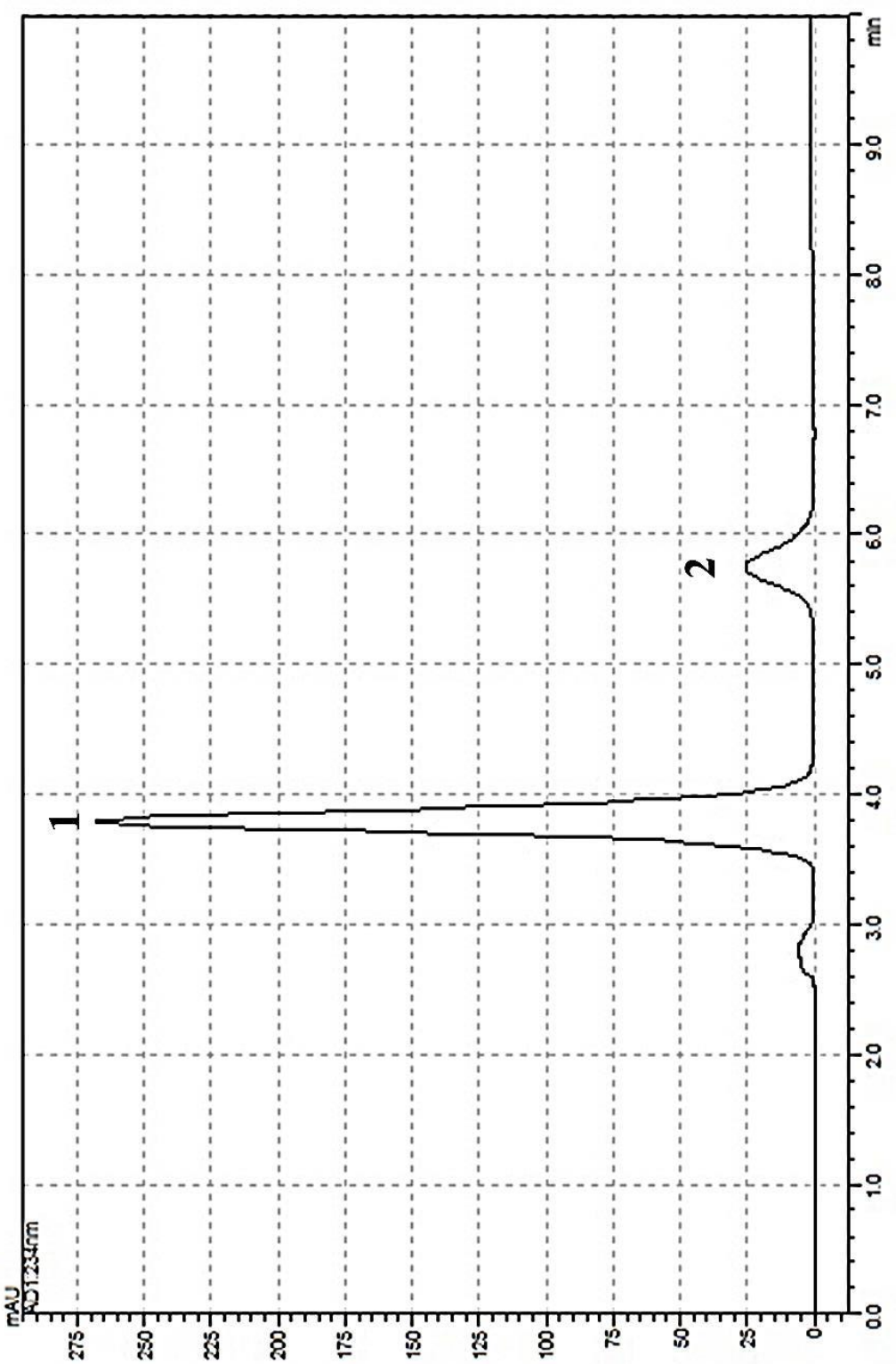

Figure 2. RP-LC chromatogram on Merck hibar 250-4.6 RP18 (5µm) column representing peaks of Aspirin (peak 1) and Rosuvastatin calcium (peak 2).

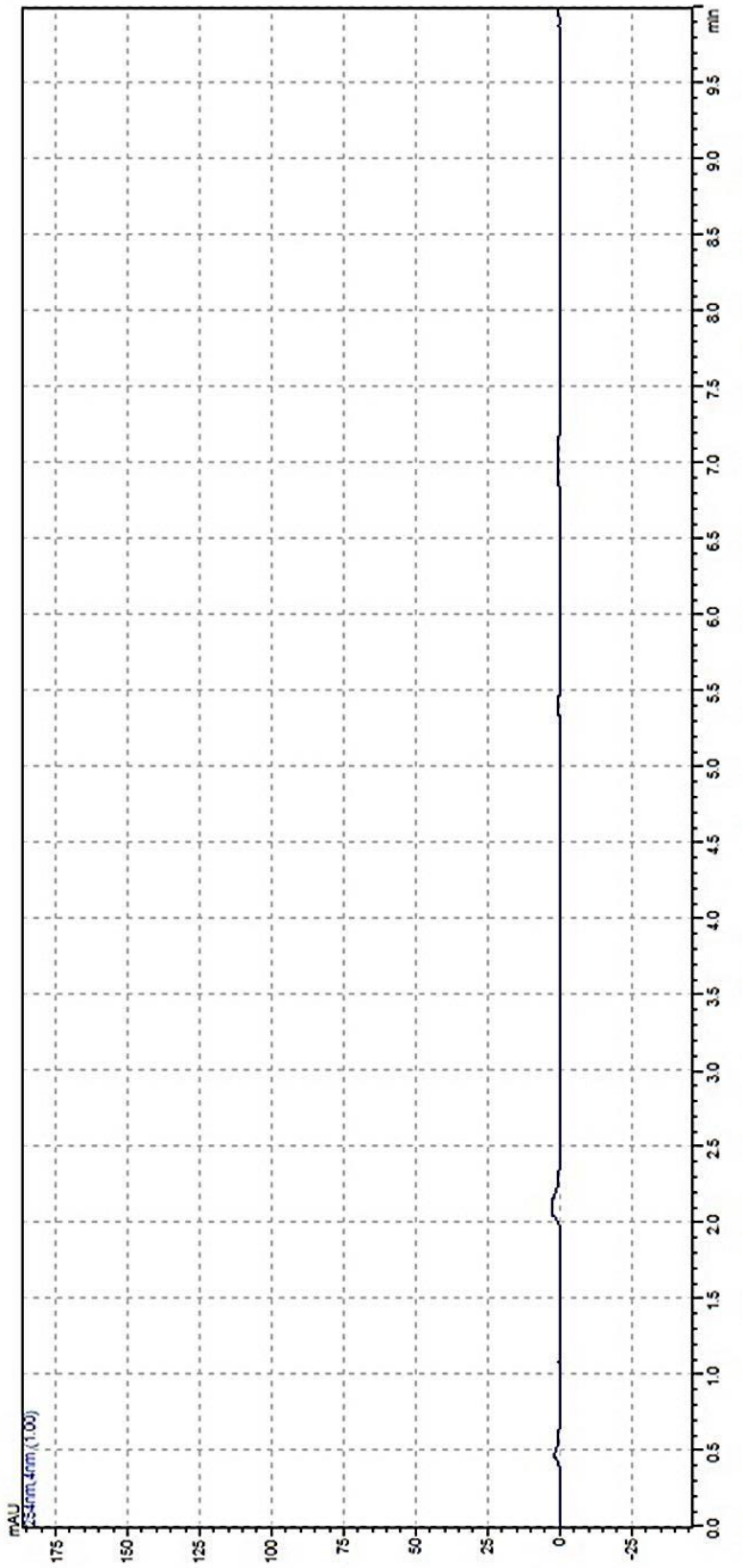

Figure 3. RP-LC chromatogram on Merck hibar 250-4.6 RP18 (5 μm) column representing blank.

Table 1. Summary of Validation parameters.

Parameters	Aspirin	Rosuvastatin calcium
Retention time (min)	3.747	5.969
Theoretical plates	2269.153	2071.9
Tailing factor	0.878	1.06
HETP	98.38	71.903
Linearity range (μg mL^{-1})	15 - 90 μg/ml	2 -12 μg/ml
Linearity Equation	y = 42923x + 69593	y = 46745x + 24019
Correlation coefficient (r)	0.9947	0.9994

2. MATERIALS AND METHODS

2. 1. Chemicals and Reagents

The reference standards of ASP and RSTC were gifted from Cadila Pharmaceuticals (Ahmedabad, India). Capsule (Unistar (10+75), Unichem Laboratories Ltd. India) containing ASP and RSTC of 75 mg and 10 mg respectively were purchased from local market. Water, methanol and ortho-phosphoric acid of HPLC grade were procured from Merck, India and Rankem, Ltd. India. The other chemicals used in experiments were of HPLC grade and purchased from local market.

2. 2. Instrumentation and Chromatographic Conditions

Analytical RP-HPLC separations were performed on Merck hibar 250-4.6 RP18 (5 μm) column (150 mm X 3.0 mm), using a Shimadzu Prominence HPLC system equipped with a Shimadzu SPD-20A detector, Rhenodyne 7725i injector with 20 μL loop, LC-20 AD pump, CBM-20 Alite controller and LC Solution software. Eluents A (20 mM KH_2PO_4) and B (Methanol) in the ratio of 30:70 were used as the mobile phases and flow rate was set at 1 mL/min.

2. 3. Preparation of stock and standard solutions

Primary stock solutions of ASP and RSTC for preparation of standard samples were prepared from separate weighing. The primary stock solutions of the analyte were prepared in methanol (1.0 mg/ml) and stored at -20 °C, which were found to be stable for one month. Appropriate dilutions were made in methanol for ASP and RSTC to produce working stock solutions (WSS) of 15, 30, 45, 60, 75, 90 μg/ml and 2, 4, 6, 8, 10, 12 μ/ml, respectively, on the day of analysis and these stocks were used to prepare calibration curve (CC).

2. 4. Sample preparation

Twenty capsules were weighed each containing 75 mg of Aspirin entric coated tablet and 10 mg of Rosuvastatin calcium granules, gelatin shell were removed and drug content was powered using mortar and pestle. An amount of pharmaceutical products powder

equivalent to 75 mg of ASP and 10 mg of RSTC were accurately weighed and transferred into a 100 ml volumetric flask and dilute with 50 ml of methanol. Solution was subjected to sonication for 15 minutes for complete extraction of drug and the solution was mark up with the methanol to get final concentration of 750 µg/ml of ASP and 100 µg/ml of RSTC respectively. Sample stock solution (SSS) was filtered through 0.45 µm PVDF filter paper (0.45 µm) before further dilution.

2. 5. Validation

Validation studies were performed using the optimized assay conditions following the principles of validation described in the ICH guideline [25]. Key analytical parameters, including, specificity, accuracy, precision, linearity, limit of detection (LOD) and limit of quantification (LOQ) were evaluated.

2. 5. 1. Selectivity/selectivity

The specificity/selectivity of the assay method was investigated by verifying the absolute separation and resolution of all the desired peaks of the analytes in mobile phase, and in mixture of excipients and standard. The interference of excipients with drug was measured by recording the retention time and % recovery.

2. 5. 2. Linearity and Range

For linearity study, six solutions at different concentrations (15, 30, 45, 60, 75, 90 µg/ml of Aspirin and 2, 4, 6, 8, 10, 12 µg/ml of Rosuvastatin calcium) were prepared using six different aliquots of WSS, and the obtained data were used for the linearity calibration plot. Lower limit of detection (LLOD) and lower limit of quantification (LLOQ) for the assay were also calculated using equation 1 and 2 and also determined experimentally by visual evaluation (Table 6).

$$\text{Lower Limit of Detection (LLOD)} = 3.3\times\sigma/s \quad \text{– Eq. 1}$$

$$\text{Lower Limit of Quantification (LLOQ)} = 10\times\sigma/s \quad \text{– Eq. 2}$$

2. 5. 3. Precision

As per the Complementary Guideline on Methodology (dated 6 November 1996 incorporated in November 2005) of ICH. Method precision was performed both in expressions of repeatability (injection and analysis) and intermediate precision (intra-day and inter-days reproducibility).

2. 5. 4. Repeatability

To determine the repeatability of assay method sample with 75 µg/ml of ASP and 10 µg/ml of RSTC were injected 6 times into HPLC system and repeatability of the retention time and peak area was determined and expressed as mean and % RSD calculated from the data obtained.

2. 5. 5. Intermediate precision

Intermediate precision (intra-day and inter-days reproducibility), were performed at three different concentration levels (45, 60, 75, µg/ml of Aspirin and 6, 8, 10, µg/ml of

Rosuvastatin calcium) were analyzed three times a day in triplicate injections over three consecutive days and expressed as mean ±SD and % RSD.

2. 5. 6. Accuracy

The study were performed in triplicates using a mixture of pure drug spiked with its formulation i. e. ASP (750 µg/ml) & RSTC (100 µg/ml) solution with three different concentrations of standards at 80 %, 100 % and 120 % (60, 75 and 90 µg/ml for ASP and 8, 10 and 12 µg/ml for RSTC respectively). Accuracy was determined in terms of percent recovery.

2. 5 .7. Robustness

The robustness of the developed assay method was studied by evaluating the manipulating small deliberate variations in procedure variables like column temperature (±1 °C), flow rate (±5 %) and pH of the mobile phase (±0.2 units).

3. RESULTS AND DISCUSSION

3. 1. Sample preparation

Numerous organic solvents were tried to prepare the stock solution of ASP and RSTC. Both ASP and RSTC were having the great solubility in methanol, thus selected as a solvent for this experiment. The corresponding working solutions of ASP and RSTC were prepared by diluting their stock solutions with methanol.

3. 2. Method development and optimization

Feasibility of different solvent system such as water-methanol, buffer-methanol, water-acetonitrile mixture in different strength, pH (2-8), and flow rate (0.8-1.2 ml/min) were experimented. Desired separations were achieved using 20 mM phosphate buffer – methanol in the ratio of 30:70 v/v (pH adjusted to 3 with ortho phosphoric acid) at a flow rate of 1 ml/min. While optimization of the ratio of different solvents in mobile phase, pH was fixed to 3.0 with ortho phosphoric acid, at a flow rate of 1 ml/min, the mobile phase composition found better resolution and separation in buffer (20 mM KH_2PO_4) – methanol (30:70 v/v). The pKa values of both RSTC and ASP were of 4.6 and 3.49 respectively. Resolution and retention of drug component depends upon the pH of the mobile phase, pH range from 2 to 8 were studied to optimize the mobile phase. Buffer : methanol ratio (30:70 v/v) and flow rate (1 ml/min.) were kept constant, pH 3 were found suitable for the current experimentation.

3. 3. Validation of the analytical method

The linear response of ASP and RSTC was determined by analyzing six independent levels of the calibration curve in the range of 15-90 µg/ml for ASP and 2-12 µg/ml for RSTC. The linearity equations and standard errors for the calibration curves of both ASP and RSTC are presented in Table 1. Average percent recoveries for ASP and RSTC were of 100.3 % and 100.03 %, respectively, while % RSD values for both ASP and RSTC were 0.607 and 0.485, which was less than 1 % and indicates accuracy of the reported method. Method specificity was verified using standard solutions of each drug alone, with excipients, and solvents shows that the resulting peaks on chromatograms at retention times of 3.747 and 5.969 min coincided with ASP and RSTC respectively with no interference by other substances.

Table 2. System precision and method precision.

System precision				Method precision			
Aspirin		Rosuvastatin Calcium		Aspirin		Rosuvastatin Calcium	
Injection no.	Area counts (lV s)	Injection no.	Area counts (lV s)	Injection no.	Assay (% claim)	Injection no.	Assay (% claim)
1	3532926	1	493145	1	80.808	1	9.996064
2	3537304	2	493299	2	81.078	2	10.10525
3	3533012	3	493012	3	80.985	3	10.15923
4	3530784	4	492812	4	80.688	4	10.09548
5	3536452	5	493437	5	81.022	5	10.07004
6	3529372	6	492948	6	80.781	6	10.04726
Mean	3533308.3	Mean	492812	Mean	80.688	Mean	9.996064
SD	3096.739	SD	231.7407	SD	0.155	SD	0.055431
% RSD	0.087	%RSD	0.047	%RSD	0.192	%RSD	0.554529

The average plate numbers over the concentration range were 2269.153 and 2071.9 for ASP and RSTC respectively. The chromatograms of blank and the mobile phase do not show any interference at the retention time of Aspirin and Rosuvastatin calcium as it can be seen from the respective chromatograms (Figure 2, 3).

System precision experiment was performed by preparing the standard solution of ASP (75 µg/ml) and RSTC (10 µg/ml) for six times and analyzed as per the ICH guideline (Table 2). Method precision experiment was performed by preparing the test solution of ASP (75 µg/ml) and RSTC (10 µg/ml) for six times from different capsule units and analyzed (Table 2). As per the ICH guideline, it expresses within laboratory variations as on different days analysis or equipment within the laboratory. The Intra-day precision was determined for standard solution of ASP (75 µg/ml) and RSTC (10 µg/ml) for different hours in same day, (like 0, 2, 4, 6, 8 and 10 Hours) and results were found 0.1 % and 0.2 % respectively, which were within the limit prescribed by ICH (Table 3). The Inter-day precision was determined for standard solution of ASP (75 µg/ml) and RSTC (10 µg/ml) for different days, (like 1, 2, 3, 4, 5 and 6 Days) (Table 3). The accuracy of the method was determined by recovery studies and the percentage recovery was calculated, overall mean percentage recovery was found 99.966 % and 99.347 % for ASP and RSTC respectively (Table 4). Minor deliberate changes in different experimental parameters such as flow rate (±5 %), pH (±0.2 units) and mobile phase ratio did not significantly affect area under the curve and retention time of both ASP and RSTC indicating that the proposed method is robust (Table 5). The LOD for ASP and RSTC standard solution were found to be 0.0445 µg/ml and 0.0046 µg/ml respectively, while LOQ were found to be 0.134697 µg/ml and 0.01386 µg/ml respectively (Table 6).

Table 3. Intra-day precision and Inter-day precision.

Intra-day precision				
Hours	Aspirin		Rosuvastatin Calcium	
	Concentration (ppm)	AUC	Concentration (ppm)	AUC
0	75	3532261	10	493285
2	75	3539762	10	493389
4	75	3531523	10	492912
6	75	3529431	10	492932
8	75	3537213	10	493743
10	75	3530785	10	493678
Mean		3529431	Mean	492912
STDEV		4058.831	STDEV	354.972
% RSD		0.115	% RSD	0.072

Table 3(continue). Intra-day precision and Inter-day precision.

Inter Day				
Day	Aspirin		Rosuvastatin Calcium	
	Concentration (ppm)	AUC	Concentration (ppm)	AUC
1	75	3572364	10	494326
2	75	3586704	10	495284
3	75	3579736	10	490926
4	75	3554987	10	490826
5	75	3563512	10	491295
6	75	3564987	10	492048
Mean		3554987	Mean	490826
STDEV		11585.7	STDEV	1897.71
% RSD		0.3259	% RSD	0.386636

Table 4. Accuracy Study data.

Drugs	Recovery levels	Amount added (mg)	Amount recovered (mg)	% Recovery	Mean % Recovery	SD	%RSD
Aspirin	Level 1	8	7.999	100	100	0.750555	0.751
		8	8	100			
		8	8.1	101.3			
	Level 2	10	9.998	100	100	0.57735	0.577
		10	10.1	101			
		10	10	100			
	Level 3	12	12	100	99.9	0.493288	0.494
		12	11.99	99.9			
		12	12.1	100.8			
	Overall				99.966	0.607	0.607
Rosuvastatin Calcium	Level 1	8	7.99	99.9	99.875	0.832	0.833
		8	8.00	100.0			
		8	8.11	101.4			
	Level 2	10	10.00	100.0	99	1	1.01
		10	9.90	99.0			
		10	10.10	101.0			
	Level 3	12	12.00	100.0	99.166	0.481	0.485
		12	11.90	99.166			
		12	12.00	100.0			
	Overall				99.347	0.481	0.485

Table 5. Robustness study data.

Robustness of Aspirin				Robustness of Rosuvastatin calcium			
Chromatographic changes							
Factor	Level	Mean AUC	% RSD	Factor	Level	Mean AUC	% RSD

Flow rate (ml/min)				Flow rate (ml/min)			
0.9	-0.1	3350549.7	0.1	0.9	-0.1	486929.7	0.6
1.0	0	3361486.7	0.1	1.0	0	496407.7	0.3
1.1	+0.1	3149075.0	0.4	1.1	+0.1	484091	0.6
Mobile phase ratio (Buffer: Methanol)				Mobile phase ratio (Buffer: Methanol)			
28:72	-2:2	3350549.7	0.1	28:72	-2:2	496929.7	0.3
30:70	0:0	3353153.3	0.1	30:70	0:0	497421.3	0.3
32:68	2:-2	3347486.7	0.1	32:68	2:-2	495387.7	0.2
pH				pH			
2.8	-0.2	3472598.7	0.3	2.8	-0.2	493054.3	0.5
3.0	0	3350549.7	0.1	3.0	0	496929.7	0.2
3.2	+0.2	3322326.7	0.4	3.2	+0.2	494721	0.2
Wavelength (nm)				Wavelength (nm)			
232	+2	3345820.0	0.1	232	+2	496487.3	0.2
234	0	3350549.7	0.1	234	0	496929.7	0.1
236	-2	3358686.7	0.2	236	-2	497411.6	0.3

Table 6. LOD and LOQ.

Drug	Based on visual evaluation		Based on the Standard Deviation of the Response and the Slope	
	LOD	LOQ	LOD	LOQ
Aspirin	1 ppm	2 ppm	0.01535	0.3097
Rosuvastatin	0.5 ppm	1 ppm	0.01358	0.1063

4. CONCLUSION

Proposed method is simple, rapid and cost effective. Method is validated as per the guideline of ICH and shows satisfactory results. Previously reported methods are either for alone ASP and RSTC for with other combination the present validated method allows scientist to perform simultaneous estimation of both ASP and RSTC at a time. Further this method can be employed for the human clinical pharmacokinetic study of the combination of ASP and RSTC in future. In summary, the described method provides high throughput for

simultaneous quantification of ASP and RSTC with outstanding accuracy, precision, selectivity and reproducibility.

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