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Development and Validation of Uv Spectroscopic Method For Simultaneous Estimation of Dapagliflozin and Rosuvastatin in Synthetic Mixture

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ABSTRACT-
sensitive, precise, accurate and simple UV spectrophotometric methods have been developed for Analytical Method for Simultaneous Estimation of Dapagliflozin and Rosuvastatin In
Synthetic Mixture. Here, simultaneous equation method is used.The two wavelengths 224.60 nm (224.60for Dapagliflozin) and 244.20 nm (244.20 for Rosuvastatin) were selected for the formation of Simultaneous equations.Linearity was observed in the concentration range of 5-25 mcg/ml for Dapagliflozinand 5-25 mcg/ml for Rosuvastatin by this method.Regression equation forDapa and Rosu are given respectively y = 0.0343x + 0.065
y=0.0399x + 0.0122% RSD for precision, Interday,Intraday,Repetability found within the limit. Recovery study was performed to confirm the accuracy of the methods. The methods were validated as per ICH guidelines.
Keywords-Dapagliflozin, Rosuvastatin, Syntheticmixture, Vlidation, Simultaniious Estimtion.

I. INTRODUCTION-

The purpose ofAnalysis is to identify substances, purify them, separate them, quantify them, determine the molecular structures of chemical compounds that make up pharmaceuticals, and determine how these compounds are combined to make up a pharmaceutical product[1].It's Mainly done by Chemical analysis of drug molecules or agents and their metabolites.[2]

Dapagliflozin is mainly used to treat type 2 diabetes. It can also be used to treat heart failure. Dapagliflozin is especially wont to treat sort two polygenic disorder. It can even be wont to treat failure. Dapagliflozin was approved by EU in 2012 to treat DM-2 and vessel connected illness. Dapagliflozin was approved for medical use within the us in Jan 2014.By inhibiting SGLT2, dapagliflozin blocks organic process of filtered aldohexose within the urinary organ, increasing urinary aldohexose excretion and reducing blood sugar levels. Its mechanism of action is freelance of exocrine gland exocrine gland cell perform and modulation of hypoglycemic agent sensitivity.[3]dapagliflozin structure mainly contains C-glycosyl comprising beta-D-glucose in which the anomeric hydroxy group is replaced by a 4-chloro-3-(4-ethoxybenzyl) phenyl group.3

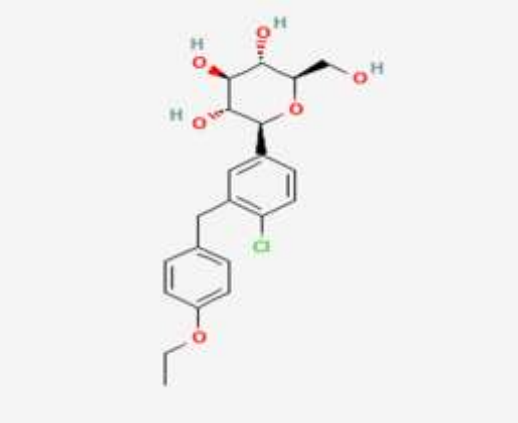

Fig 1 structure of Dapagliflozin

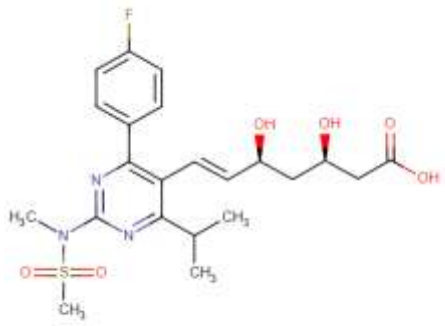

Fig 2 structure of Rosuvastatin

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Methodology-

SIMULTANEOUS EQUATION METHOD

Simultaneous equation method uses for the absorbance at two selected wavelengths, and both being the λmax of both drugs. To determine wavelength for measurement, standard spectra of Dapagliflozin and Rosuvastatinwas scanned between 200-800nm in Methanol. The method was based on the measurement of absorbance of Dapagliflozinat 244.20 nm and Rosuvastatinat 220nm and in both wavelengths.

EXPERIMENTAL WORK

Apparatus and Instrument

- A double beam UV/Visible spectrophotometer (Shimadzu UV-1700) with spectral width of 2 nm, 1 cm path length quartz cells was used to measure absorbance of all the solutions.
- Spectra obtain by UV-Probe 2.50 software.
- An Electronic analytical balance (Shimadzu AUW-220D) was used for weighing the samples.
- Sonicator (TRANS-O-SONIC)
- Volumetric flask (10,25,50,100ml) were used (Borosillicte)
- Pipettes-1,2,5,10ml (Borosillicate)
- All instruments and glass wares were calibrated.

Reagents and Material

- Rosuvastatin calciumAPI (Globela Pharma Pvt.Ltd)
- Dapagliflozin(Zydus Pharma Ltd)

Simultaneous Equation Conditions

- Mode : Spectrum
- Scan speed : Fast
- Wavelength range : 200-800 nm
- Derivative order : Zero
- Base line correction: Methanol

Solvent selection for UV-method:

- The solubility of DAP and ROS in methanol. For the UV method common solvent selected for both. So, methanol was selected as solvent for analysis of DAP and ROS.

Preparation of Standard Solutions

Standard Solutions of **Dapagliflozin(DAP)**

- **Preparation of stock solution of DAP (1000 µg/ml):** Accurately weighed quantity of DAPAGLIFLOZIN25 mg was transferred to 25 ml volumetric flask, add some methanol, and sonicate for 10min and diluted up to the mark with methanol to give a stock solution having strength of 1000µg/ml.
- **Preparation of stock solution of DAP (100 µg/ml):** Aliquot of 2.5 ml from above standard stock solution was pipette out into 25ml of volumetric flask and diluted up to the mark with methanol to give a stock solution having strength of 100µg/ml.

Standard solution of Rosuvastatin calcium (ROS)

- **Preparation of stock solution of ROS (1000 µg/ml):** Accurately weighed quantity of Rosuvastatin calcium 25mg was transferred to 25ml volumetric flask, dissolved, and diluted up to mark with methanol to give a stock solution having strength of 1000µg/ml.
- **Preparation of stock solution of ROS (100 µg/ml):** Aliquot of 2.5ml from above standard stock solution and transferred to 25ml of volumetric flask and diluted up to the mark with methanol to give a stock solution having strength of 100µg/ml.
- Preparation of standard mixture solution:From the above standard stock solution(100µg/ml) of DAP take 1.5ml and from stock solution of ROS take 1.5ml and transferred in to 10ml volumetric flask and diluted up to mark with methanol to give a solution having strength of DAP was 15µg/ml and ROS was 15µg/ml.
- **Preparation of test solution** Take synthetic mixture equivalent to 10mg DAP and 10mg ROS in 100ml volumetric flask and add methanol up to the mark give solution strength (100µg/ml of DAP and 100µg/ml of ROS) sonicate for 10min. Take 1ml from above solution and transferred in 10ml volumetric flask and make the volume up to mark with methanol give solution strength (10 µg/ml of DAP and 10 µg/ml of ROS).

Procedure for Determination of Wavelength for Measurement

1.5ml of stock solution of DAP (100µg/ml) and 1.5 ml of stock solution of ROS (100µg/ml) were pipette out into two separate 10 ml volumetric flasks and volume was adjusted to the mark with methanol to get 15 µg/ml of DAP and 15 µg/ml of ROS. Each solution was scanned between 200-800 nm against methanol as a blank reagent. The spectrum of each solution was obtained. The wavelength maximums were found to be 224.60nm for DAP and 244.20 nm for ROS respectively.

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VALIDATION OF METHOD:

Preparation for synthetic mixture:

Take synthetic mixture equivalent to 10mg DAP and 10mg ROS in 100ml volumetric flask and add methanol up to the mark give solution strength (100 µg/ml of DAP and 100 µg/ml of ROS) and sonicate for 10min. Take 1ml from above solution and transferred in 10ml volumetric flask and make the volume up to mark with methanol give solution strength (10 µg/ml of DAP and 10 µg/ml of ROS).

Table 2:Formulation of synthetic mixture:

Sr.no	Ingredient	Quantity (mg)	Role
1	Dapagliflozin (DAP)	100	Reducing blood glucose levels
2	Rosuvastatin calcium (ROS)	100	Lower "bad" cholesterol
3	Hydroxypropyl methylcellulose	300	Binder
4	Polly vinaylpyrrolidone	200	Diluent
5	Magnesium stearate	25	Lubricant
6	Talc	10	Glidant
7	Starch	1400	binder, diluent, and disintegrant.

II. RESULT AND DISCUSTION

The simultaneous equation method based on the absorbance of both drug DAP and ROS at their λmax.There are two wavelengths selected for development of simultaneous equation method λmax of the Dapagliflozin and Rosuvastatinat 224.60nm and 244.20 nm respectively in methanol. The wavelength maximums were found to be 224.60nm for DAP and 244.20 nm for ROS respectively.

Selection of wavelength for simultaneous Estimation of DAP and ROS:

To determine wavelength, standard spectra of Dapagliflozin and Rosuvastatinwere scanned between 200-800nm by using methanol. Absorbance maxima were obtained 224.60nm for DAP and 244.20 nm for ROS respectively.

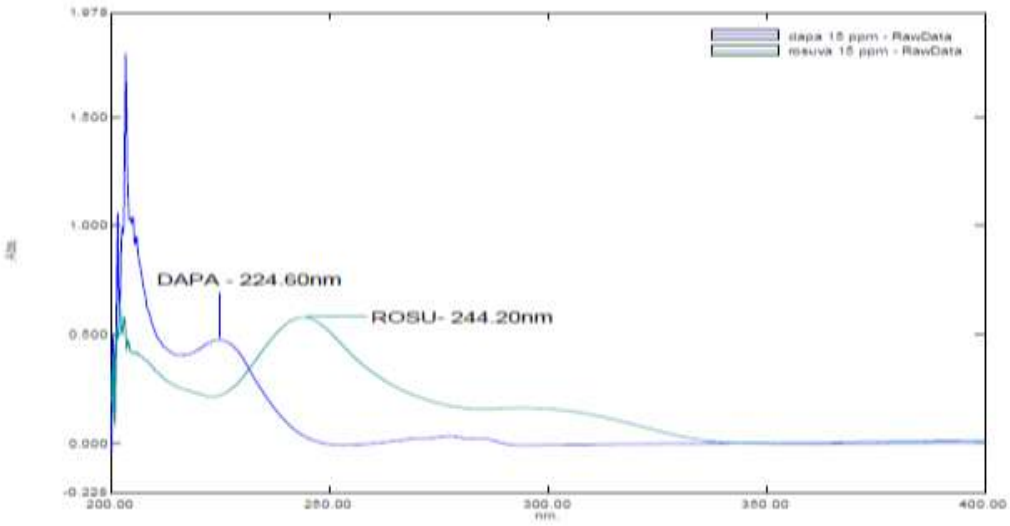

Figure 3 Overlay of 224.60nm for DAP and 244.20 nm for ROS

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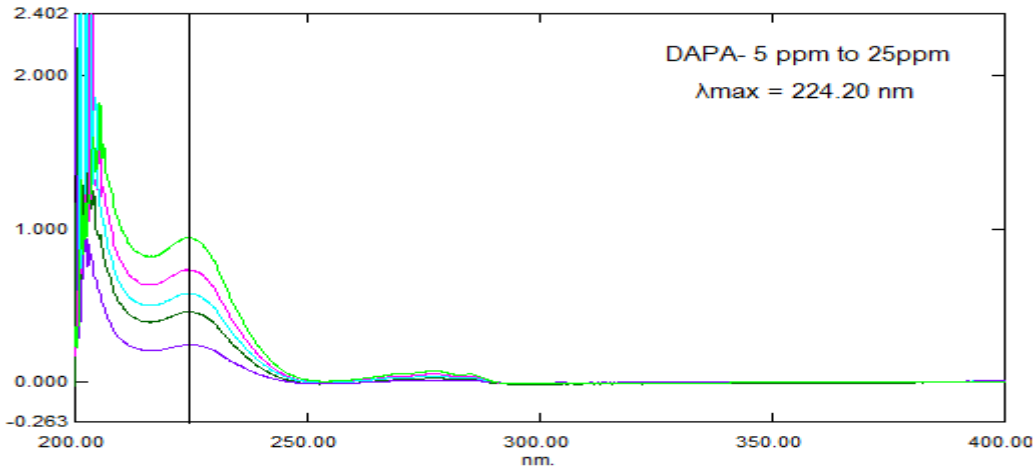

Fig 4 Overlay Spectra of DAP of different concentration (5-25µg/ml) at 224.20 nm

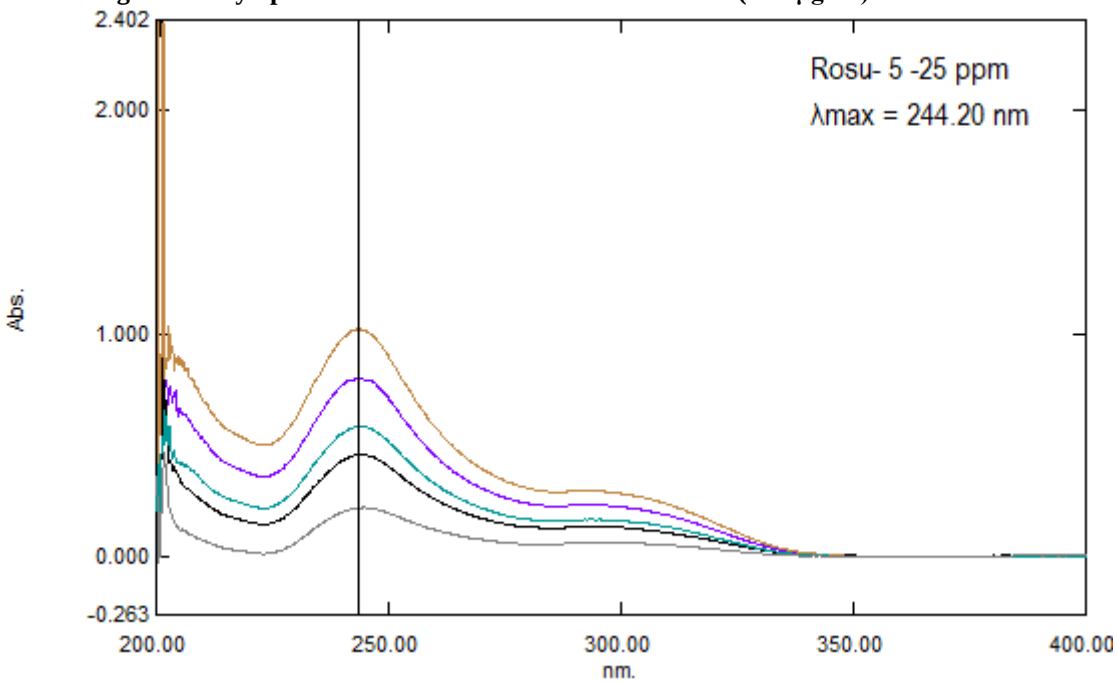

Fig. 5 Overlay Spectra of ROS of different concentration (5-25µg/ml) at 244.20 nm

VALIDATION PARAMETERS FOR THE DEVLOPED METHOD

1. Linearity and Range

The Different concentrations of DAP (5-25µg/ml) and ROS (5-25µg/ml) were prepared from respective stock solutions (100µg/ml). The absorbance was observed at 224.20 nm and 244.20nm. At the wavelengths 224.20 nm and 244.20nm good linearity was observed and hence these wavelengths were fixed for their simultaneous estimation.

The absorbance of DAP and ROS Measure at both wavelengths. The absorptivity were calculated for Dapagliflozin and Rosuvastatin at 224.60nm and 244.20 nm respectively in methanol.

The Correlation coefficient (r2) for calibration curve of DAP and ROS was found to be 0.999 and 0.999, respectively.

The regression line equation for DAPA and ROSU are as following,

y = 0.0343x + 0.0655 at 224.20nm for DAP_____ (1)

y = 0.0029x - 0.0082 at 245 nm for DAP _____ (2)

y = 0.0399x + 0.0122 for ROS at 245 nm _____ (3)

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y = 0.0234x - 0.1014 for ROS at 224.20 nm _____ (4)

Simultaneous equation

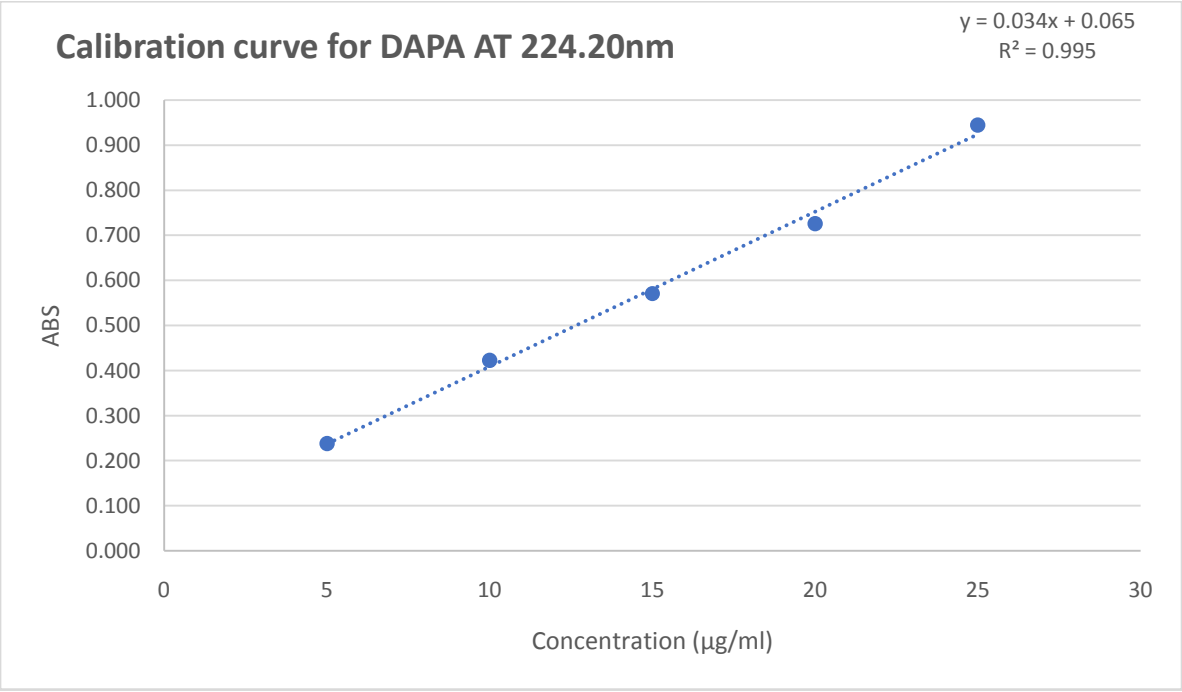
Cx = (A2*ay1 – A1*ay2 / ax2*ay1 – ax1*ay2)
Cy = (A1*ax2 – A2*ax1 / ay1*ax2 – ay2*ax1)
Where, Cx = Concentration of DAPA
Cy = Concentration of ROSU
A 1 = Absorbance of test at λ1 (λmax of DAP)
A2 = Absorbance of test at λ2 (λmax of ROS)
ax1 = Absorptivity of x drug (DAP) at λ1
ax2 = Absorptivity of x drug (DAP) at λ2
ay1 = Absorptivity of y drug (ROS) at λ1
ay2 = Absorptivity of y drug (ROS) at λ2

Table:3Linearity Dataof DAPA and ROSUat 224.60 nm and 244.20nm

DAP			ROS		
Conc. µg/ml	**Mean Abs.* At 224.20nm**	**Mean Abs.* At 245nm**	**Conc. µg/ml**	**Mean Abs.* At 224.20nm**	**Mean Abs.* At 245nm**
5	0.238 ±0.0045	0.007±0.0001	5	0.218±0.0045	0.0140±0.0003
10	0.423±0.0044	0.0204±0.0003	10	0.428±0.0079	0.140±0.0022
15	0.571±0.0077	0.0370±0.0001	15	0.579±0.0103	0.2445±0.0045
20	0.726±0.0115	0.215±0.0015	20	0.810±0.0078	0.3565±0.0053
25	0.945±0.0163	0.276±0.0025	25	1.021±0.0174	0.4907±0.0047

Table 4Absorptivity at 224.60 nm and 244.20nm

At 224.20 nm		At 244.60 nm	
ax1	0.0343	**ax2**	0.00290
ay1	0.0234	**ay2**	0.0399



Calibration curve for DAPA AT 224.20nm

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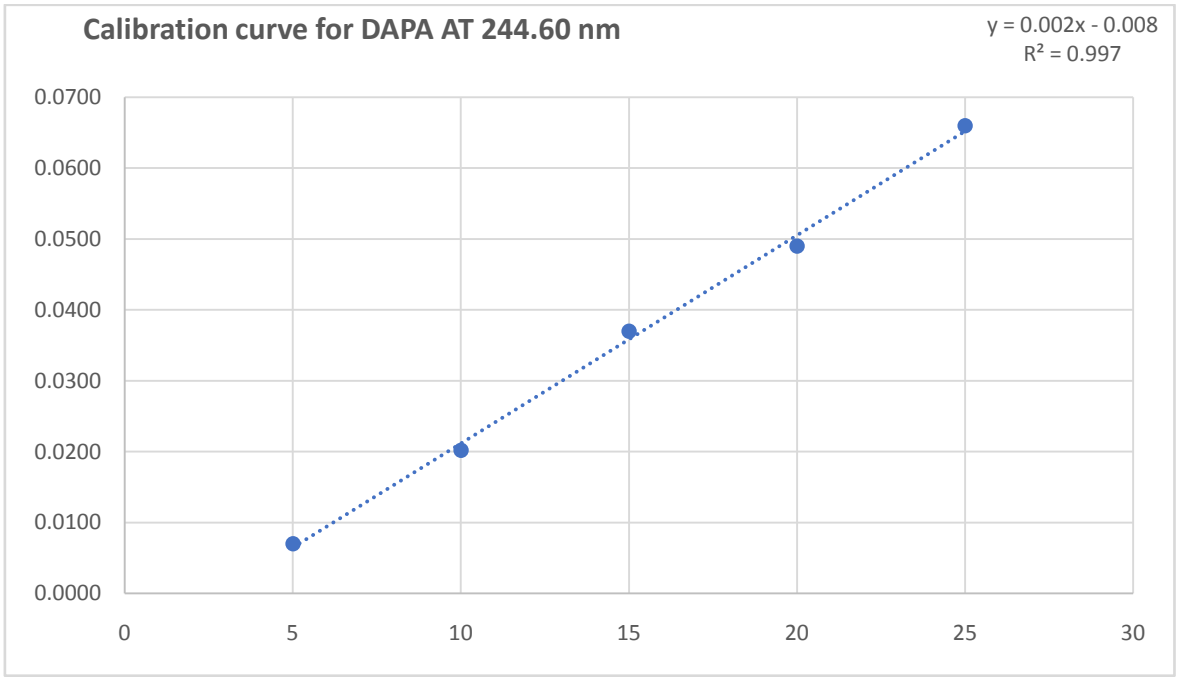
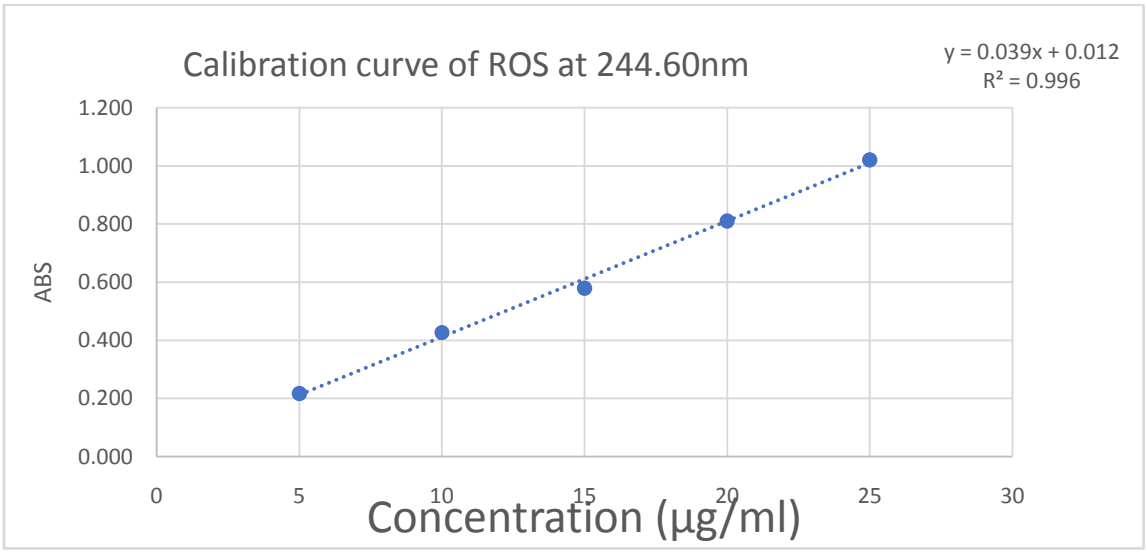


Calibration curve for DAPA AT 244.60 nm



Calibration curve for ROSU at 244.60 nm

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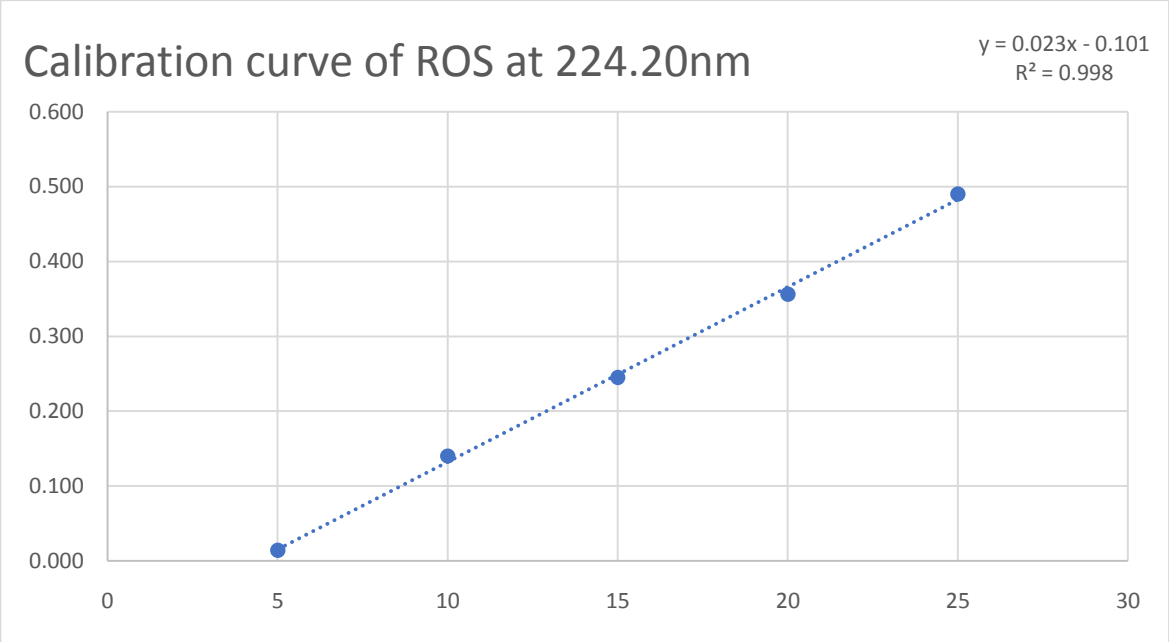

Calibration curve for ROSU AT 224.20 nm

2. Precision

i. Repeatability

The concentration of solutions 15µg/ml of DAPA and 15µg/ml of ROS solution was analyzed at 224.60nm and 244.20nm for seven time (n=7) and % R.S.D was calculated. These value was found to be less than ±2.0 indicated that the method is precise. (Table6.6)

Table 6.6: Repeatability data for estimation of DAPA and ROS (n=7)

Sr.no	**Mean Abs.**	**Mean Abs.**
	DAPA	ROSU
1	0.567	0.575
2	0.565	0.572
3	0.561	0.569
4	0.574	0.589
5	0.578	0.567
6	0.581	0.585
7	0.569	0.589
Average	0.571	0.578
Std. Dev.	0.0072	0.0095
RSD	1.27	1.63

ii. Intraday precision

The concentration of Solutions 5, 15, 25 µg/ml for DAPA and 5, 15, 25 µg/ml for ROS respectively series were analyzed three times on the same day using developed spectroscopic method and %RSD was calculated. The % RSD was found to be 1.17-1.75% for DAPA and 1.12- 1.49% for ROSU. These %RSD value was found to be less than ±2.0 indicated that the method is precise. (Table 6.7)

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Table 5: Intraday precision data for estimation of DAPA and ROSU (n=3)

Conc. (µg/ml)	Mean Abs. ±SD	% RSD	Conc. (µg/ml)	Mean Abs. ±SD	% RSD
DAPA			**ROSU**		
5	0.2383 ± 0.0042	1.75	5	0.2173 ± 0.0025	1.16
15	0.5667 ± 0.0067	1.17	15	0.5717 ± 0.0064	1.12
25	0.9527 ± 0.0129	1.35	25	1.0180 ± 0.0151	1.49

iii. Interday precision

The concentration of solutions 5, 15, 25 µg/ml for DAPA and 5, 15, 25 µg/ml for ROS respectively series were analyzed three times on the different day using developed spectroscopic method and %RSD was calculated. The % RSD was found to 1.17-1.75% for DAPA and 1.12-1.49% for ROSU. These %RSD value was found to be less than ±2.0 indicated that the method is precise.(Table 6.8)

Table 6.7: Interlay precision data for estimation of DAPA and ROSU(n=3)

Conc. (µg/ml)	Mean Abs. ±SD	% RSD	Conc. (µg/ml)	Mean Abs. ±SD	% RSD
DAPA			**ROSU**		
5	0.2377 ± 0.0046	1.94	5	0.2167 ± 0.0038	1.75
15	0.5760 ± 0.0062	1.08	15	0.5817 ± 0.0095	1.62
25	0.9463 ± 0.0132	1.40	25	1.0273 ± 0.0187	1.82

3. Accuracy

The developed UV spectroscopic method was checked for the accuracy. It was determined by calculating the recovery of DAPA and ROSU. The spiking was done at three levels 50 %, 100 % and 150 %.

Percentage recovery for DAPA and ROSUby this method was found in the range of 100.51 to 100.88% and 99.97 to 101.33%, respectively.

Table 6: Recovery data of DAPA (n=3)

Level	Conc. of DAPA from Synthetic mixture (µg/ml)	Amount of Std. DAPA added (µg/ml)	Total amount of DAPA (µg/ml)	Total amount of DAPA Recovered (µg/ml) Mean ± SD	% Recovery
0%	10	0	10	10.17 ± 0.029	101.75 %
50%	10	5	15	**14.95 ± 0.10**	99.64 %
100%	10	10	20	19.56 ± 0.09	97.81%
150%	10	15	25	25.46 ± 0.10	101.85%

Table 7: Recovery data of ROSU *(n=3)

Level	Conc. of ROSU from Synthetic mixture (µg/ml)	Amount of Std. ROSU added (µg/ml)	Total amount of ROSU (µg/ml)	Total amount of ROSU Recovered (µg/ml) Mean ± SD	% Recovery
0%	10	0	10	10.23 ±0.11	102.29
50%	10	5	15	14.72 ±0.05	98.16
100%	10	10	20	20.02 ±0.21	100.10
150%	10	15	25	25.44 ±0.47	101.77

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4. Limit of Detection and Quantitation

The LOD for DAPA and ROSU was obtained 0.0501µg/ml and 0.0334µg/ml, respectively. The LOQ for DAPA and ROSU was obtained 0.152µg/ml and 0.1014µg/ml, respectively. The obtained LOD and LOQ results are presented in Table 6.11.

Table 8: LOD and LOQ data of DAPA and ROSU (n=5)

	DAPA (µg/ml)	ROSU(µg/ml)
LOD	0.89	0.38
LOQ	2.71	1.17

Application of the proposed method for analysis of DAPA and ROSU in synthetic mixture.

A simultaneous equation was usedfor estimation of DAPA and ROSU. The test solution was recorded and measures the absorbance at 224.20 nm and 244.60nm for estimation of DAPA and ROSU. The concentrations of DAPA and ROSU in synthetic mixture were determined using the simultaneous equation. The % assay values are given in Table 6.12

Table 9: Analysis data of synthetic formulation (n=3)

Sr. No	Drug	Mean Concentration (µg/ml)	% Assay ± SD
1	DAPA	15.27	101.80 ±0.44
2	ROSU	15.22	101.44 ±0.55

Table 10: Summary of validation parameters

PARAMETERS	DAPA	ROSU
Concentration range(µg/ml)	5-25	5-25
Wavelength(nm)	224.20	244.60
Regression equation	y = 0.0343x + 0.065	y=0.0399x + 0.0122
Correlation Coefficient(r2)	0.9952	0.9966
Accuracy(%Recovery) (n=3)	100.51-100.88	98.16 -102.29
Repeatability (%RSD) (n=7)	1.27	1.63
Intra-day Precision (%RSD) (n=3)	1.17 -1.75	1.12 -1.49
Inter-day precision (%RSD) (n=3)	1.08 -1.94	1.62 -1.82
LOQ(µg/ml)	0.89	0.38
LOD(µg/ml)	2.71	1.17

III. CONCLUSION

The proposed methods for simultaneous estimation of and ROSU in Synthetic mixture dosage form were found to be accurate, simple and rapid which can be well understood from validation data. The % R.S.D. was found to be less than 2, which indicates the validity of method. Linearity was observed by linear regression equation method for DAPA and ROSU in different concentration range. The Correlation coefficient of these drugs was found to be close to 1.00, indicating good linearity. The assay results obtained by proposed methods are in fair agreement, hence it can be used for routine analysis of two drugs in synthetic mixture. There was no interference from tablet excipients was observed in these methods. It can be easily and conveniently adopted for routine quality control analysis. accurate, simple, rapid, precise, reliable, sensitive, reproducible and economic and are validated as per ICH guidelines.

A simple UV spectrophotometric method was developed for the simultaneous determination of DAPA and ROSU in tablet formulation without any interference from the excipients. To the best of our knowledge, the present study is the first report for the purpose. The present methods succeeded in adopting a simple sample preparation that achieved satisfactory extraction recovery and facilitated its application in formulated formulation. The results of our study indicate that the proposed UV spectroscopic methods are simple, rapid, precise and accurate.Statistical analysis proves that, these methods are repeatable and selective for the analysis of PARA and CAF. It can therefore be

concluded that use of these methods can save much time and money and it can be used in small laboratories with accuracy.

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