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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF DAPAGLIFLOZIN AND ROSUVASTATIN IN SYNTHETIC MIXTURE

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ABSTRACT: In the present study, a specific, precise, accurate and robust high-performance liquid chromatographic method for analysis of Dapagliflozin (DAPA) and Rosuvastatin (ROS) was developed and validated according to ICH guidelines. For UV wavelength for dapa and rosu used 224.20,244.60 respectively. Correlation Coefficient(r2) 0.99520.9966 Found within the limit. The HPLC column C18 5µm, 250 × 4.6 mm, was used for developed method and mobile phase Acetonitile: Methanol: phosphate buffer - adjust pH 3.5 with ortho phosphoric acid (30:35:35) and flow rate 1ml/min. Detection Wavelength is 244nm.Linearity range used 5-25 µg/ml for both Drugs. %recovery found 98.85-102.62%,99.06 % - 101.58 % For DAPAGLIFLOZIN, ROSUVASTATIN Respectively. Repeatability, Intraday precision (RSD) Interday precision (RSD), Robustness Found within the limit. LOD limits found 0.41 µg/ml,0.22 µg/ml Respectively for dapa, rosu. LOQ limit found 1.25 µg/ml,0.66 µg/ml for dapa and rosu respectively.

[**KEYWORDS:**DAPAGLIFLOZIN, ROSUVASTATIN, SYNTHETICMIXTURE, VLIDATION, SIMULTANIIOUS ESTIMTION.]

INTRODUCTION-

The purpose of Analysis 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. High Performance Liquid Chromatography is the most common analytical separation tool, components by distributing between mobile phase and stationary phase.

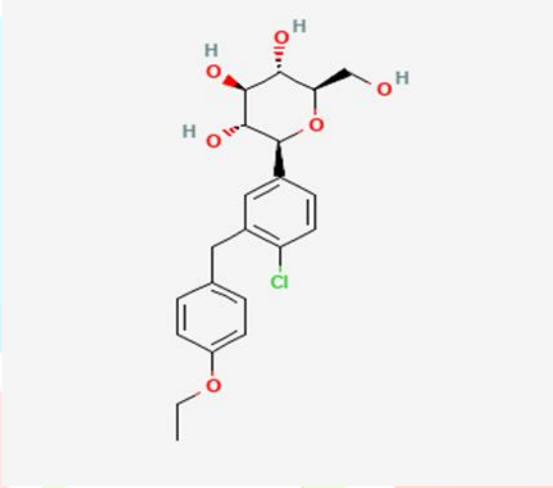

FIG 1. STRUCTURE OF DAPAGLIFLOZIN

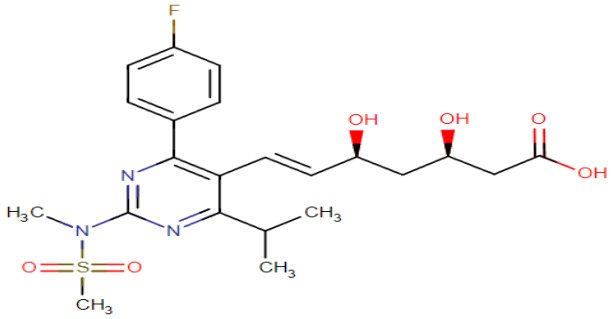

FIG 2. STRUCTURE OF ROSUVASTATIN

METHODOLOGY:

Establishment of Optimum Condition for HPLC Method development.Various conditions used during the development of analytical methods should be optimizing for developing sensitive, accurate and reproducible method.

EXPERIMENTAL WORK:

APPARATUS AND INSTRUMENT: HPLC Shimadzu P series integrated HPLC was equipped with a quaternary gradient unit, a LC-20 AD solvent delivery unit, DGU-20AR degassing unit, detector, a CTO-10ASVP column oven, SPD-M40 PDA detector and a SIL-20AC programmable auto sampler controlled by LAB SOLUTION software. The Shimpack ODS C18 column 25 cm (4.6 mm x 250mm, 5 um) was used as a stationary phase. For filtration of solution, a Nylon-66 membrane filter was used.

MATERIALS AND REAGENTS:

- Rosuvastatin calcium API (Globela Pharma Pvt.Ltd).
- Dapagliflozin (Zydus Pharma Ltd).

SELECTION OF DILUENT:

- Based on solubility, Dapagliflozin (DAPA) and Rosuvastatin (ROS) was soluble in water. Hence, methanol was selected as diluent.

PREPARATION OF STANDARD SOLUTIONS:

STANDARD SOLUTIONS OF DAPAGLIFLOZIN (DAP)

- **Preparation of stock solution of DAP (1000 µg/ml):** Accurately weighed quantity of DAPAGLIFLOZIN 25 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 (100 µg/ml of DAP and 100 µg/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).

SELECTION OF MOBILE PHASE:

The water, buffer, pH of the buffer, organic solvent, and buffer-to-solvent ratio were all factors in the mobile phase selection process. The HPLC technique selection is influenced by the sample's nature, physicochemical properties, molecular weight, and solubility. pH management necessitates the use of a buffer. The pH of the acidic component is kept low, while the pH of the base is kept high. Separation, peak purity, tailing factor, theoretical plate, and other parameters were used to optimize the mobile phase for HPLC system. Various mobile phases in various compositions and pH levels were tried to achieve a sharp peak of Dapagliflozin (DAPA) and Rosuvastatin (ROS).

SELECTION OF WAVELENGTH:

An ideal wavelength is the one that gives Maximum response for the drugs that was to be detected. For selection of wavelength U.V spectrophotometer is used or using HPLC assisted with UV detector, UV overlay spectra Dapagliflozin (DAPA) and Rosuvastatin (ROS) were obtained. For High Performance Liquid Chromatography 244 nm was selected wavelength where both drugs show good absorbance.Preparation of Buffer: Dissolve 3.40 g of potassium dihydrogen phosphate R inn900 ml of water R. Adjust to pH 3.5 with phosphoric acid R and dilute to 1000.0 ml with water R. Mix well and sonicate. Filter through 0.45 µm membrane filter paper.

SELECTION OF COLUMN:

Dapagliflozin (DAPA) and Rosuvastatin (ROS) are polar in nature. So, C18 analytical column were selected for HPLC method. The column was used Shimpack ODS C18 column (250 mm × 4.6 mm, 5 µm) was used for the development of the method.

VALIDATION OF METHOD:

Optimization Of Mobile Phase Composition

Based on review of literature, several mobile phases were selected on the basis of solubility of drug in the solvents. Various solvents and mixtures of solvents was tried using methanol, acetonitrile, HPLC grade water and phosphate buffer of different pH and their combinations. And the best result was obtained by using Acetonitrile: Methanol: phosphate buffer - adjust pH 3.5 with ortho phosphoric acid in the volume ratio 30:35:35 v/v/v (Trial 7) having good peak shape and resolution of greater than 2 as well as theoretical plate more than 2000. Flow rate was 1 ml/min monitor at 223nm. Stationary phase was Shimpack ODS C18 column 25 cm (4.6 mm x 250mm, 5 um) and Injection Volume was 10 µl. Retention time of Dapagliflozin (DAPA) 7.510 min and Rosuvastatin (ROS) 8.799 were obtained.

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 1: 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 vinayl pyrrolidone	200	Diluent
5	Magnesium stearate	25	Lubricant
6	Talc	10	Glidant
7	Starch	1400	binder, diluent, and disintegrant.

RESULTS AND DISCUSSION:

SYSTEM SUITABILITY TEST: -

- The system suitability parameters were calculated and all system suitability parameter are within the acceptable range.

Table 2. System Suitability Parameter

Parameter	Dapagliflozin (DAPA)	Rosuvastatin (ROS)
Retention Time(min)	7.510 min	8.860 min
Resolution	0.00	3.087
Theoretical plate	35901	38664
Symmetric Factor	1.21	1.19
Peak Area	680817	487937

METHOD VALIDATION

Linearity

Linear responses were obtained in concentration range of 5 – 25 ppm (5, 10, 15, 20, 25) for DAPA and 5 – 25 ppm (5, 10, 15, 20, 25) for ROS. The data for linearity has shown in table 6.8 for Dapagliflozin (DAPA) and Rosuvastatin (ROS). The calibration curve for Dapagliflozin (DAPA) and Rosuvastatin (ROS) was given in fig no.4 and fig no.5.

Table 3. Linearity Data of Dapagliflozin (DAPA) and Rosuvastatin (ROS)

Dapagliflozin (DAPA)			Rosuvastatin (ROS)		
Conc.	Peak Area	RSD	Conc.	Peak Area	RSD
5	136587	0.79	5	97114.50	0.67
10	275045	1.12	10	193252.17	1.27
15	419097	1.81	15	291424	1.35
20	542100	0.65	20	392028	1.21
25	684544	0.85	25	486768	0.61

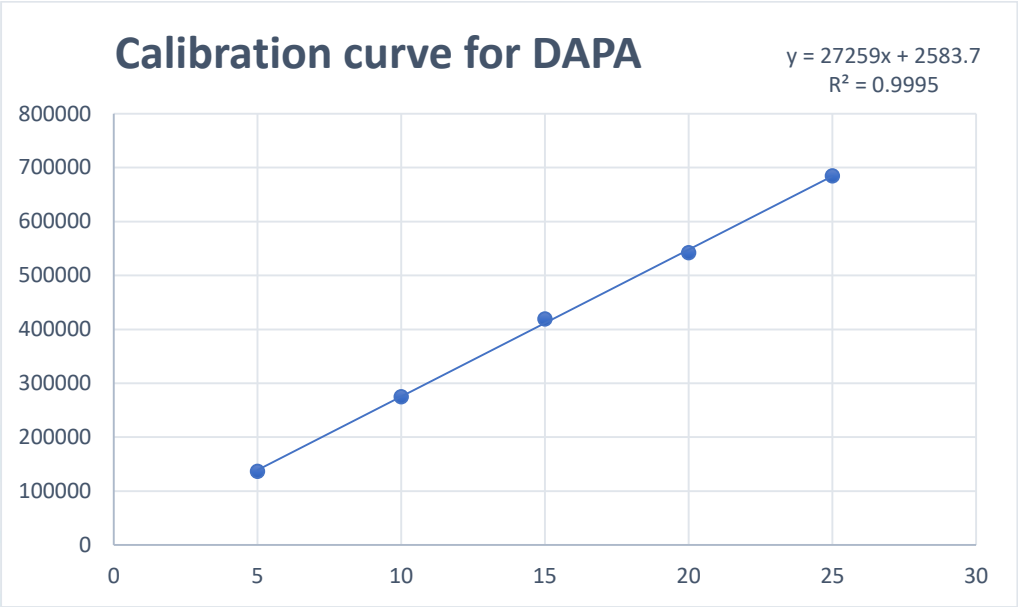

Fig no-3 Calibration Curve of 5 – 25 ppm (5, 10, 15, 20, 25) for DAPA

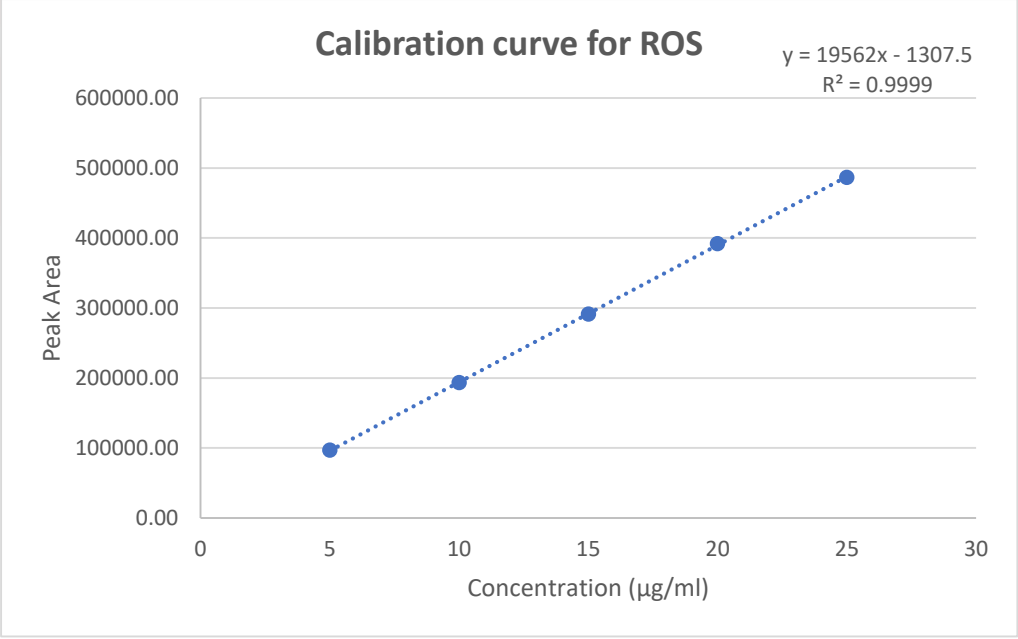

Fig no-4. Calibration Curve of 5 – 25 ppm (5, 10, 15, 20, 25) for ROS

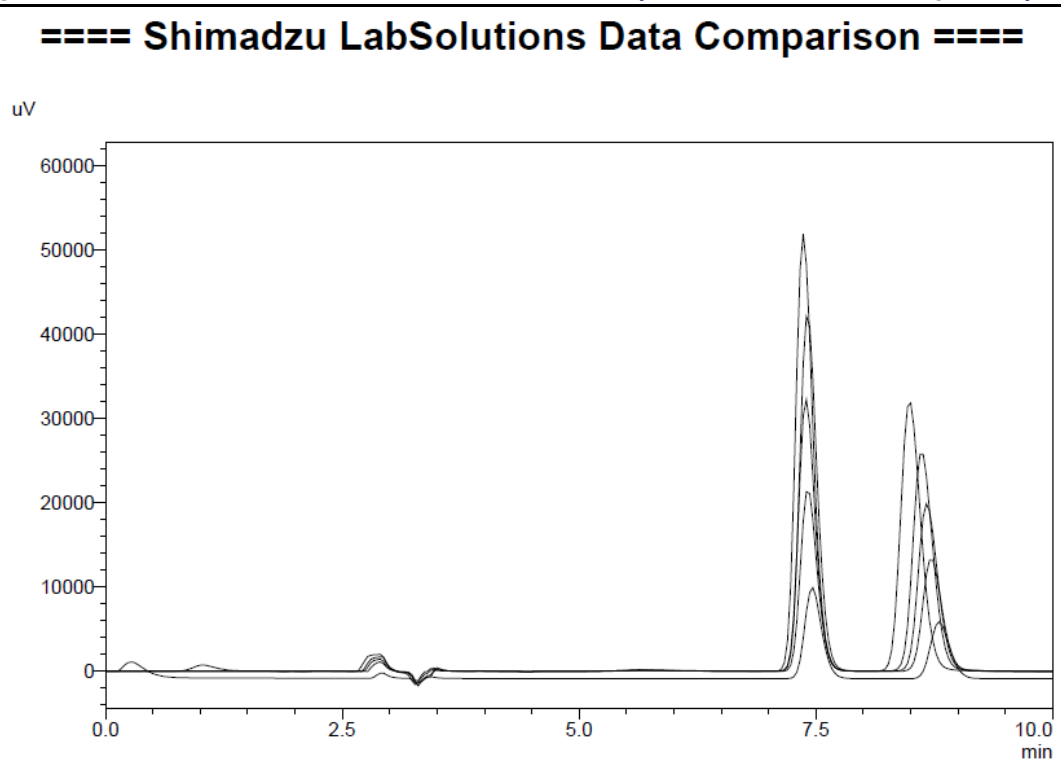

Fig no-5 Overlay Chromatogram of 5 – 25 ppm for DAPA and 5 – 25 ppm for ROS respectively.

Accuracy

Accuracy of method was carried out at three levels (50 %, 100 % and 150 %). % Recovery for Dapagliflozin (DAPA) was found to be in range of 97.23 – 102.60 %, while for and Rosuvastatin (ROS) it was found to be in range of 97.10 -102.86 % are shown in Table 4.

Table 4. Accuracy data

Level (%)	Target Conc. (µg/ml)	Spiked Conc. (µg/ml)	Total Conc. (µg/ml)	Area	Conc. Found (µg/ml)	% Recovery
Dapagliflozin (DAPA)						
0	10	0	10	272680.00	9.91	99.03
50	10	5	15	422201.67	15.39	102.62
100	10	10	20	541477.33	19.77	98.85
150	10	15	25	691153.00	25.26	101.04
Rosuvastatin (ROS)						
0	10	0	10	192478.67	9.91	99.06
50	10	5	15	296747	15.24	101.58
100	10	10	20	395958.33	20.31	101.54
150	10	15	25	485266.33	24.87	99.49

Precision

- For precision RSD was found to be less than 2 revels that the proposed method is acceptable shown in Table 5,6.

Table 5. Repeatability Data of Dapagliflozin (DAPA) and Rosuvastatin (ROS)

Dapagliflozin (DAPA)			Rosuvastatin (ROS)		
Sr. No	Conc. (µg/ml)	Area	Sr. No	Conc. (µg/ml)	Area
1	10	271705	1	10	193881.00
2	10	272757	2	10	195275.00
3	10	273578	3	10	189280.00
4	10	274745	4	10	195512.00
5	10	279705	5	10	195281.00
6	10	277778	6	10	192284.00
Average	275045		Average	193586	
SD	3092.49		SD	2439.63	
% RSD	1.12		RSD	1.26	

Table 6. Intraday and Interday precision of method

Dapagliflozin (DAPA)				
Conc	Intraday precision		Interday precision	
	Peak Area (Mean ± SD)^n	%RSD	Peak Area (Mean ± SD)^n	%RSD
10	137015.67 ± 847.52	0.62	136058.00 ± 1039.13	0.76
30	420401.67 ± 5324.63	1.27	419792.00 ± 7311.25	1.74
60	685604.67 ± 8016.45	1.17	686819.67 ± 4352.71	0.63
Rosuvastatin (ROS)				
10	97490.33 ± 636.95	0.65	95705.33 ± 2645.75	1.32
30	288098.67 ± 1180.97	0.41	294748.33 ± 1985.10	0.67
60	485266.33 ± 3207.71	0.66	488937.00 ± 2645.75	0.54

LOD and LOQ

- LOD & LOQ of Dapagliflozin (DAPA) and Rosuvastatin (ROS) were determined by equation according to ICH guideline calculation of these was given in Table 7

Table 7. LOD and LOQ of Dapagliflozin (DAPA) and Rosuvastatin (ROS)

Drug	Dapagliflozin (DAPA)	Rosuvastatin (ROS)
Limit of detection (LOD)	0.41 µg/ml	0.21 µg/ml
Limit of quantification (LOQ)	1.24 µg/ml	0.66 µg/ml

Robustness: Deliberate change in different parameters like Flow rate, Wavelength and buffer ratio showed Relative standard deviation of peak area less than 2 %, indicating that the method was robust. Results, presented in table 8 indicate that the selected factors remained unaffected by small variation of these parameters.

Table 8. Robustness study for Dapagliflozin (DAPA) and Rosuvastatin (ROS)

Sr.no	Factor	Drug	Level	Mean area (n=3) ± S.D	%RSD
1	Change in the Flow Rate	DAPA	0.9 ml/min	275689.67 ± 3093.34	1.12
			1.1 ml/min	273689.33 ± 998.77	0.36
		ROS	0.9 ml/min	196022.00 ± 1096.95	0.56
			1.1 ml/min	189015.33 ± 668.52	0.35
2	Change in wavelength	DAPA	240 nm	271357.00 ± 1989.22	0.73
			248 nm	268370.00 ± 2629.12	0.98
		ROS	240 nm	191022.33 ± 2045.81	1.07
			248 nm	191355.67 ± 1801.48	0.94
3	Change in mobile phase ratio	DAPA	35,25,40	273356.00 ± 2607.73	0.95
			35,30,35	273301.33 ± 1616.64	0.59
		ROS	35,25,40	192028.67 ± 3008.65	1.57
			35,30,35	193858.67 ± 1455.94	0.75

7.10 ASSAY OF SYNTHETIC MIXTURE

- Synthetic mixture of Dapagliflozin (DAPA) and Rosuvastatin (ROS) containing 10 mg and 10 mg when analysed using the developed method, showed 100.80 % assay for Dapagliflozin (DAPA) and 98.96 % assay for Rosuvastatin (ROS). Chromatogram was given in fig no. 6 and % assay was given in Table 9.

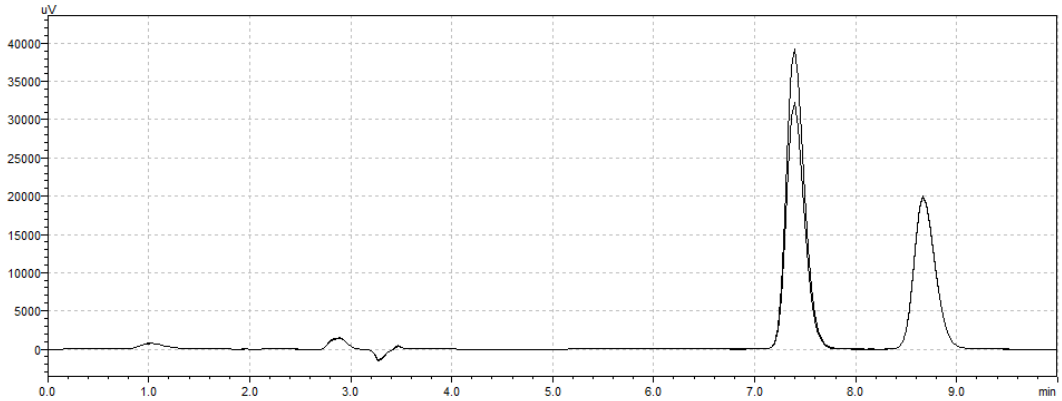

Fig no-6 Chromatogram for Analysis of Synthetic mixture

Table 9. Analysis of Synthetic mixture

Drugs	Conc.	% Assay
Dapagliflozin (DAPA)	10 mg	100.80 ± 1.75
Rosuvastatin (ROS)	10mg	98.96 ± 0.54

Developed method was validated as per the ICH Q2R1 guideline. Summary of validation parameter is shown in Table 10.

Sr. No.	Parameter	Dapagliflozin (DAPA)	Rosuvastatin (ROS)
1	Linearity	5-25 µg/ml	5-25 µg/ml
2	Correlation coefficient	0.9995	0.9999
3	Accuracy (% Recovery)	98.85 % - 102.62%	99.06 % - 101.58 %
4	Repeatability	% RSD found should be less than 2	
5	Intraday precision (RSD)	% RSD found should be less than 2	
6	Interday precision (RSD)	% RSD found should be less than 2	
7	Robustness	% RSD found should be less than 2	
8	LOD (µg/ml)	0.41 µg/ml	0.22 µg/ml
9	LOQ (µg/ml)	1.25 µg/ml	0.66 µg/ml

CONCLUSION:

the present study, a specific, precise, accurate and robust high-performance liquid chromatographic method for analysis of Dapagliflozin (DAPA) and Rosuvastatin (ROS) was developed and validated according to ICH guidelines. For UVwavelength

For dapa and rosu used 224.20,244.60 respectively. Correlation Coefficient(r2) 0.99520.9966 Found within the limit. The HPLC column C18 5µm, 250 × 4.6 mm, was

used for developed method and mobile phase Acetonitile: Methanol: phosphate buffer

- adjust pH 3.5 with ortho phosphoric acid (30:35:35) and flow rate 1ml/min. Detection

Wavelength is 244nm.Linearity range used 5-25 µg/ml for both Drugs. %recovery found 98.85-102.62%,99.06 % - 101.58 % For DAPAGLIFLOZIN, ROSUVASTATIN

Respectively. Repeatability, Intraday precision (RSD) Interday precision (RSD), Robustness Found within the limit. LOD limits found 0.41 µg/ml,0.22 µg/ml Respectively for dapa, rosu. LOQ limit found 1.25 µg/ml,0.66 µg/ml for dapa and rosu

respectively.

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