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Validation of an In-house Method for Analysis and Dissolution of Empagliflozin in Film Coated Tablets

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Abstract: ***There is no pharmacopoeial specific monograph for chemical analysis of empagliflozin. HPLC method had been developed inhouse. A column C-18, Thermo-scientific Hypersil 4.6 mm × 150 mm of 5 µm pores was used. The detector is UV detector at 224 nm. The flow rate is 1ml/min for injection volume of 20 µL at 30 ℃ and run time is 10 minutes. The mobile phase is Orthophosphoric acid buffer and Acetonitrile 70: 30. The method was then validated for system suitability, specifity, accuracy, range linearity, the system precision; repeatability, and robustness by changing the flow rate wavelength and speed of the paddle. All the results were compared with the USP acceptable criteria for each test and were found to satisfactory. The method stands validated and can be used for routine quality control and stability studies.***

Keywords: Empagliflozin method of Analysis, Empagliflozin method validation, in-house dissolution method validation, method validation procedure

1. Introduction

Empagliflozin is $C_{23}H_{27}ClO_7$ of molar weight 450.91 gm/mol having the following structural formula[1] (2S, 3R, 4R, 5S, 6R)-2-[4-chloro3-({4-[(3S)nn-oxolan3-yloxy] phenyl} methyl)phenyl]-6-(hydroxymethyl) oxane-3,4,5-triol.

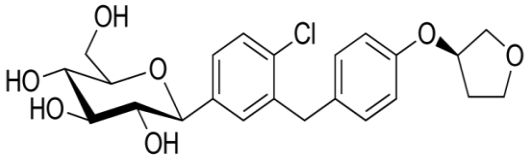

Figure 1

Empagliflozin reversibly and selectively inhibit Sodium-Glucose-Co-Transporter 2 (SGLT2) which is found exclusively in the renal proximal convoluted tubule [2].

There is no pharmacopoeial monograph for Empagliflozin. Refereeing to ICH Guidelines; Text on Validation of analytical procedure and Validation of analytical procedures; Methodology Q2 (R1) [3] and USP ⟨1226⟩: Text on Validation of Compendial Methods[4], the objectives was proposed to validate an in-house method of analysis of empagliflozin by HPLC. Many studies had been published, one method used RP-HPLC method using the buffer and acetonile 50:50 and wavelength 210 nm [5],

2. Materials and Methods

2.1 Materials

Empagliflozin reference standard SNV-190305 99.5% China, Empagliflozin 10 mg film coated tablets Sudan, purified Water HPLC grade, Sodium Phosphate dibasic dihydrateAR , Sodium phosphate monobasic anhydrousAR grade, AcetonitrileHPLC grade, Sodium hydroxideAR grade, Hydrochloric acid AR grade, Orthophosphoric acid AR grade, Glass waresClass 'A' grade.

Instruments: HPLC-Prominence (PDA), HPLC Column, Dissolution Apparatus, Analytical Balance, Purified water system, Rotatory shaker, pH meter.

2.2 Analytical Method

Dissolution conditions:
Apparatus: Dissolution type 2, paddle mixer USP. Speed of rotation: 75 rpm. Dissolution time: 30 minutes. Temperature: 37ºC ± 0.5ºC. Dissolution Volume: 900ml

Dissolution medium: **0.05M phosphate Buffer pH 6.8:**
To 400 ml of water add 3.28 gm of Sodium phosphate dibasic dihydrate [$Na_2HPO_4.2H_2O$] and 1.3546 gm of Sodium phosphate monobasic anhydrous [NaH_2PO_4]. Adjust the pH to 6.8 using sodium hydroxide or hydrochloric acid and make up the volume to 1000 ml with water.

Chromatographic conditions
Column: Thermo Scientific BDS Hypersil-C18, 4.6-mm x15-cm, 5µm; Detector: at wavelength UV 224 nm; Flow rate: 1.0 ml/min. Injection Volume: 20µl; Column Temperature: 30^0C; Run time: 10 minutes.

Buffer Preparation:
Dilute 1.0 ml of orthophosphoric acid and dilute to 1000 ml with water.

Mobile phase Preparation:
Prepare a filtered and degassed mixture of Acetonitrile and Buffer (30:70). Filter this solution through 0.45µ filter and degas by sonication.

Standard Preparation:
Weigh and transfer 10.0 mg of Empagliflozin Working Standard into a 100 ml volumetric flask, add 50 ml of mobile phase and sonicate with intermittent shaking to dissolve the content, make up the volume with mobile phase. Mix well. Pipette out 5 ml of this solution into 50 ml volumetric flask and make up the volumes with dissolution medium mix well. Pass the sample through a syringe tip filter of 0.45-µm pore size (The concentration of Empagliflozin Working Standard is 0.01 mg/ml)

Sample Preparation:
Take 6 Tablets and introduce one Tablet each into 6 different dissolution vessel containing 900 ml of dissolution medium (previously equilibrated at 37ºC ± 0.5ºC). After 30 minutes time interval withdraw about 12 ml of sample from zone midway between the surface of dissolution medium and top of the rotating paddle not less than 1cm from the wall of vessels. Filter each about 12 ml sample through 0.45 µ membrane filter and collect after discarding the first few ml of the filtrate. (The concentration of Empagliflozin is about 0.0111 mg/ml)

Procedure: Equilibrate the column with mobile phase for sufficient time until stable baseline is obtained. Inject 20µl of Blank and standard solution as per the sequence of injections. If the system suitability criteria meet the requirements then inject the Sample preparation as per the sequence and record the chromatograms. The sequence of injection is one for the blank, 6 for the standard and one for each of the six samples.

Release Percentage Calculation: Calculate the percentage of the labeled amount of Empagliflozin dissolved by using the following expressions:
Result = $(r_U/r_S) \times (C_S/L) \times V \times 100$
r_U = peak response of Empagliflozin Sample solution
r_S = peak response of Empagliflozin Standard solution
C_S = concentration of Empagliflozin WS in the *Standard solution* (mg/mL)
L = Label claim of tablet; 10mg
V= Volume of medium; 900ml

Methods for Validation Parameters
Validation parameters includes system suitability, specificity, accuracy, linearity and range, system precision, repeatability precision, intermediate precision, robustness[6].

System suitability
System suitability was demonstrated by making six replicate injections of standard solution as per the specification method. The peak area of Empagliflozin for six replicates injections was recorded. The precision was evaluated by computing the relative standard deviation for the analyte peak area of these replicate injections.

Acceptance Criteria:
1) In the chromatogram of blank, there should be no interference at the retention time of Empagliflozin.
2) Theoretical plates for the peak due to Empagliflozin from standard solution should not be less than 2000.
3) Tailing factor for the peak due to Empagliflozin obtained from standard solution should not be more than 2.0
4) The relative standard deviation of peak area responses of Empagliflozin for six replicate injections of the standard solution should not be more than 2.0%

Specificity:
Standard solution, and sample solution were prepared as per the methodology. Inject diluent and placebo. Injections of standard solutions and sample solution of product by using the chromatographic system described in the methodology
Acceptance Criteria:
1) System should meet the system suitability criteria as specified in method of analysis
2) There should be no potentially interfering peaks in blank and placebo which can adversely affect the quantitation of Empagliflozin peak in the sample [4].

Accuracy:
A placebo of product that is inclusive of all ingredients will be spiked with Empagliflozin Working Standard from levels corresponding the approximately 80%, 100%, and 120% of the nominal sample solution concentration in triplicate. The recovery samples were prepared in triplicate for each level.

Preparation of Placebo:
Weigh and transfer 246.5 mg of placebo powdered into dissolution vessel containing 900 ml of dissolution medium (previously equilibrated at 37ºC ± 0.5ºC).Operate the instrument of dissolution as mentioned in methodology. After 30 minutes time interval withdraw about 12 ml of sample from zone midway between the surface of dissolution medium and top of the rotating paddle not less than 1 cm from the wall of vessels. Filter each about 12 ml sample through 0.45 µ membrane filter and collect after discarding the first few ml of the filtrate.

Preparation of Accuracy levels
Weigh and transfer 246.5 mg of placebo powdered into dissolution vessel containing 900 ml of dissolution medium (previously equilibrated at 37ºC ± 0.5ºC).Spike 8.0 mg of Empagliflozin standard in same vessel. Operate the instrument of dissolution as mentioned in methodology. After 30 minutes time interval withdraw about 12 ml of sample from zone midway between the surface of dissolution medium and top of the rotating paddle not less than 1 cm from the wall of vessels. Filter each about 12 ml sample through 0.45 µ membrane filter and collect after discarding the first few ml of the filtrate. (Prepare in triplicates). Follow the same procedure for second & third Accuracy levels.

Preparation of Accuracy levels Triplicates:
Preparing triplicates of the three levels, using 246.5 mg placebo and 8,10 and a2 mg of Empagliflozin standard and diluted to 900 ml. the concentration of level 1, 2 and three will be 80, 100 1nd 120% respectively.

Acceptance Criteria:
The mean percentage recovery of Empagliflozin peak should be 98.0 – 102.0% at each level [4].

Linearity and Range:
Prepare a standard stock solution of Empagliflozin. From this solution diluted to level approximately 20% of the

nominal sample solution concentration to approximately 180% of the nominal sample solution concentration at 9 levels from 20%, 40%, 60%, 80%, 100%, 120%, 140%, 160%, and 180% respectively. Plot a graph of concentration (at X-axis) versus average peak area of analyte (at Y-axis). Evaluate the correlation coefficient. (r

Preparation of Linearity Stock solution:
Weigh and transfer 10.0 mg of Empagliflozin WS into a 100 ml volumetric flask, add 50 ml of mobile phase and sonicate with intermittent shaking to dissolve the content, make up the volume with mobile phase.

Preparation of Linearity Levels 1: Pipette out 1 ml of this solution into 50 ml volumetric flask and make up the volumes with dissolution medium mix well.

Table 1: Preparation of Linearity Levels

Linearity levels	Volume of Linearity stock solution (ml)	Diluted up to volume with dissolution medium, ml	Concentration of Empagliflozin (mg/ml)	Working St Concentration % working level
1	1	50	0.002	20
2	2	50	0.004	40
3	3	50	0.006	60
4	4	50	0.008	80
5	5	50	0.01	100
6	6	50	0.012	120
7	7	50	0.014	140
8	8	50	0.016	160
9	9	50	0.018	180

Acceptance Criteria:
1) The Linearity regression co-efficient of average peak area of Empagliflozin for replicate determination plotted against respective concentration should not be less than 0.999
2) The % y- intercept as obtained from the linearity data (without extrapolation through origin 0, 0) should be between ± 2.0
3) The % RSD of peak area of Empagliflozin for Linearity Level 1 and Level 9 should not be more than 2.0 [4

System Precision:
System suitability was demonstrated by making six replicate injections of standard solution as per the specification method. The peak area of Empagliflozin for six replicates injections was recorded. The precision was evaluated by computing the relative standard deviation for the analyte peak area of these replicate injections.

Acceptance Criteria:
1) The relative standard deviation of peak area responses of Empagliflozin for six replicate injections of the standard solution should not be more than 2.0%
2) Theoretical plates for the peak due to Empagliflozin obtained from standard solution should NLT 2000.
3) Tailing factor for the peak due to Empagliflozin obtained from standard solution should not be more than 2.0 [4].

Method Precision (Repeatability):
Perform dissolution on six units as per the sample preparation procedure in methodology and inject once. Determine % RSD of six units for % released.

Acceptance criteria:
The % RSD for the % released of Empagliflozin of six units should not be more than 2 % with all the individual values within limit.

Intermediate Precision:
To demonstrate the intermediate precision, prepare six dissolution sample solutions in the same way as per sample preparation in repeatability. (Analysis was performed by different analyst on different day and on different instrument.) Compare the results against repeatability experiment.

Instrument Details:
Prominence-i, Shimadzu high performance liquid chromatography system is used.

Acceptance criteria:
1) % RSD of each analyst should be $\leq$ 3.0%
2) % RSD of mean data of two analysts should not be more than 3.0% [4].

Robustness:
Three parameters were deliberately changed to evaluate the robustness of the method. In each parameter prepare six dissolution sample solutions in the same way as per sample preparation in methodology.
a) By changing the flow rate of mobile phase from 1.0 ml to 0.8 ml
b) By changing the flow rate of mobile phase from 1.0 ml to 1.2 ml
c) By changing the wavelength from 224 nm to 222 nm
d) By changing the wavelength from 224 nm to 226 nm
e) By changing the RPM of paddle from 75 to 77 rpm/min.
f) By changing the RPM of paddle from 75 to 73 rpm/min.

Acceptance Criteria
The % variation of drug release should not be more than 3% for changed parameters when Compared to normal parameter **[4].**

3. Results and Discussion

System Suitability

Table 2: Results of System Suitability

Details	EMPAGLIFLOZIN	
	RT (min)	Area
Average	7.611	510254
SD	0.0013	190.8
%RSD	0.02	0.04
Theoretical plate	64139	
Tailing factor	1.061	

Inference:
1) In the chromatogram of blank, there is no interference at the retention time of Empagliflozin.

2) Theoretical plates for the peak due to Empagliflozin obtained from standard solution are 64139
3) Tailing factor for the peak due to Empagliflozin obtained from standard solution are 1.061
4) The relative standard deviation of peak area responses of Empagliflozin for six replicate injections of the standard solution is 0.04%.

Thus, the method comply the acceptable criteria for system suitability.

Specificity

Table 3: Results for Specificity Test

Details	Empagliflozin	
	RT (min)	Area
Blank	No interference	No interference
Placebo	No interference	No interference
Standard-1	7.61	514579
Standard-2	7.608	511523
Standard-3	7.604	510996
Standard-4	7.607	511512
Standard-5	7.603	510287
Standard-6	7.604	510330
Test solution of Empagliflozin Tablets 10mg	7.599	520470
	7.602	516928

Inference:
1) The system meets the system suitability criteria as specified in method of analysis
2) There are no potentially interfering peaks in blank and placebo which can adversely affect the quantitation of Empagliflozin peak in the sample. Thus, the method complies the acceptable criteria.

Accuracy:

Table 4: Results of Accuracy

Accuracy level	Conc %	Mean of recovery
1	80	100.2%
2	100	100.4%
3	120	100.5%

Recovery% is the percentage of the amount found to the amount added.

Inference:
The mean percentage recovery of Empagliflozin is found within 98.0 – 102.0% at each level, thus comply the acceptable criteria.

Linearity and Range

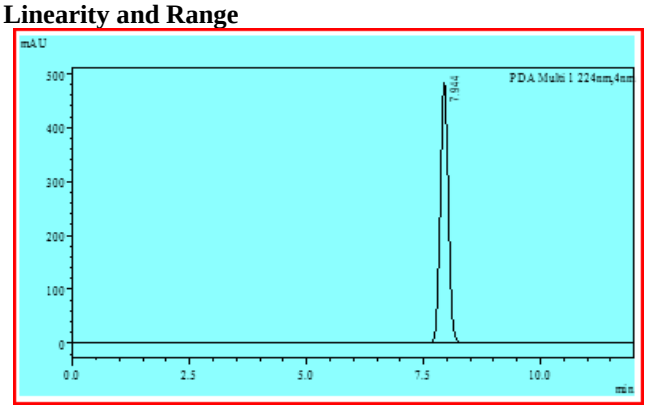

Figure 2: Peak of Empagliflozin Standard

Table 4: Peak Area response of Empagliflozin

Concentration	Average Area of 6 injection	SD	RSD
20%	101432	82.3	0.08
40%	208195	164.0	0.08
60%	307738	306.9	0.10
80%	415287	309.7	0.07
100%	516825	266.6	0.05
120%	617182	1932.5	0.31
140%	720599	334.5	0.05
160%	827218	33.9	0.00
180%	924400	2366.1	0.26

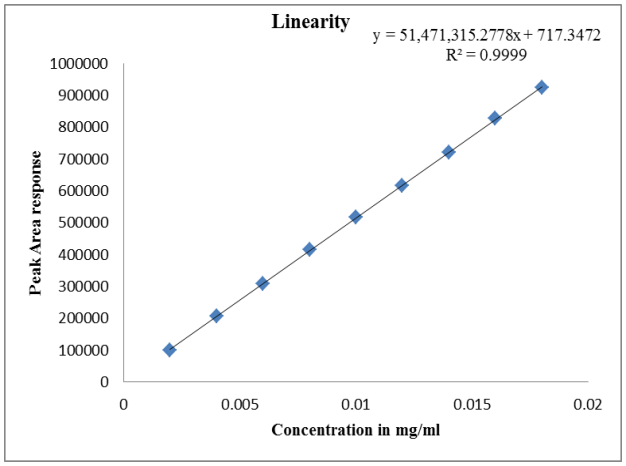

Figure 3: Linearity Graph

% y intercept = 0.014 **%.**

The correlation Coefficient is 0.9999, the Y intercept is 717.3472222 and the slope is 51473115.28.

Inference:
1) The correlation coefficient is 0.9999 within the limit.
2) The % y-intercept as obtained from the linearity data is 0.014% found within the limit
3) The % RSD of peak area of Empagliflozin for Linearity Level 1 is 0.08% and Level 9 is 0.26%.

Precision

System Precision

Table 5: Results of System Suitability

Details	Empagliflozin	
	RT (min)	Area
Mean of 6 injections	7.607	515088.0
SD	0.0013	367.7
%RSD	0.017	0.071
Theoretical plate	63832	
Tailing factor	1.06	

The relative standard deviation of peak area responses of Empagliflozin for six replicate injections of the standard solution are 0.071%. Theoretical plates for the peak due to Empagliflozin obtained from standard solution are 63832. Tailing factor for the peak due to Empagliflozin obtained from standard solution are 1.06

Method Precision

Table 6: The repeatability results

Area of Empagliflozin	% Release of Empagliflozin
Mean of six tablets	101.0
SD	0.94
%RSD	0.93

Inference: % RSD for six units in % dissolution was found 0.93%, hence met the acceptance criteria.

Intermediate Precision:

Table 7: Results of System Suitability: (Analyst-1)

Details	Empagliflozin	
	RT (min)	Area
Mean of 6 tablets	7.614	512376.8
SD	0.0015	154.5
%RSD	0.019	0.030
Theoretical plate	63680	
Tailing factor	1.062	

Results of the ruggedness: Analyst 2
The mean of release percentage for analyst 2 is 100.8% with SD 0.68.

Cumulative Data of Intermediate Precision
The cumulative mean of release percentage for anayst 1 and 2 is 100.93% with SD 0.8.

Inference
The %RSD for analyst – 1 is 0.93% & Analyst – 2 is 0.67% and the overall % RSD of both analysts is 0.79%. Thus meet the acceptance criteria.

Robustness

1) System Suitability

A- Changing flow rate from 1.0 ml/min to 0.8 ml/min& Change in flow rate from 1.0 ml/min to 1.2 ml/min

Table 8: Results of changing the flow rate

Flow rate 0.8 ml/min				Flow rate 1.2 ml/min		
Working Standard			Tablets	Working Standard		Tablets
Parameter	R T mins	Area	Release %	R T mins	Area	Release %
Mean of 6 injections	9.519	649174.0	99.7	6.377	423838.2	101.0
SD	0.0045	510.2	0.83	0.0024	464.2	0.72
RSD	0.047	0.079	0.83	0.038	0.110	0.71
Theoretical plates	74001			56400		
Tailing factor	1.06			1.07		

B-Changing the Wavelength from 224 nm to 222 nm & Changing Wavelength from 224 to 226 nm

Table 9: Results of Changing the wavelngth

Wavelength 222mn				Wavelength 226 nm		
Working Standard			Tablets	Working Standard		Tablets
Parameter	R T mins	Area	Release %	R T mins	Area	Release %
Mean of 6 injections	7.624	523493.7	100.2	7.624	7.629	100.3
SD	0.0014	276.4	0.83	0.0014	0.0013	1.08
RSD	0.018	0.053	0.83	0.018	0.018	1.07
Theoretical plates	64379			64114		
Tailing factor	1.056			1.060		

C- Change in RPM of Paddle from 75 to 77 rpm & Change in RPM of Paddle from 75 to 73 rpm

Table 10: Results of changing the paddle speed

Parameter	77 rpm	73 rpm
Mean of release %	99.7	99.7%
SD	1.16	0.47
RSD %	1.17	0.47%

Table 11: The results of robustness study on different parameters are shown in Table

No	Robustness Parameter	Limits	Mean % Release	% Variation
1	Flow rate	Nominal: 1.0 ml/min	101.0	Nil
		Upper Limit: 1.2 ml/min	101.0	Nil
		Lower Limit: 0.8 ml/min	99.7	-1.28 %
2	Wave length	Nominal: 224 nm	101.0	Nil
		Upper Limit: 226 nm	100.3	-0.69%
		Lower Limit: 222 nm	100.2	- 0.79
3	RPM	Normal: 75	101.0	Nil
		Upper Limit: 77	99.7	-1.28%
		Lower Limit: 73	99.7	-1.28 %

Inference:
System suitability criteria are met. No significant variation in the % release of the sample. The difference in the percent release of changed parameter was not more than 3.0% when compared with the normal parameter.

Comments on Results of Validation:

Accuracy
There is no interference in the chromatogram at the retention time of empagliflozin , the recovery percentage at level 1,2,3 were 100.2, 100.4, 100.5 respectively and the mean is 100.36 so complying the acceptance criteria which states that the mean should be 98% - 102%.

Linearity
On plotting the average peak area for the replicates against the respective concentration, the regression coefficient is 0.9999 which is more than 0.999, thus comply the acceptance criteria. The Y% intercept value is 0.014 less than ±2, the relative standard deviation of the peak area response at level 1,2 is 0.08% and 0.26% respectively which is less than 2%, thus the method complies the acceptance criteria for linearity.

Precision

a) The theoretical plate is 63832, more than 2000, thus, complying the acceptance criteria indicating column efficiency. The tailing factor is 1.06, that is less than 2, the relative standard deviation for the six replicates is 0.071%, less than 2 and the relative standard deviation for the release % is 0.93% , less than 2, thus, the method complies the acceptance criteria for precision.
b) Intermediate precision: there is no interference in the chromatogram of empagliflozin retention time, the theoretical plates is 63680 in the chromatogram of the standard preparation which is less than 2000 indicating column efficiency. The tailing factor is 1.062 less than 2 and the relative standard deviation is 0.03 for the peak area of the six replicates and that of the release % is 0.79%. thus the method complies the intermediate precision acceptable criteria.

Robustness

a) Robustness for changing the flow rate from 1 ml/min to 0.8 ml/min
b) There is no interference in the chromatogram at the retention time of empagliflozin. The theoretical plates is 74001, the tailing factor is 1.06 and the relative standard deviation for the six replicates peak area is 0.079% and that of the release % is 0.83 with variation of -1.28 in the six replicates which is less than 3%. Thus, the method complies the acceptance criteria.
c) Robustness for changing the flow rate from 1 ml/min to 1.2 ml/min

There is no interference in the chromatogram at the retention time of empagliflozin. The theoretical plates is 56400, the tailing factor is 1.07 and the relative standard deviation for the six replicates peak area is 0.11% and that of the release % is 0.71% with no variation. Thus, the method complies the acceptance criteria for robustness by changing the flow rate.

a) Robustness for changing the wave length from 224 to 222 nm

There is no interference in the chromatogram at the retention time of empagliflozin. The theoretical plates is 46379, the tailing factor is 1.056 and the relative standard deviation for the six replicates peak area is 0.053% and that of the release % is 0.83 with variation of -0.79% in the six replicates which is less than 3%. Thus, the method complies the acceptance criteria.

b) Robustness on changing the wave length from 224 to 226 nm

There is no interference in the chromatogram at the retention time of empagliflozin. The theoretical plates is 64114, the tailing factor is 1.60 and the relative standard deviation for the six replicates peak area is 0.058% and that of the release % is 1.071 with variation of -0.69% in the six replicates which is less than 3%. Thus, the method complies the acceptance criteria.

c) Robustness on changing the RPM from 75 to 77 rpm

There is no interference in the chromatogram at the retention time of empagliflozin. The theoretical plates is 64307, the tailing factor is 1.056 and the relative standard deviation for the six replicates peak area is 1.056% and that of the release % is 0.47 with variation of -.28% in the six replicates which is less than 3%. Thus, the method complies the acceptance criteria.

d) Robustness on changing the RPM from 75 to 73 rpm

There is no interference in the chromatogram at the retention time of empagliflozin. The relative standard deviation is 1.17 and variation % is 1.28. thus the method complies the acceptance criteria on changing the RPM.

4. Conclusion

The analytical method used for determination of Dissolution test of Empagliflozin content in Empagliflozin-10mg film coated. Tablets complies with the acceptance criteria of the analytical parameters such as System suitability, Specificity, Accuracy, Linearity and range, Precision, Reproducibility (intermediate precision), & Robustness without deviation. The method was similar to study done by Shayamala et.al[7] but using the detection wavelength 233 nm. The use of 224 nm gave better results. Hence, method stands validated. The method can be used for routine Quality control and Stability study analysis.

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