Research Article

DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF ASPIRIN AND ROSUVASTATIN CALCIUM IN BULK DRUG AND PHARMACEUTICAL DOSAGE FORM BY AREA UNDER CURVE METHOD

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ABSTRACT

The work deals with the simultaneous estimation of aspirin and rosuvastatin calcium in bulk drug and pharmaceutical dosage form. A specific, rapid and simple UV spectrophotometric method with good sensitivity was developed and validated. Method was based on application of area under curve at selected wavelength range, 221-230 nm and 241-253 nm. Methanol was used as a solvent for this method. The calibration curve was linear for both the drugs in the range 5 - 20 µg/ml and 3.5 - 17.5 µg/ml for ASP and ROSU, respectively. The method was validated according to ICH guideline and recovery studies were carried out. Method was found to be accurate, precise and reproducible. This method was applied to the assay of the drugs in marketed formulation, which were found to be in the range of 101 to 102% of the labeled value for both ASP and ROSU. Hence, the method herein described can be successfully applied in quality control of combined pharmaceutical dosage forms.

Keywords: UV spectrophotometry, Aspirin (ASP), Rosuvastatin Calcium (ROSU), Area under curve method.

INTRODUCTION

Aspirin is also known as acetylsalicylic acid, and it is a salicylate drug, often used as an analgesic, antipyretic, anti-inflammatory and also has an antiplatelet effect by inhibiting the production of thromboxane, which under normal circumstances binds platelet molecule together to create a patch over damage of the walls within blood vessels. Chemically it is 2-acetoxybenzoic acid (Figure 1) and is a non-steroidal anti-inflammatory drug (NSAIDs) and shows inhibition of the enzyme cyclooxygenase and it is official in Indian Pharmacopoeia[4], The United States Pharmacopeia[5] and British Pharmacopoeia[6]. Rosuvastatin Calcium is official in indian pharmacopoeia[4]. It is chemically [(E)-(3R,5S)-7-{4 - (4-fluorophenyl) - 6 - isopropyl - 2 - {methyl (methylsulphonyl amino)] pyrimidin -5-yl}-3,5-dihydroxyhepten-6-oic acid calcium (Figure 2). It is used as a lipid lowering agent act by inhibition of 3-hydroxy-3-methylglutaryl-coenzymeA (HMG-CoA) reductase. Rosuvastatin is orally administered as calcium salt[1-3].

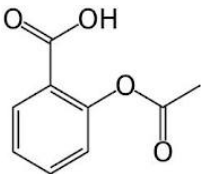

Figure 1: Chemical structure of Aspirin

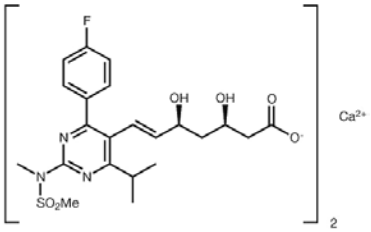

Figure 2: Chemical structure of Rosuvastatin Calcium

Literature survey revealed that there are various methods have been reported for estimation of ASP such as UV spectrophotometry, HPTLC, GC, Fluorimetry individually and in combined dosage form with other drugs[7-12]. For ROSU various analytical methods have been reported for its individual estimation and in combination include UV spectrophotometry, Stability indicating method, HPTLC and RP-HPLC methods[13-19]. Literature survey also reveals that there is no spectrophotometric method available for the determination of these analytes in combination; therefore the aim of the study was to develop simple, rapid, accurate, reproducible and economic First Order Derivative spectrophotometric method for simultaneous determination of ASP and ROSU from its formulation. The proposed methods were validated as per the International Conference on Harmonization (ICH) analytical method validation guidelines[20].

MATERIALS AND METHODS

Materials and instrument

An UV-Visible double beam spectrophotometer (hελios Alpha, Model - V 7.09) having two matched quartz cells with 10 mm light path. All weighing were done on electronic balance (Contech, Model-CA34). AR grade methanol was purchased from Chemdyes Corporation, Ahmedabad. Aspirin (% purity 99.96) reference standard was provided as gift sample by Allwyn Pharma Surgico, Baroda, Gujarat and Rosuvastatin Calcium (% purity 99.86) was provided as gift sample by Alembic Pharmaceutical Ltd., Baroda, Gujarat. The Pharmaceutical formulation of ASP and ROSU named as Unistar was procured from the market contain 75 mg of ASP and 10 mg of ROSU.

Preparation of standard stock solution and calibration curve

Standard stock solution of pure drug containing 1000 µg/ml of ASP and 1000 µg/ml of ROSU prepared separately in methanol. The working standard solutions of these drugs were obtained by dilution of the respective stock solution in methanol. Series of solution with conc. 5-20 µg/ml and 3.5-17.5 µg/ml of ASP and ROSU respectively were used for preparing calibration curve. Each solution was scanned between 200-400 nm against methanol as a reagent blank. The first order derivative spectra of each solution were obtained. The amplitude determine at respective wavelength.

Procedure for determination of wavelength for measurement

1 ml of working standard stock solution of ASP (100 µg/ml) and 1.0 ml of working standard stock solution of ROSU (100 mg/ml) were pipette out into two separate 10 ml volumetric flask and volume was adjusted to the mark with methanol to get a concentration of 10 µg/ml of ASP and 10 µg/ml of ROSU.

Each solution was scanned between 200-400 nm against methanol as a reagent blank. Area under curve of each solution was measured at 221 - 230 nm and 241 - 253 nm. The graph of area under curve verses respective concentration was plotted at selected wavelength.

Method

Area under curve method

The two-wavelength range selected should be such that there is negligible change in absorbance of drug. Within these wavelength ranges peak area of both drugs were measured and used in calculations. The area under curve of said concentrations for both the drugs were noted at selected analytical wavelength ranges. These area under curve were then divided by concentration in gm/lit to get $X1$ and $X2$ values

Following equations were used for calculations.

Determination of 'X' values

$$X = \frac{AUC\ of\ component\ between\ selected\ wavelength\ ranges}{Conc.\ of\ that\ component\ in\ g/l}$$

The concentration of drug was then calculated by using following equation,

Area under curve of each solution was measured at 221 – 230 nm and 241 – 253 nm.

The concentration of ASP and ROSU can be obtained as,

$$C_{ASP} = \frac{AUC_{241-253} \times X_{ROSU_{221-230}} - AUC_{221-230} \times X_{ROSU_{241-253}}}{X_{ASP_{241-253}} \times X_{ROSU_{221-230}} - X_{ASP_{221-230}} \times X_{ROSU_{241-253}}}$$

$$C_{ROSU} = \frac{AUC_{241-253} \times X_{ASP_{221-230}} - AUC_{221-230} \times X_{ASP_{241-253}}}{X_{ASP_{241-253}} \times X_{ROSU_{221-230}} - X_{ASP_{221-230}} \times X_{ROSU_{241-253}}}$$

$$X = \frac{AUC\ of\ component\ between\ selected\ wavelength\ ranges}{Concentration\ of\ that\ component\ in\ g/l}$$

$AUC_{241-253}$ = Area under curve of sample at 241 – 253 nm

$AUC_{221-230}$ = Area under curve of sample at 221 – 230 nm

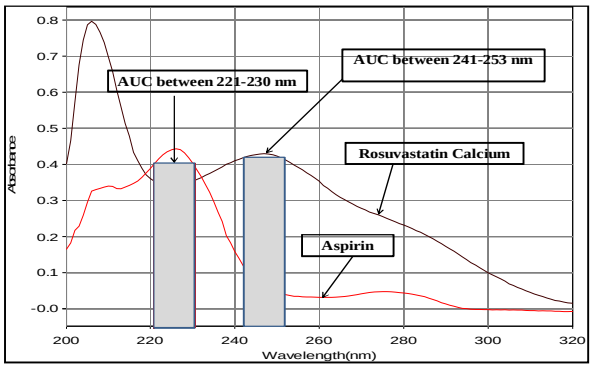

Figure 3: Overlay spectra of ASP and ROSU showing selection of wavelength for measurement of AUC

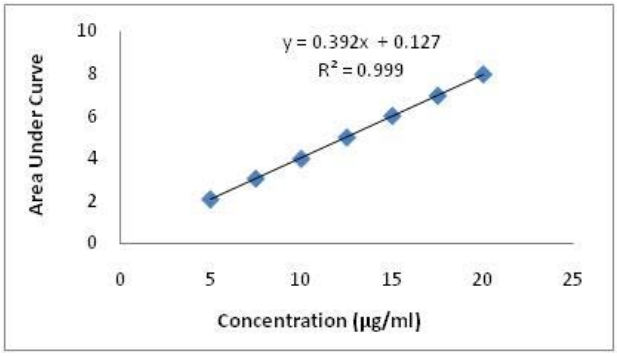

Figure 4: Calibration curve for ASP at 221 - 230 nm in methanol

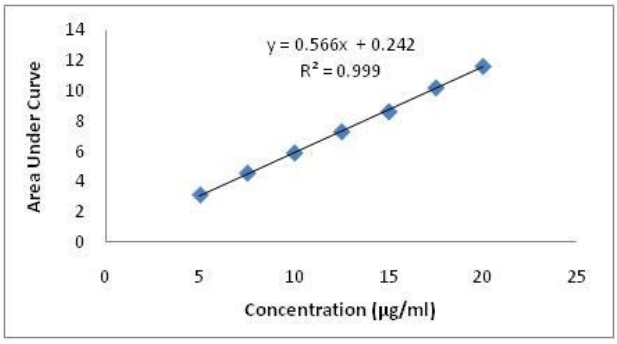

Figure 5: Calibration curve for ROSU at 241 - 253 nm in methanol

RESULTS AND DISCUSSION

Linearity

The linearity range for ASP & ROSU was found to be in the range of 5 – 20 µg/ml and 3.5 – 17.5 µg/ml, respectively. Correlation co-efficient for calibration curve of ASP and ROSU was found to be 0.999 and 0.999, respectively.

Accuracy

The accuracy of the method was performed by conducting the recovery studies (80, 100 and 120%) of pure drugs from marketed formulation, by standard addition method. The actual and measured concentrations were then compared.

Precision

The intraday precision of the developed method was evaluated by analyzing combined samples of different

concentrations of ASP and ROSU three times on the same day and %RSD was calculated. The inter day precision was evaluated from the combined concentration of ASP and ROSU on three different days and %RSD was calculated. The repeatability was evaluated by standard solutions of ASP (5-20 µg/ml) and ROSU (3.5-17.5 µg/ml) were prepared and analyzed three time on the same day.

LOD (Limit of Detection)

The LOD is estimated from the set of 5 calibration curves used to determine method linearity.

The LOD may be calculated as

LOD = 3.3 x (SD / Slope)

Where, SD = the standard deviation of Y- intercept of 5 calibration curves.

Slope = the mean slope of the 5 calibration curves.

LOQ (Limit of Quantification)

The LOQ is estimated from the set of 5 calibration curves used to determine method linearity.

The LOQ may be calculated as

LOD = 10 x (SD / Slope)

Where, SD = the standard deviation of Y- intercept of 5 calibration curves.

Slope = the mean slope of the 5 calibration curves.

Table 1: Repeatability data of ASP at 221 – 230 nm and ROSU at 241-253 nm

Conc. (µg/ml)	Area Under Curve Mean ± Std. Deviation (n=3) At 221-230 nm	% R.S.D	Conc. (µg/ml)	Area Under Curve Mean ± Std. Deviation (n=3) At 241-253 nm	% R.S.D
5	2.080 ± 0.01153	0.5544	3.5	2.232 ± 0.03253	1.4577
7.5	3.056 ± 0.009848	0.3222	5	3.088 ± 0.02621	0.8487
10	3.992 ± 0.007505	0.1879	7.5	4.524 ± 0.02451	0.5418
12.5	4.996 ± 0.003511	0.07028	10	5.667 ± 0.02753	0.4692
15	6.008 ± 0.0104	0.1732	12.5	7.276 ± 0.02516	0.3458
17.5	6.956 ± 0.01014	0.1459	15	8.600 ± 0.02350	0.2732
20	7.945 ± 0.01352	0.1700	17.5	10.200 ± 0.01650	0.1617

Table 2: Intraday precision data of ASP at 221 – 230 nm and ROSU at 241-253 nm

Conc. (µg/ml)	Area Under Curve Mean ± Std. Deviation (n=3) At 221-230 nm	% R.S.D	Conc. (µg/ml)	Area Under Curve Mean ± Std. Deviation (n=3) At 241-253 nm	% R.S.D
7.5	3.078 ± 0.02868	0.9320	7.5	4.536 ± 0.03157	0.6961
10	4.010 ± 0.02250	0.5610	10	5.882 ±0.035	0.5950
12.5	5.016 ± 0.0199	0.3982	12.5	7.289 ±0.03108	0.4264

Table 3: Interday precision data of ASP at 221 – 230 nm and ROSU at 241-253 nm

Conc. (µg/ml)	Area Under Curve Mean ± Std. Deviation (n=3) At 221-230 nm	% R.S.D	Conc. (µg/ml)	Area Under Curve Mean ± Std. Deviation (n=3) At 241-253 nm	% R.S.D
7.5	3.084 ± 0.03477	1.1274	7.5	4.544 ± 0.03837	0.8444
10	4.015 ± 0.02685	0.6688	10	5.890 ± 0.04252	0.7219
12.5	5.018 ± 0.02203	0.4389	12.5	7.295 ± 0.03723	0.7219

Table 4: Statistical Validation Data for Accuracy Study

Level of % Recovery	Mean (n=5) (% Recovery)		% RSD	
	ASP	ROSU	ASP	ROSU
80	101.11 ± 0.0222	101.68 ± 0.2262	0.2197	0.2225
100	101.56 ± 0.2787	10198 ± 0.01681	0.2745	0.1649
120	100.76 ± 0.1524	102.24 ± 0.09325	0.1513	0.09121

Table 5: Result of LOD and LOQ of ASP and ROSU

Parameter	ASP	ROSU
S.D.	0.019876	0.03623
Mean Slope	0.392	0.5617
LOD(µg/ml)	0.1521	0.193
LOQ(µg/ml)	0.5070	0.645

Table 6: Analysis of marketed formulation

Capsule	mg/capsule		Assay (% of label claim*)	
	ASP	ROSU	%ASP	%ROSU
Unistar	75	10	102.26	103.14

*Average of five estimations

CONCLUSION

The proposed method was found to be simple, sensitive, selective, accurate, precise and economical and can be used for determination of Aspirin and Rosuvastatin Calcium in bulk drug and pharmaceutical dosage form in a routine manner.

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