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Research article

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A new validated RP-HPLC-photo diode array (PDA) method for the simultaneous estimation of dapagliflozin and saxagliptin in bulk form and pharmaceutical tablet dosage form

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ABSTRACT

A new, simple, precise, accurate and reproducible RP-HPLC method for Simultaneous estimation of Dapagliflozin and Saxagliptinin bulk and pharmaceutical formulations. Separation of Dapagliflozin and Saxagliptin was successfully achieve dona Phenomenex Luna C18 (4.6×250mm, 5µm) particle size or equivalentin an isocratic mode utilizing Acetonitrile: Phosphate Buffer (pH-4.6) (45:55 v/v) at a flow rate of 1.0mL/min an delutes was monitored at 245nm, with are tention time of 2.102 and 3.537 minutes for Dapagliflozin and Saxagliptin respectively. The method was validated and the response was found to be linear in the drug concentration range of 6µg/mL to 14µg/mL for Dapagliflozin and 18µg/mL to 42µg/mL for Saxagliptin. The values of the slope and the correlation coefficient were found to be 77824 and 0.999 for Dapagliflozin and 10515 and0.999 for Saxagliptin respectively. The LOD and LOQ for Dapagliflozin were found to be 0.6µg/mL and1.8µg/mL respectively. The LOD and LOQ for Saxagliptin were found to be 0.8µg/mL and 2.4µg/mL respectively. This method was found to be good percentage recovery for Dapagliflozin and Saxagliptin were found to be 100.351 and 100.93 respectively indicates that the proposed method is highly accurate. The specificity of the method shows good correlation between retention times of standard with the sample so, the method specifically determines the analytes in the sample without interference from excipients of tablet dosage forms. The method was extensively validated according to ICH guidelines for Linearity, Range, Accuracy, Precision, Specificity and Robustness.

Keywords: Dapagliflozin and Saxagliptin, RP-HPLC, Accuracy, Precision.

INTRODUCTION

Analytic method development and validation are key elements of any pharmaceutical development program. HPLC analysis method is developed to identify, quantity or purifying compounds of interest. This technical brief will focus on development and validation activities as applied to drug products.

Method development

Effective method development ensures that laboratory resources are optimized, while methods meet the objectives required at each stage of drug development. Method validation, required by regulatory agencies at certain stages of the drug approval process, is defined as the “process of demonstrating that analytical procedures are suitable for their intended use” [1-2]. Understanding of the physical and chemical characteristics of drug allows one to select the most appropriate high performance liquid chromatography method development from the available vast literature. Information concerning the sample, for example, molecular mass, structure and functionality, pKa values and UV spectra, solubility of compound should be compiled. The requirement of removal of insoluble impurities by filtration, centrifugation, dilution or concentration to control the concentration, extraction (liquid or solid phase), derivatization for detection etc. should be checked. For pure compound, the sample solubility should be identified whether it is organic solvent soluble or water soluble, as this helps to select the best mobile phase and column to be used in HPLC method development.

Method development in HPLC can be laborious and time consuming. Chromatographers may spend many hours trying to optimize a separation on a column to accomplish the goals. Even among reversed phase columns, there is astonishing diversity, owing to differences in both base silica and bonded phase characteristics. Many of these show unique selectivity. What is needed is a more informed decision making process for column selection that may be used before the chromatographer enters the laboratory. The method of column selection presented here involves a minimal investment in time initially, with the potential of saving many hours in the laboratory.

Analytic methods are intended to establish the identity, purity, physical characteristics and potency of the drugs that we use. Methods are developed to support drug testing against specifications during manufacturing and quality release operations, as well as during long-term stability studies. Methods that support safety and characterization studies or evaluations of drug performance are also to be evaluated. Once a stability-indicating method is in place, the formulated drug product can then be subjected to heat and light in order to evaluate the potential degradation of the API in the presence of formulation excipients [3, 4].

The three critical components for a HPLC method are: sample preparation (% organic, pH, shaking/sonication, sample size, sample age) analysis conditions (%organic, pH, flow rate, temperature, wavelength, and column age), and standardization (integration, wavelength, standard concentration, and response factor correction). During the preliminary method development stage, all individual components should be investigated before the final method optimization. This gives the scientist a chance to critically evaluate the method performance in each component and streamline the final method optimization [5]. The percentage of time spent on each stage is proposed to ensure the scientist will allocate sufficient time to different steps. In this approach, the three critical components for a HPLC method (sample preparation, HPLC analysis and standardization) will first be investigated individually [6-8]. The degraded drug samples obtained are subjected to preliminary chromatographic separation to study the number and types of degradation products formed under various conditions [9]. Scouting experiments are run and then conditions are chosen for furtheroptimization [10]. Resolving power, specificity, and speed are key chromatographic method attributes to keep in mind during method development [11]. Selectivity can be manipulated by combination of different factors like solvent composition, type of stationary phase, mobile phase, buffers and pH. Changing solvents and stationary phases are the most comfortable approaches to achieve the separation. The proper range of pH is an important tool for separation of ionizable compounds. Acidic compounds are retained at low pH while basic compounds are more retained at higher pH. The neutral compounds remain unaffected. The pH range 4-8 is not generally employed because slight change in pH in this range would result in a dramatic shift in retention time. However, by operating at pH extremes (2-4 or 8-10), not only is there a 10-30 fold difference in retention time that can be exploited in method development but also the method can be made more robust which is a desirable outcome with validation in minutes [12,13].

MATERIALS AND METHODS

HPLC method development

Trails

Preparation of standard solution

Accurately weigh and transfer 10 mg of Dapagliflozin and Saxagliptin working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 0.1ml of the above Dapagliflozin and 0.3ml of the Saxagliptin stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure

Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Mobile Phase Optimization

Initially the mobile phase tried was Methanol: Water and Water: Acetonitrile and Methanol:Phosphate Buffer: ACN with varying proportions. Finally, the mobile phase was optimized to Acetonitrile: Phosphate Bufferin proportion 45:55 v/v respectively.

Optimization of Column

The method was performed with various columns like C18 column, Symmetry and Zodiac column. Phenomenex Luna C18 (4.6×250mm, 5µm) particle size was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

Optimized chromatographic conditions

Instrument used : Waters HPLC with auto sampler and PDADetector 996 model.
Temperature : 35ºC
Column : Phenomenex Luna C18 (4.6×250mm, 5µm) particle size
Buffer : Dissolve 6.8043 of potassium dihydrogen phosphate in 1000 ml HPLC water and adjust the pH 4.6 with diluted orthophosphoric acid. Filter and sonicate the solution by vacuum filtration and ultrasonication.
pH : 4.6
Mobile phase : Acetonitrile: Phosphate Buffer(45:55 v/v)
Flow rate : 1ml/min
Wavelength :245 nm
Injection volume: 10 µl
Run time : 7 min

Validation

Preparation of buffer and mobile phase

Preparation of potassium dihydrogen phosphate (kh2po4) buffer (ph-4.6)

Dissolve 6.8043 of potassium dihydrogen phosphate in 1000 ml HPLC water and adjust the pH 4.6 with diluted orthophosphoric acid. Filter and sonicate the solution by vacuum filtration and ultrasonication.

Preparation of mobile phase

Accurately measured 450 ml (45%) of Methanol, 550 ml of Phosphate buffer (55%) were mixed and degassed in digital ultra sonicater for 15 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation

The Mobile phase was used as the diluent.

Validation parameters

System suitability

Accurately weigh and transfer 10 mg of Dapagliflozin and 10mg of Saxagliptin working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.1ml of the above Dapagliflozin and 0.3ml of the Saxagliptin stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Procedure

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Precision

Repeatability

Preparation of Dapagliflozin and Saxagliptin Product Solution for Precision

Accurately weigh and transfer 10 mg of Dapagliflozin and 10mg of Saxagliptin working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.1ml of the above Dapagliflozin and 0.3ml of the Saxagliptin stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Intermediate precision

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different days by maintaining same conditions.

Procedure

Day 1

The standard solution was injected for Six times and measured the area for all Six injections in HPLC. The %RSD for the area of Six replicate injections was found to be within the specified limits.

Day 2

The standard solution was injected for Six times and measured the area for all Six injections in HPLC. The %RSD for the area of Six replicate injections was found to be within the specified limits.

Accuracy

For preparation of 50% Standard stock solution

Accurately weigh and transfer 10 mg of Dapagliflozinand 10mg of Saxagliptin working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.05ml of the above Dapagliflozin and 0.15ml of the Saxagliptin stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

For preparation of 100% Standard stock solution

Accurately weigh and transfer 10 mg of Dapagliflozin and 10mg of Saxagliptin working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.1ml of the above Dapagliflozin and 0.3ml of the Saxagliptin stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

For preparation of 150% Standard stock solution

Accurately weigh and transfer 10 mg of Dapagliflozinand 10mg of Saxagliptin working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.15ml ofDapagliflozin and 0.45ml of Saxagliptin from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure

Inject the Three replicate injections of individual concentrations (50%,100%,150%) were made under the optimized conditions. Recorded the chromatograms and measured the peak responses. Calculate the Amount found and Amount added for Dapagliflozin and Saxagliptin and calculate the individual recovery and mean recovery values.

Robustness

The analysis was performed in different conditions to find the variability of test results. The following conditions are checked for variation of results. .

For preparation of Standard solution

Accurately weigh and transfer 10 mg of Dapagliflozin and 10mg of Saxagliptin working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.1ml of the above Dapagliflozin and 0.3ml of the Saxagliptin stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Effect of Variation of flow conditions

The sample was analyzed at 0.9 ml/min and 1.1 ml/min instead of 1ml/min, remaining conditions are same. 10µl of the above sample was injected and chromatograms were recorded.

Effect of Variation of mobile phase organic composition

The sample was analyzed by variation of mobile phase i.e.

Acetonitrile: Phosphate Buffer was taken in the ratio and 50:50, 40:60 instead (45:55), remaining conditions are same. 10µl of the above sample was injected and chromatograms were recorded.

RESULTS AND DISCUSSION

Trails

Trail 1

Mobile phase : ACN: Water (80:20%v/v)
Column : Symmetry C18 (4.6 ×150mm, 5µm particle size) Make; waters
Flow rate : 0.8ml/min
Wavelength : 245 nm
Column temp : 40ºC
Injection Volume : 10 µl
Run time : 4 minutes

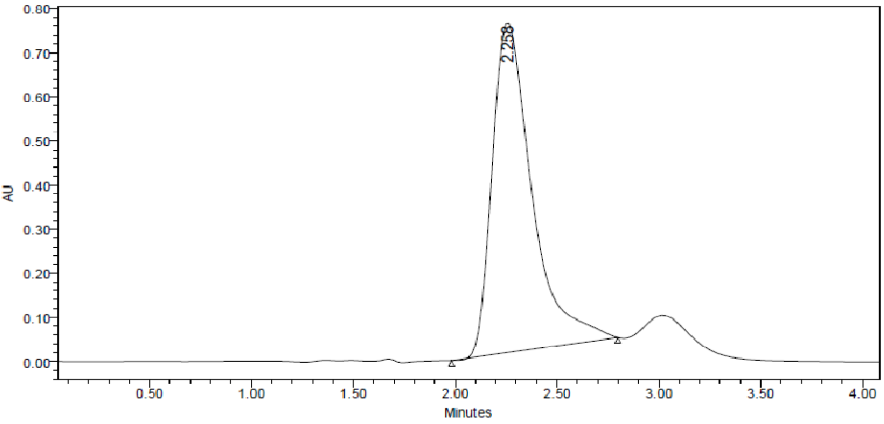

Figure1 : Chromatogram for Trail 1

Observation

In a separation of Dapagliflozin and Saxagliptin peak was obtained only for one compound because there may be less solubility. So, we go for further trails.

Trail 2

Mobile phase : Water: Methanol (40:60v/v)
Column : Zodiac C18 (4.6×250mm) 5µ
Flow rate : 0.8 ml/min
Wavelength : 245 nm
Column temp : 40ºC
Injection Volume : 8 µl
Run time : 2 minutes

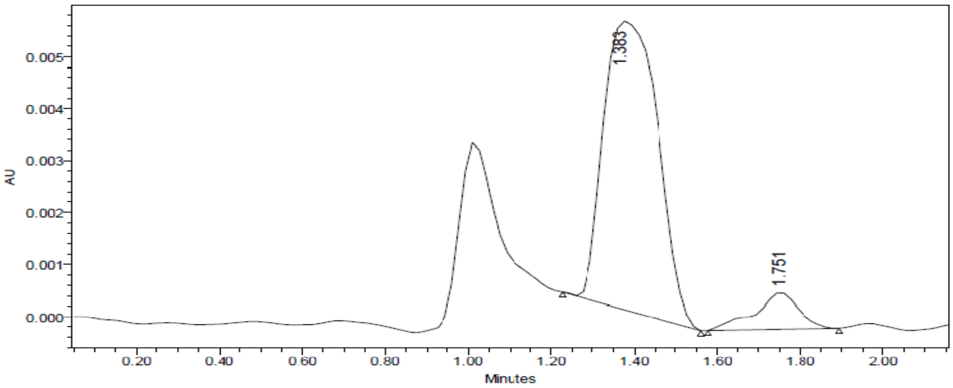

Figure 2: Chromatogram for trail 2

Observation

The separations of two compounds were obtained but improper separation is obtained and resolution is not good. So, we go for further trails.

Trail 3

Mobile phase : Methanol: TEA Buffer pH 3.2 (40:60% v/v)
Column : Zodiac C18 (4.6×250mm 5µm)
Flow rate : 1.0 ml/min

Wavelength : 245 nm
Column temp : 40ºC
Injection Volume : 10 µl
Run time : 10 minutes

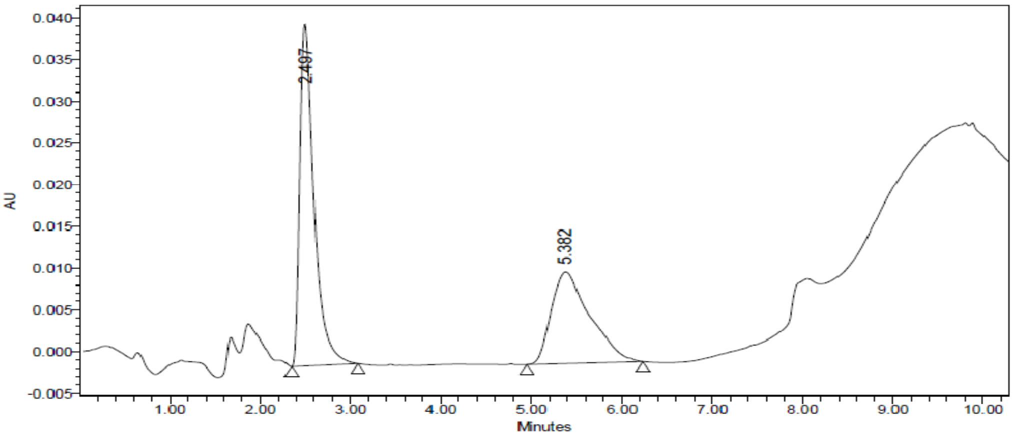

Figure 3: - Chromatogram for trail 3

Observation: The separations of two compounds were obtained but base line noise was very high, peak symmetry was not available. We go for further trail as the void peaks are obtained.

Trail 4

Mobile phase : Acetonitrile: Phosphate Buffer (pH-3.6) (30:70 v/v)
Column : Phenomenex Luna C18 (4.6×250mm, 5µm) particle size
Flow rate : 1 ml/min
Wavelength : 245 nm
Column temp : 40ºC
Injection Volume : 10 µl
Run time : 7 minutes

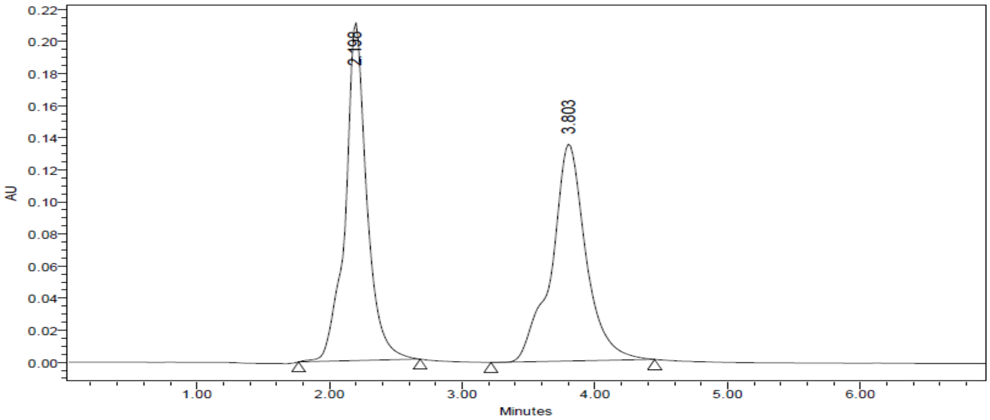

Figure 4: - Chromatogram for trail 3

Observation: After repeating the same conditions got proper peaks, base line is good and we can go for repeatability. Therefore separations of two peaks, base line, peak symmetry, resolution are proper.

Optimized Chromatogram (Standard)

Mobile phase : Acetonitrile: Phosphate Buffer (pH-4.6) (45:55 v/v)
Column : Phenomenex Luna C18 (4.6×250mm, 5µm) particle size
Flow rate : 1 ml/min
Wavelength : 245 nm
Column temp : 35ºC
Injection Volume : 10 µl
Run time : 7 minutes

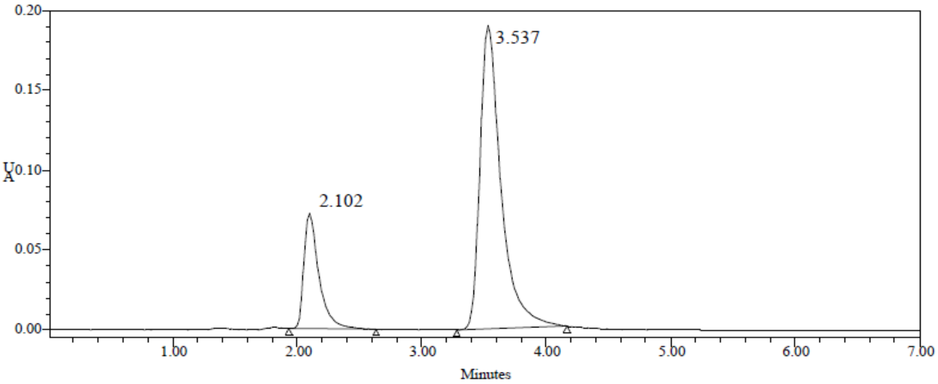

Figure 5: Optimized Chromatogram

Observation: From the above chromatogram it was observed that the Dapagliflozin and Saxagliptin peaks are well separated and they shows proper retention time, resolution, peak tail and plate count. So it's optimized trial.

Optimized Chromatogram (Sample)

Mobile phase	:	Acetonitrile: Phosphate Buffer (pH-4.6) (45:55 v/v)
Column	:	Phenomenex Luna C18 (4.6×250mm, 5µm) particle size
Flow rate	:	1 ml/min
Wavelength	:	245 nm
Column temp	:	35ºC
Injection Volume	:	10 µl
Run time	:	7 minutes

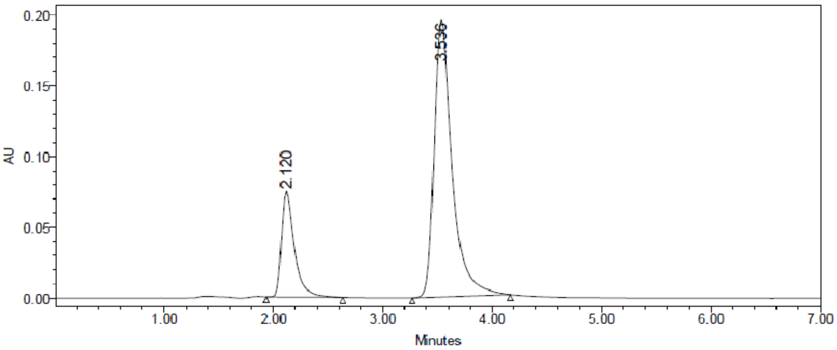

Figure 6: Optimized Chromatogram (Sample)

Acceptance criteria

- Resolution between two drugs must be not less than 2.
- Theoretical plates must be not less than 2000.
- Tailing factor must be not less than 0.9 and not more than 2.
- It was found from above data that all the system suitability parameters for developed method were within the limit.

Validation

Blank

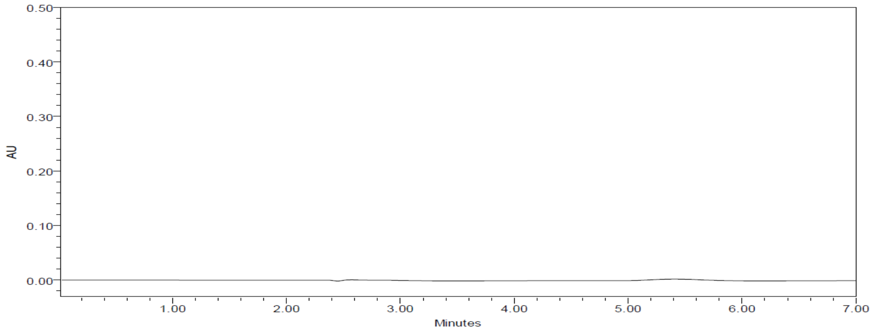

Figure 7: Chromatogram showing blank (mobile phase preparation)

System suitability

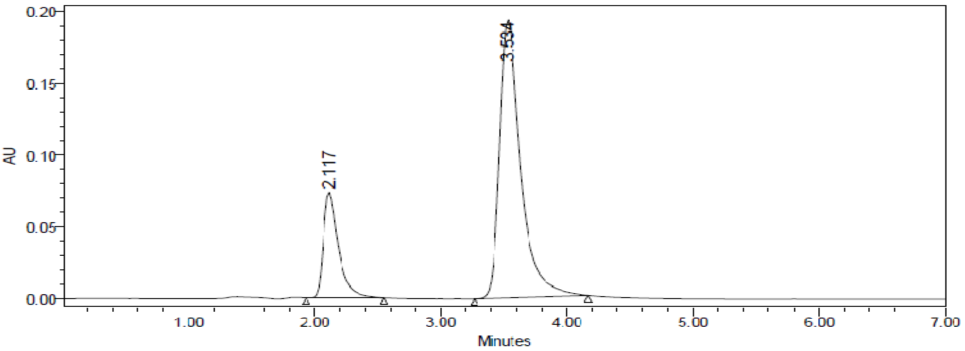

Figure 8: Chromatogram showing injection -1

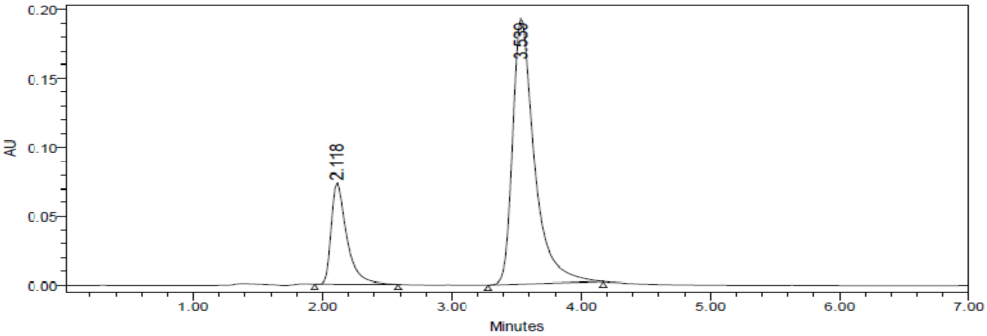

Figure 9: Chromatogram showing injection -2

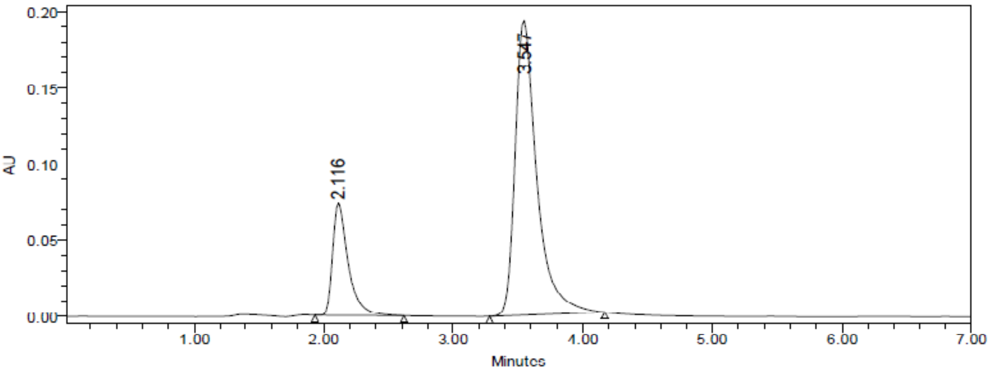

Figure 10: Chromatogram showing injection -3

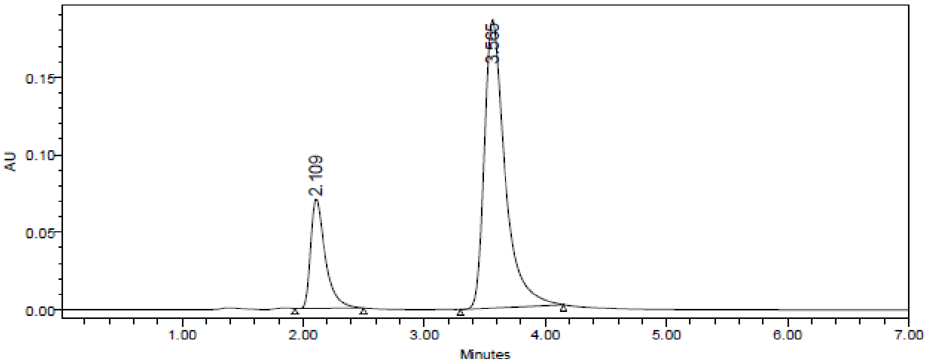

Figure 11: Chromatogram showing injection -4

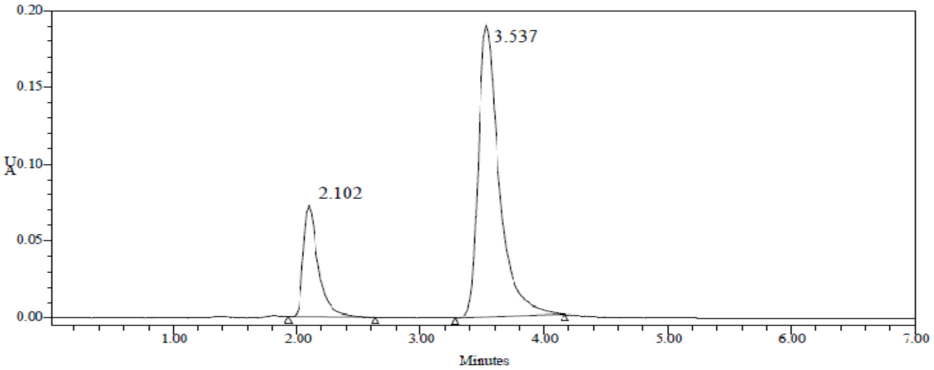

Figure 12: Chromatogram showing injection -5

Acceptance criteria

- %RSD of five different sample solutions should not more than 2
- The %RSD obtained is within the limit, hence the method is suitable.

Acceptance criteria

- %RSD for sample should be NMT 2.
- The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

Specificity

The ICH documents define specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components.

Analytical method was tested for specificity to measure accurately quantitated Dapagliflozin and Saxagliptin in drug product.

Assay (standard)

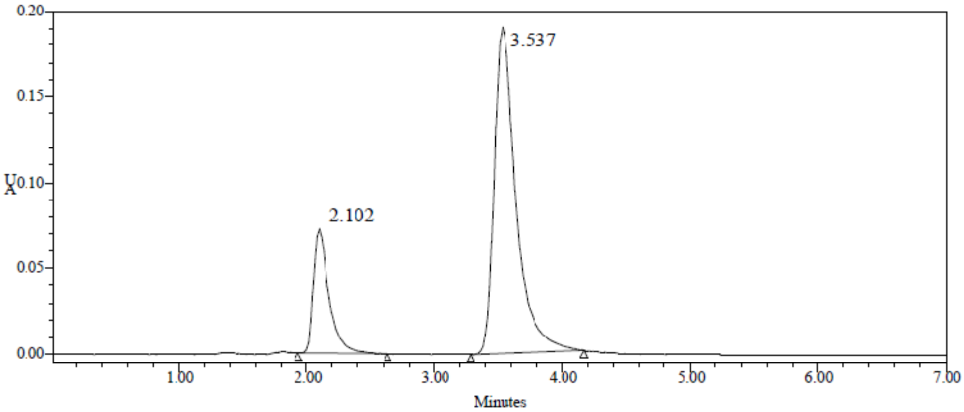

Figure 13: Chromatogram showing assay of standard injection -1

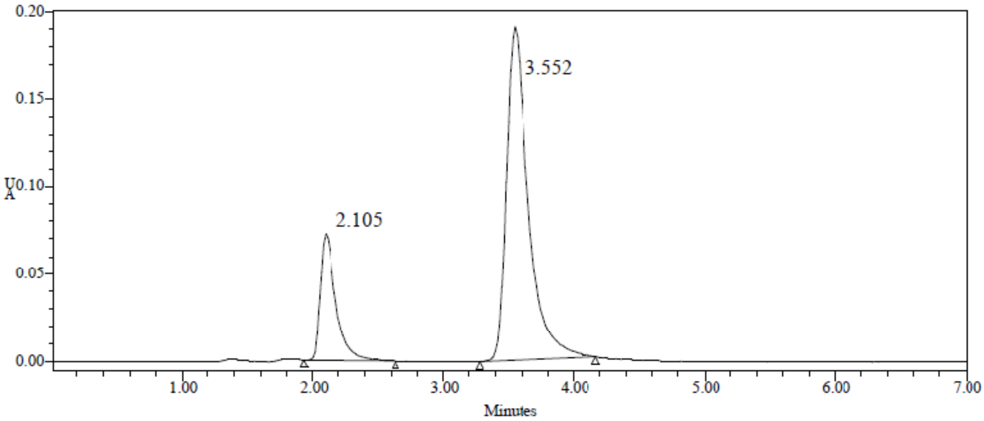

Figure 14: Chromatogram showing assay of standard injection -2

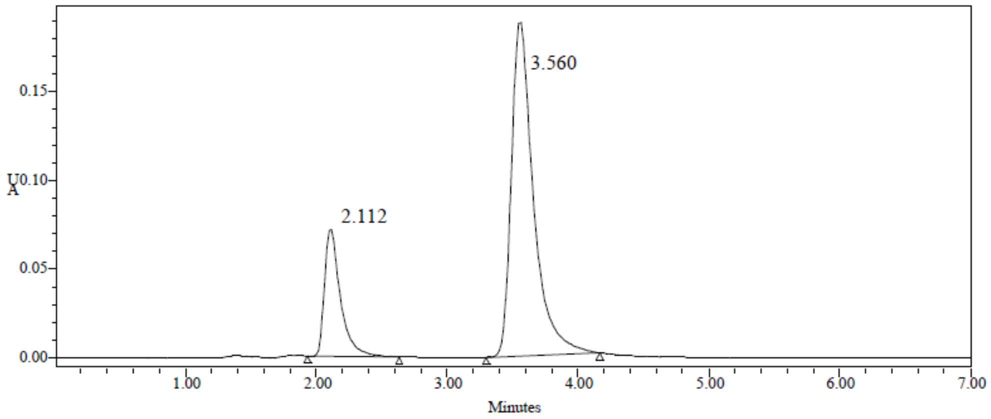

Figure 15: Chromatogram showing assay of standard injection -3

Assay (Sample)

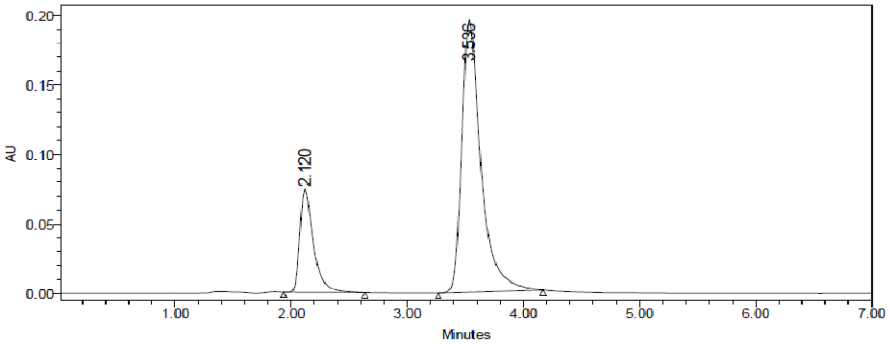

Figure 16: Chromatogram showing assay of sample injection-1

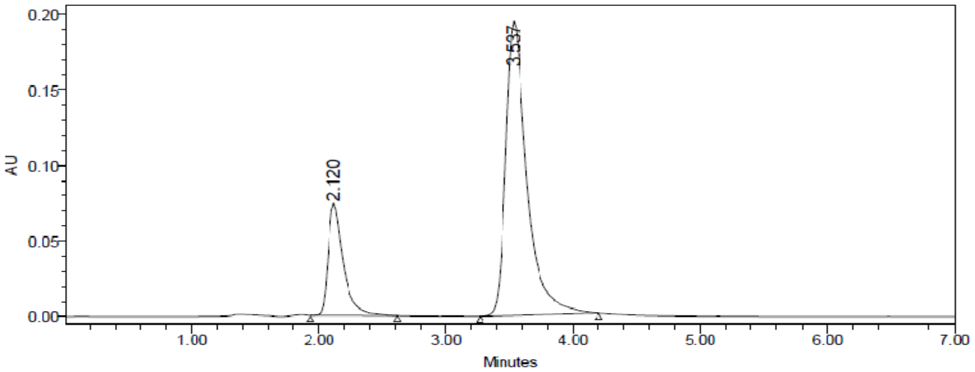

Figure 17: Chromatogram showing assay of sample injection-2

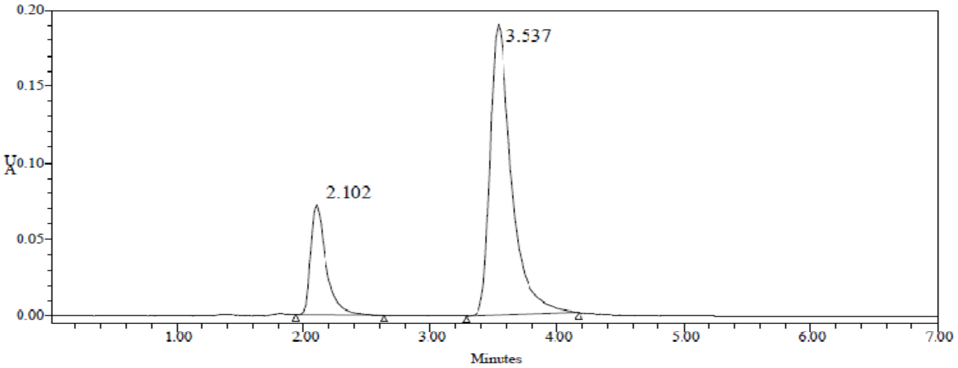

Figure 18: Chromatogram showing assay of sample injection-3

%ASSAY =

$$\frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

The % purity of Dapagliflozin and Saxagliptin in pharmaceutical dosage form was found to be 99.8%.

Linearity

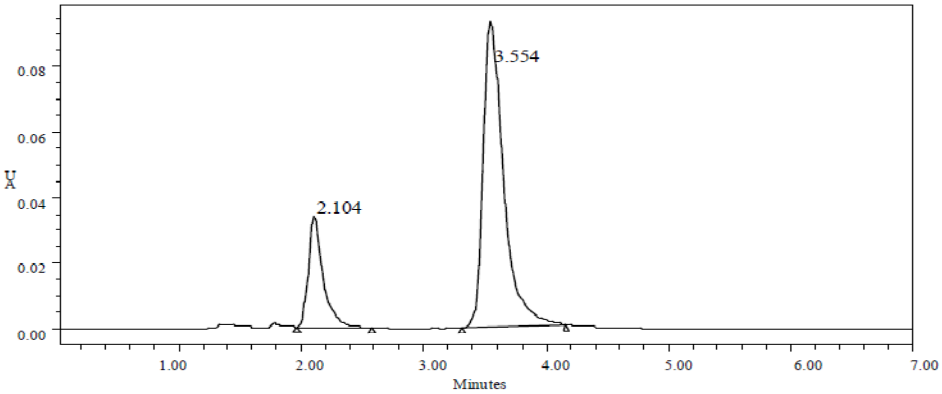

Figure 19: Chromatogram for linearity concentration-6µg/ml of Dapagliflozin &18 µg/ml of Saxagliptin

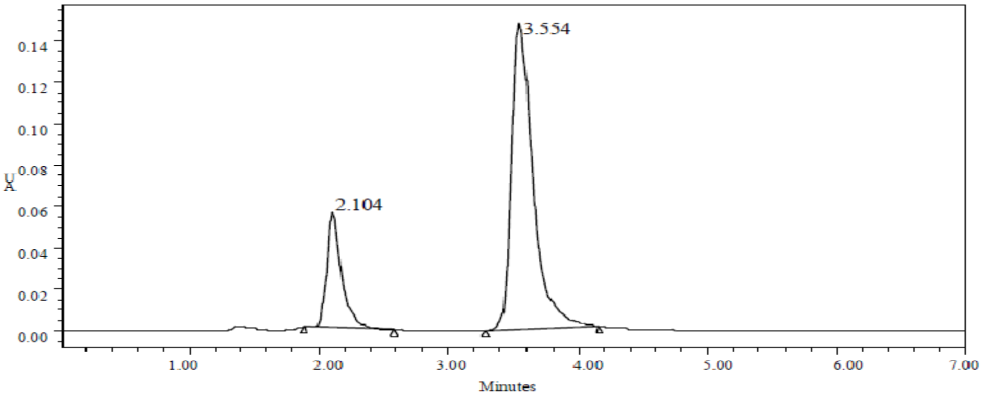

Figure 20: Chromatogram for linearity concentration-8 µg/ml of Dapagliflozin &24µg/ml of Saxagliptin

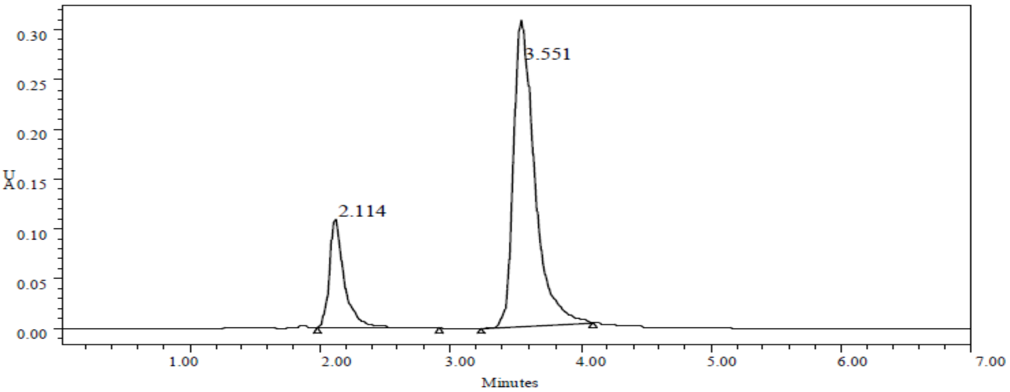

Figure 21: chromatogram for linearity concentration-10 µg/ml of Dapagliflozin &30µg/ml of Saxagliptin

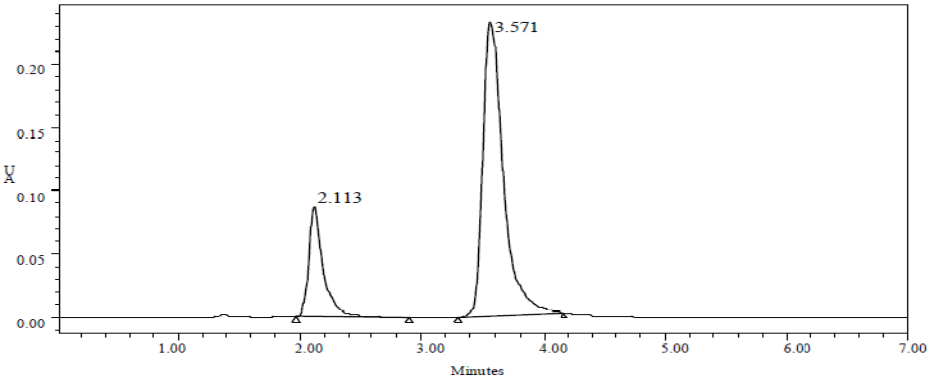

Figure 22: chromatogram for linearity concentration-12 µg/ml of Dapagliflozin &36 µg/ml of Saxagliptin

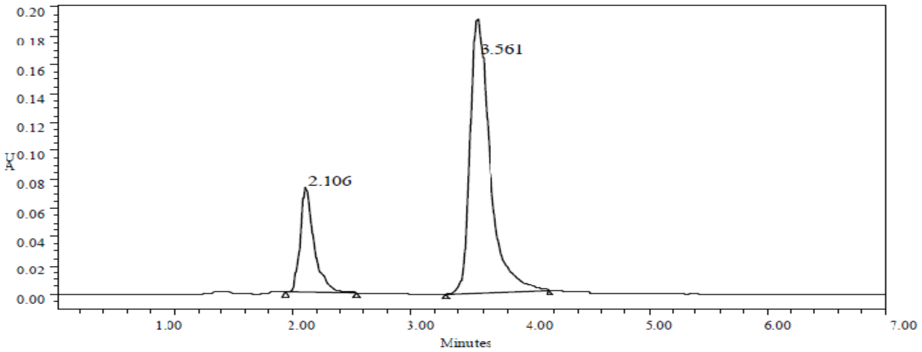

Figure 23: Chromatogram for linearity concentration-14 µg/ml of Dapagliflozin &42 µg/ml of Saxagliptin

Chromatographic data for linearity study

Dapagliflozin

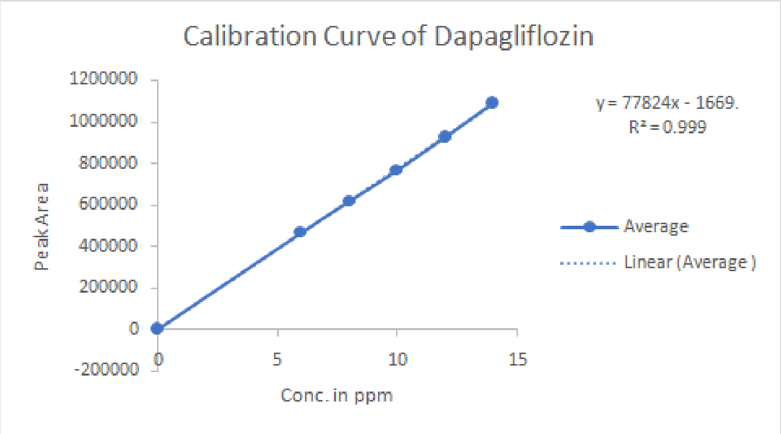

Figure 24: Calibration Graph for Dapagliflozin

Linearity plot

The plot of Concentration (x) versus the Average Peak Area (y) data of Dapagliflozin is a straight line.

Y = mx + c
Slope (m) = 77824
Intercept (c) = 1669
Correlation Coefficient (r) = 0.999

Validation criteria

The response linearity is verified if the Correlation Coefficient is 0.99 or greater.

Conclusion

Correlation Coefficient (r) is 0.99, and the intercept is 1669. These values meet the validation criteria.

Saxagliptin

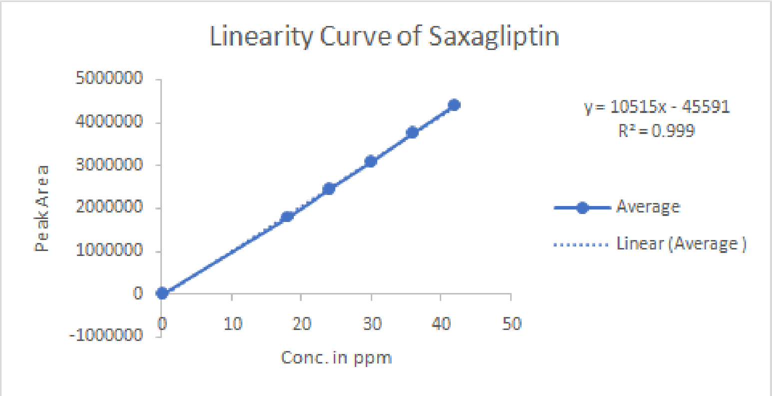

Figure 25: Calibration Graph for Saxagliptin

Linearity plot

The plot of Concentration (x) versus the Average Peak Area (y) data of Saxagliptin is a straight line.

Y = mx + c
Slope (m) = 10515
Intercept (c) = 45591
Correlation Coefficient (r) = 0.999

Validation criteria

The response linearity is verified if the Correlation Coefficient is 0.99 or greater.

Conclusion

Correlation Coefficient (r) is 0.99, and the intercept is 45591. These values meet the validation criteria.

Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.

Repeatability

Obtained Five (5) replicates of 100% accuracy solution as per experimental conditions. Recorded the peak areas and calculated % RSD.

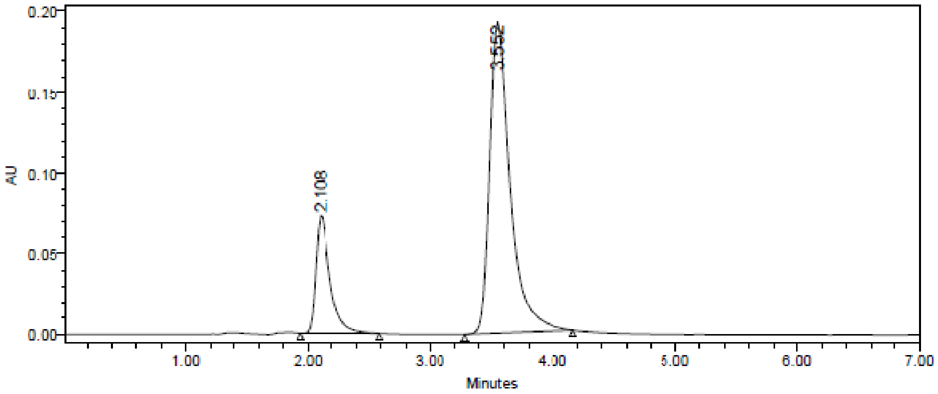

Figure 26: Chromatogram showing precision injection -1

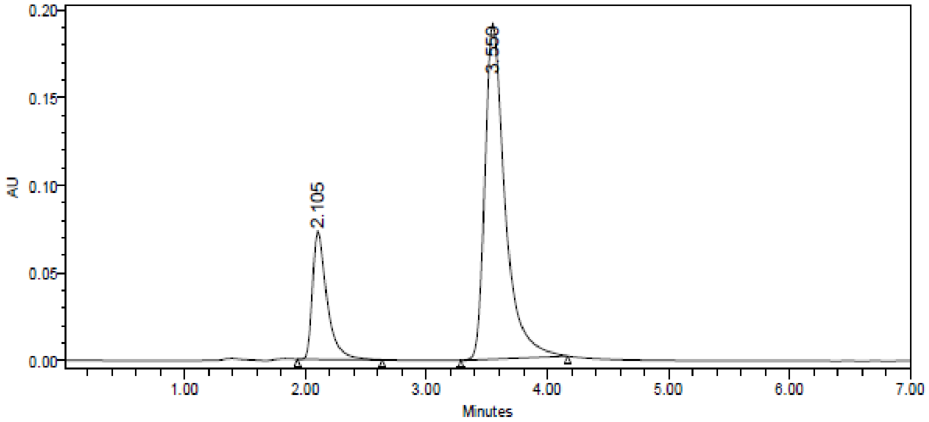

Figure 27: Chromatogram showing precision injection -2

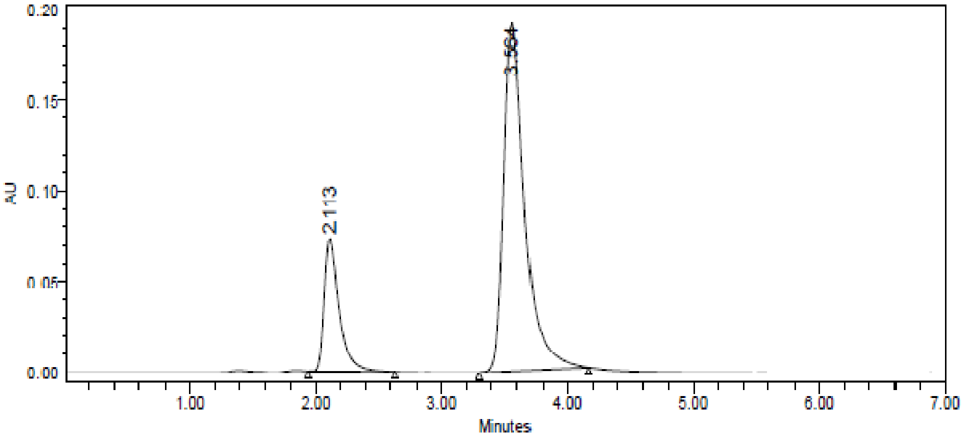

Figure 28: Chromatogram showing precision injection -3

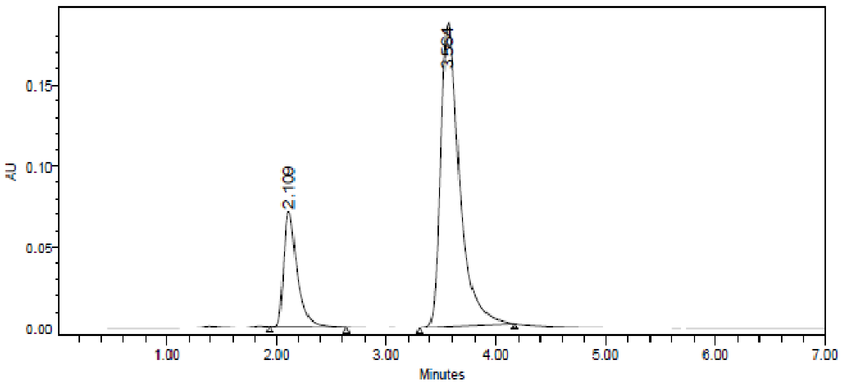

Figure 29: Chromatogram showing precision injection -4

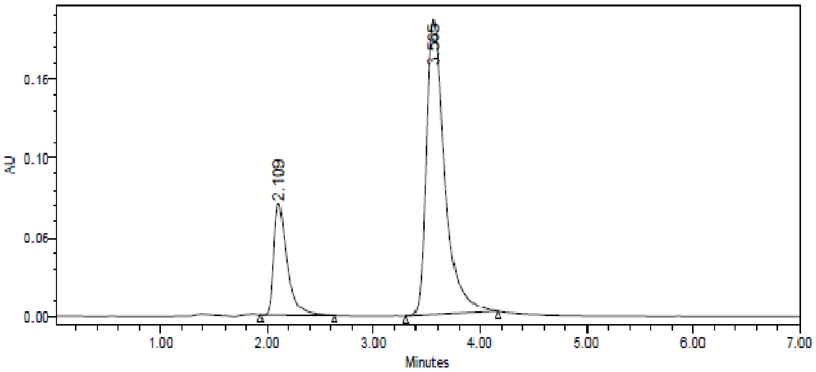

Figure 30: Chromatogram showing precision injection -5

Acceptance criteria

- %RSD for sample should be NMT 2
- The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

Acceptance criteria

- %RSD for sample should be NMT 2
- The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

Intermediate precision

Day 1

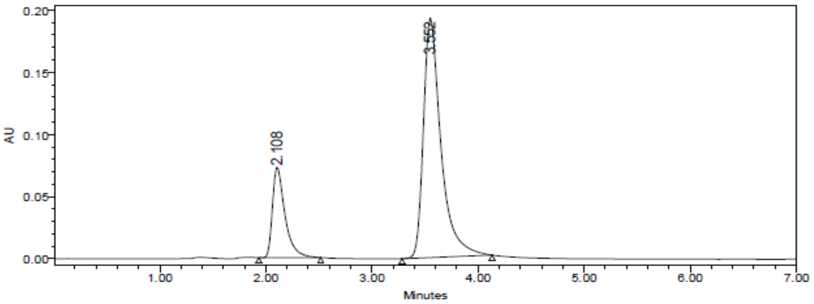

Figure 31: Chromatogram showing Day1 injection -1

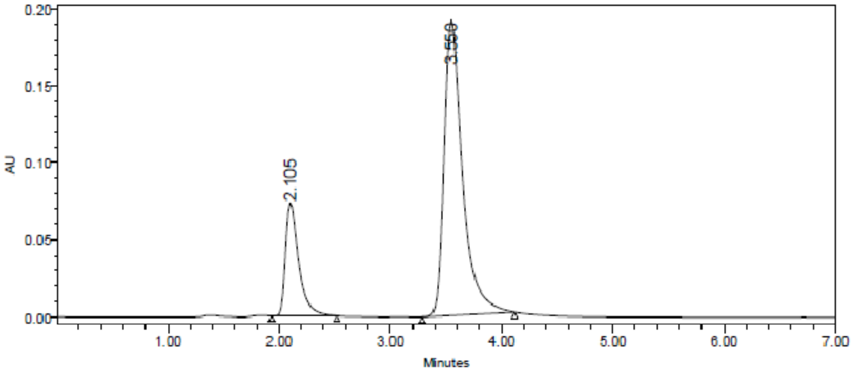

Figure 32: Chromatogram showing Day1 injection -2

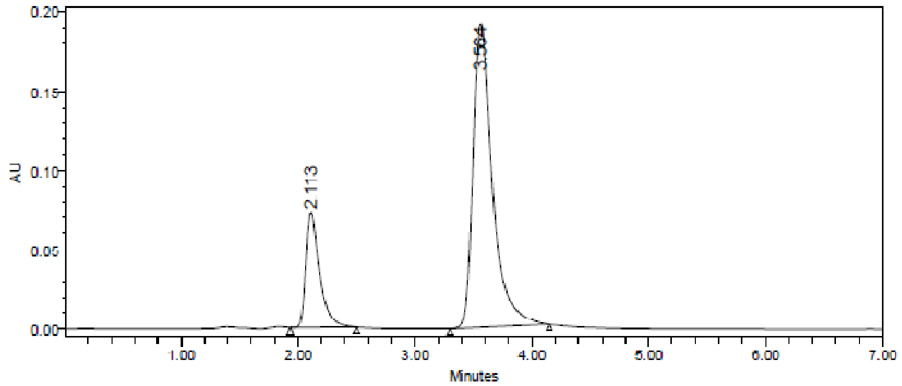

Figure 33: Chromatogram showing Day1 injection -3

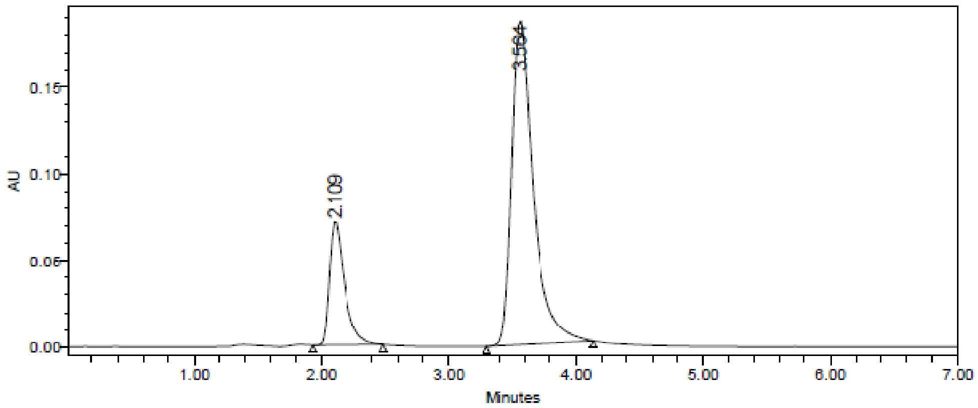

Figure 34: Chromatogram showing Day1 injection -4

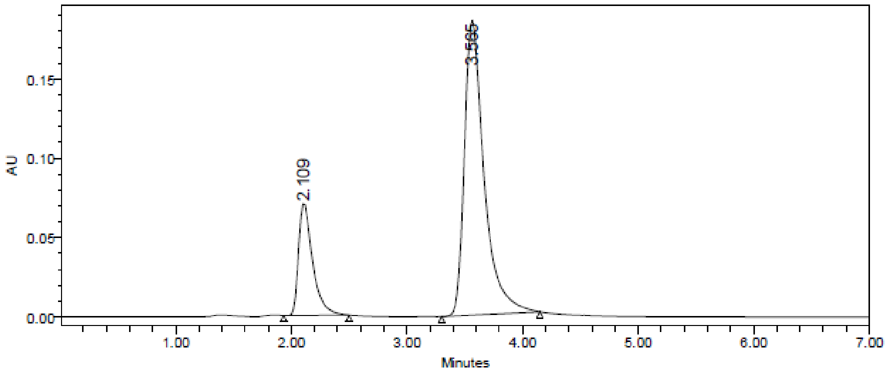

Figure 35: Chromatogram showing Day1 injection -5

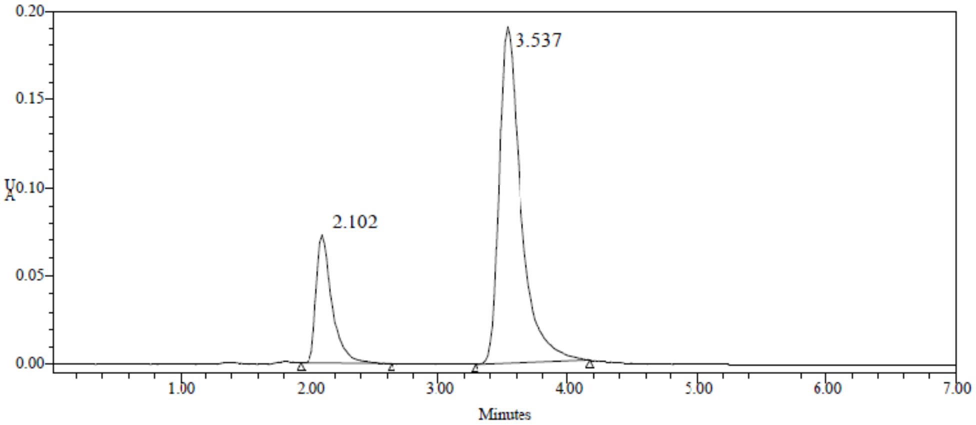

Figure 36: Chromatogram showing Day1 injection -6

Acceptance criteria

- %RSD of Six different sample solutions should not more than 2.

Acceptance criteria

- %RSD of Six different sample solutions should not more than 2.
- The %RSD obtained is within the limit, hence the method is rugged.

Day 2

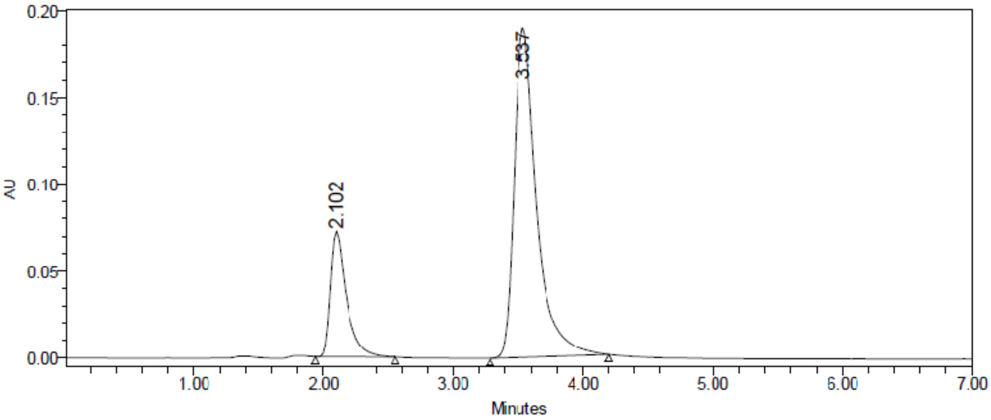

Figure 37: Chromatogram showing Day 2 injection -1

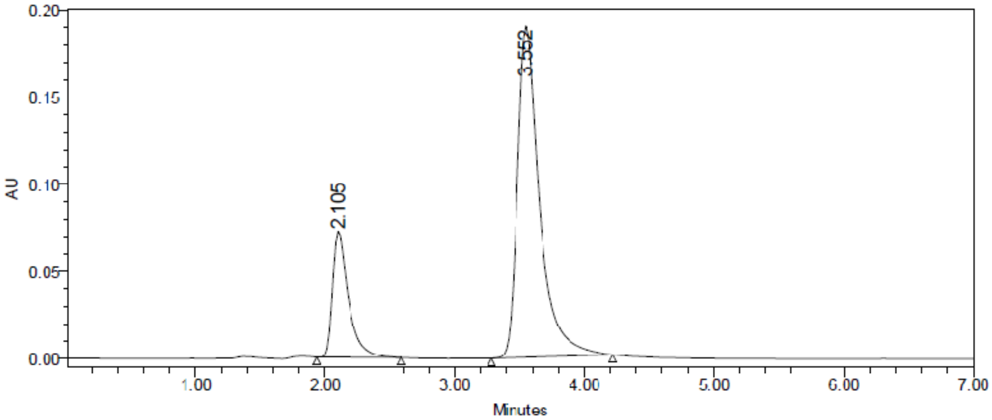

Figure 38: Chromatogram showing Day 2 injection -2

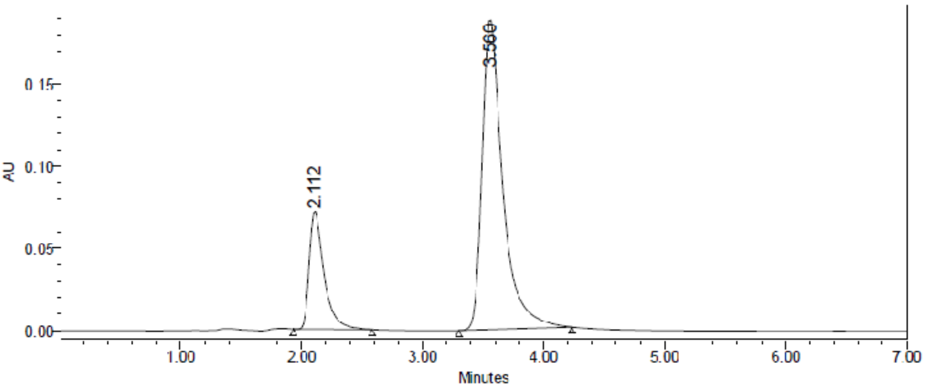

Figure 39: Chromatogram showing Day 2 injection -3

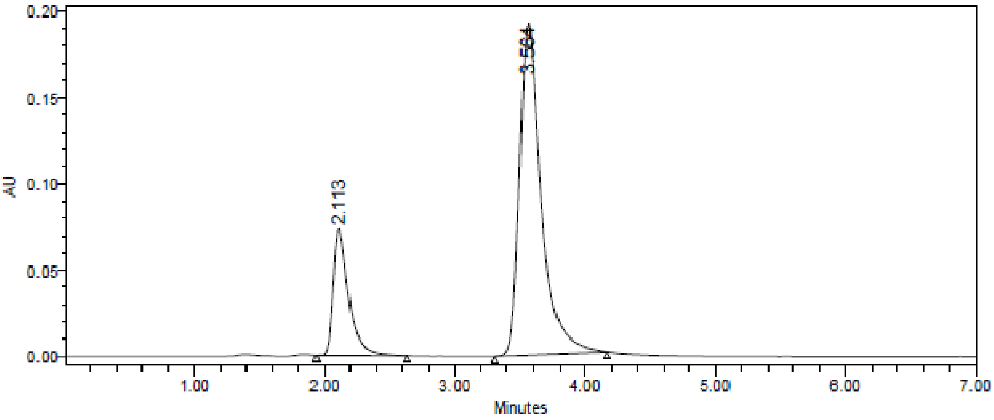

Figure 40: Chromatogram showing Day 2 injection -4

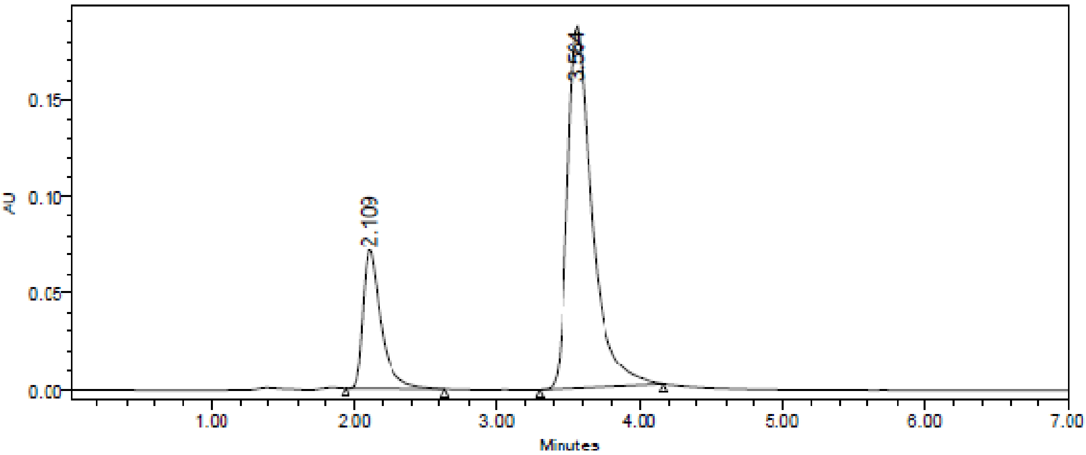

Figure 41: Chromatogram showing Day 2 injection -5

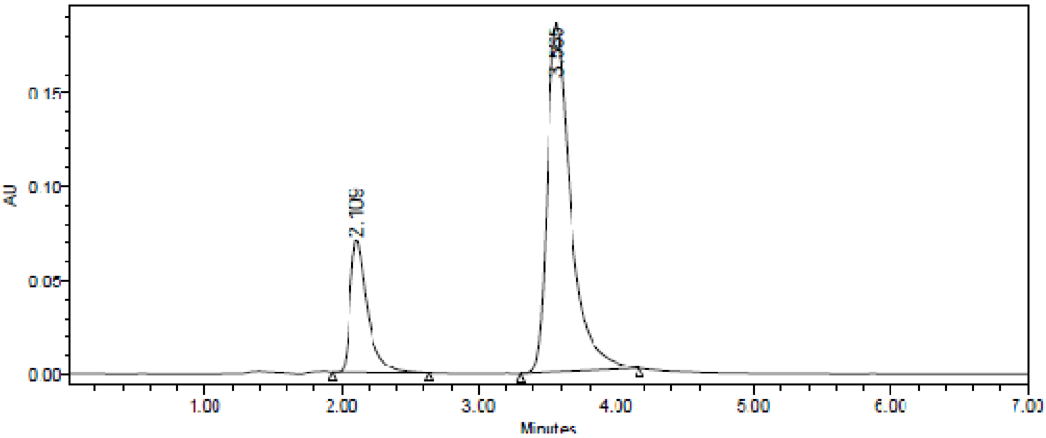

Figure 42: Chromatogram showing Day 2 injection -6

Acceptance criteria

- %RSD of Six different sample solutions should not more than 2.

Acceptance criteria

- %RSD of Six different sample solutions should not more than 2

Accuracy50%

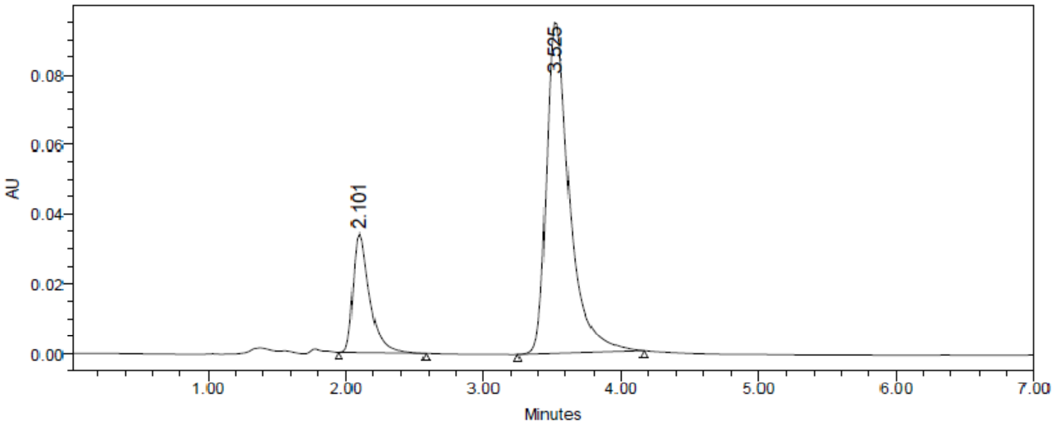

Figure 43: Chromatogram showing accuracy-50% injection-1

- The %RSD obtained is within the limit, hence the method is rugged.

Accuracy

Accuracy at different concentrations (50%, 100%, and 150%) were prepared and the % recovery was calculated.

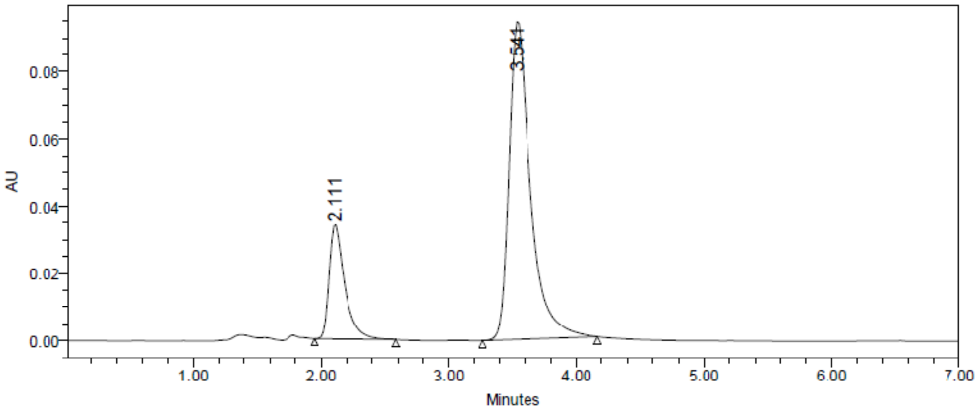

Figure 44: Chromatogram showing accuracy-50% injection-2

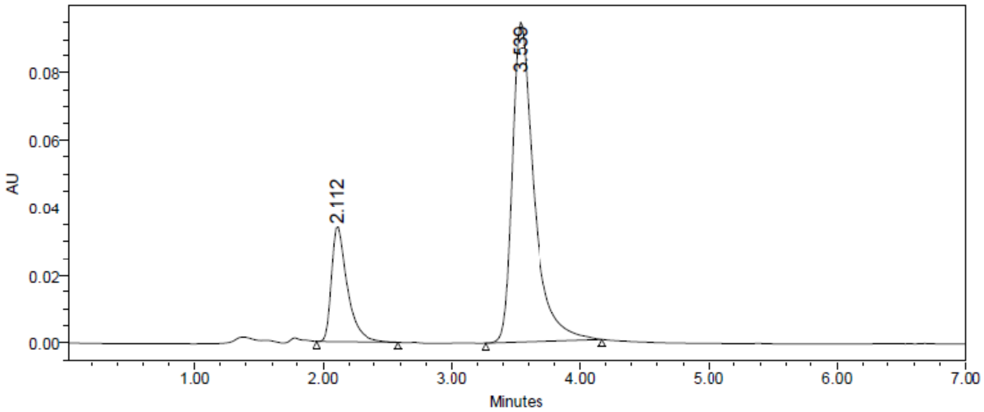

Figure 45: Chromatogram showing accuracy-50% injection-3

Accuracy100%

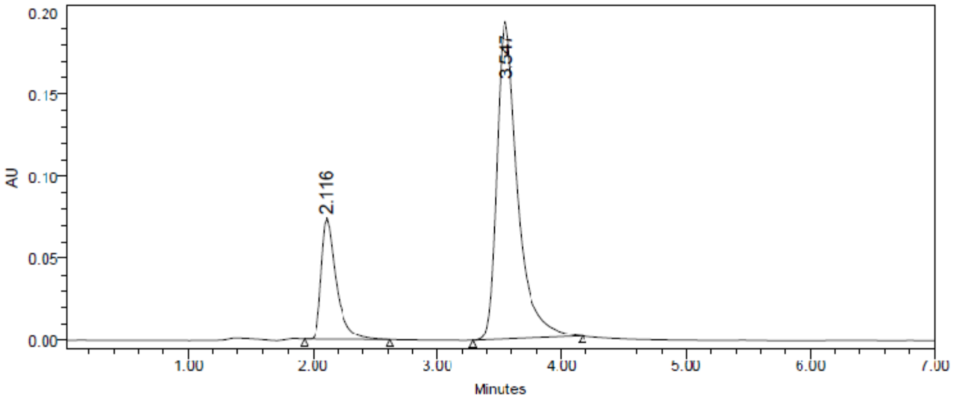

Figure 46: Chromatogram showing accuracy-100% injection-1

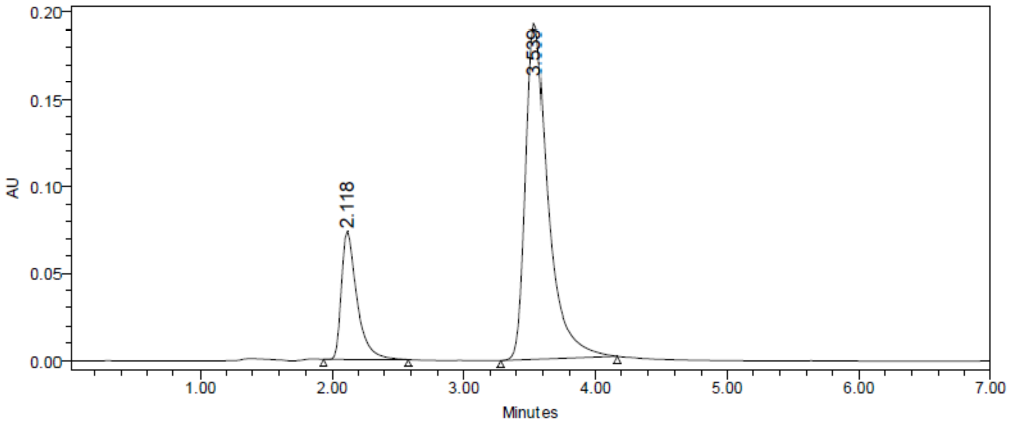

Figure 47: Chromatogram showing accuracy-100% injection-2

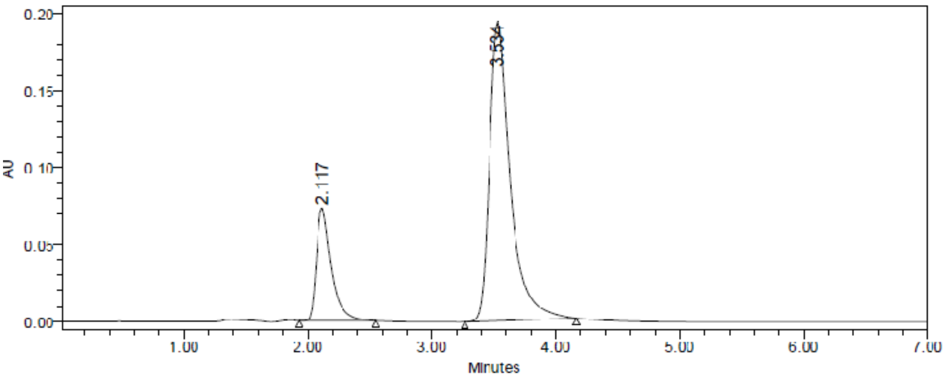

Figure 48: Chromatogram showing accuracy-100% injection-3

Accuracy150%

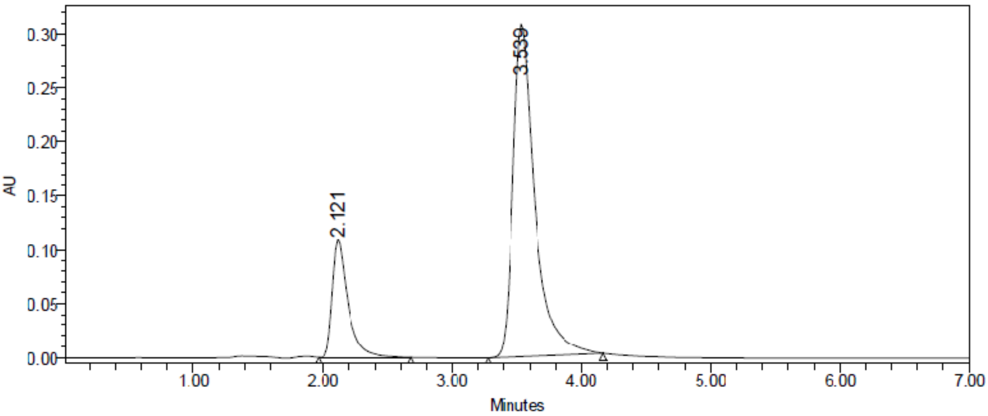

Figure 49: Chromatogram showing accuracy-150% injection-1

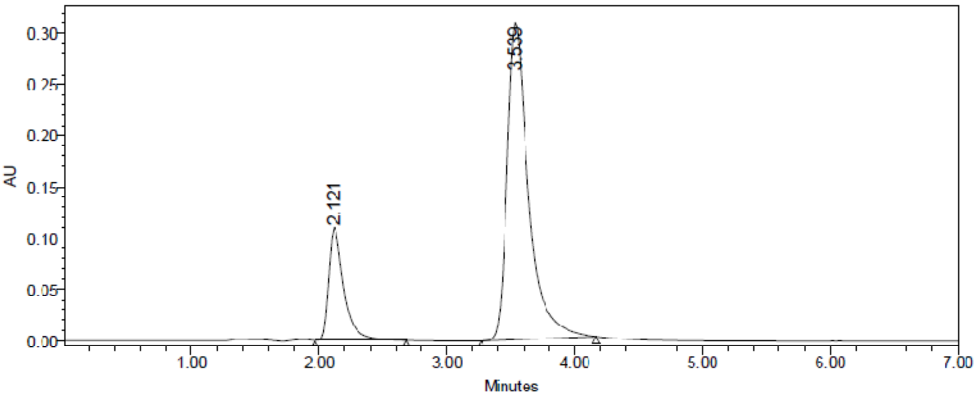

Figure 50: Chromatogram showing accuracy-150% injection-2

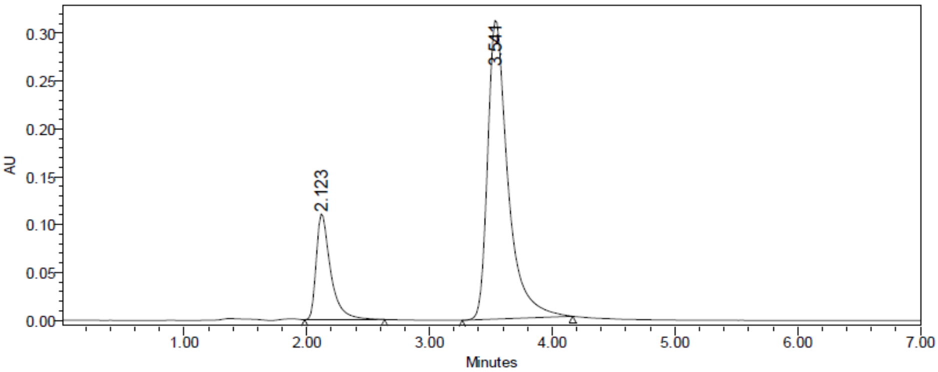

Figure 51: Chromatogram showing accuracy-150% injection-3

Acceptance criteria

- The percentage recovery was found to be within the limit (98-102%).

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

Limit of detection

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

$$LOD = 3.3 \times \sigma / s$$

Where
σ = Standard deviation of the response
S = Slope of the calibration curve

Result

Dapagliflozin

0.6µg/ml

Saxagliptin

0.8µg/ml

Limit of quantitation

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined.

$$LOQ = 10 \times \sigma / S$$

Where
σ = Standard deviation of the response
S = Slope of the calibration curve

Result

Dapagliflozin

1.8µg/ml

Saxagliptin

2.4µg/ml

Robustness

The robustness was performed for the flow rate variations from 0.9 ml/min to 1.1ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Dapagliflozin and Saxagliptin. The method is robust only in less flow condition and the method is robust even by change in the Mobile phase ±5%. The standard and samples of Dapagliflozin and Saxagliptin were injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate count.

Variation in flow

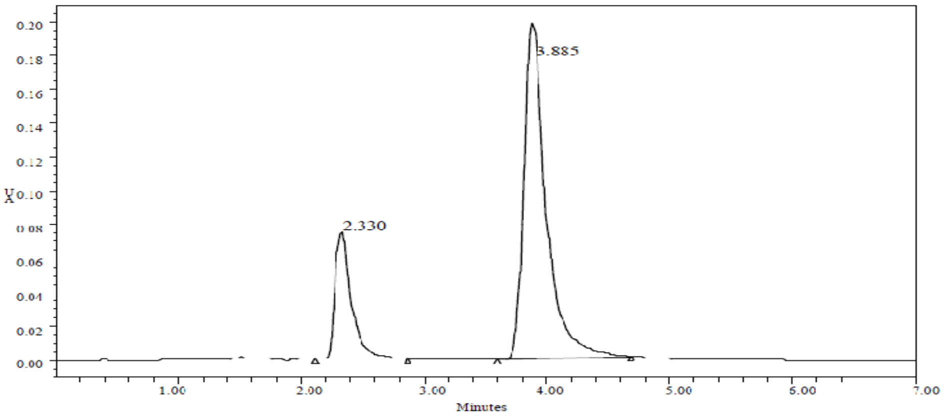

Figure 52: chromatogram showing less flow of 0.9ml/min

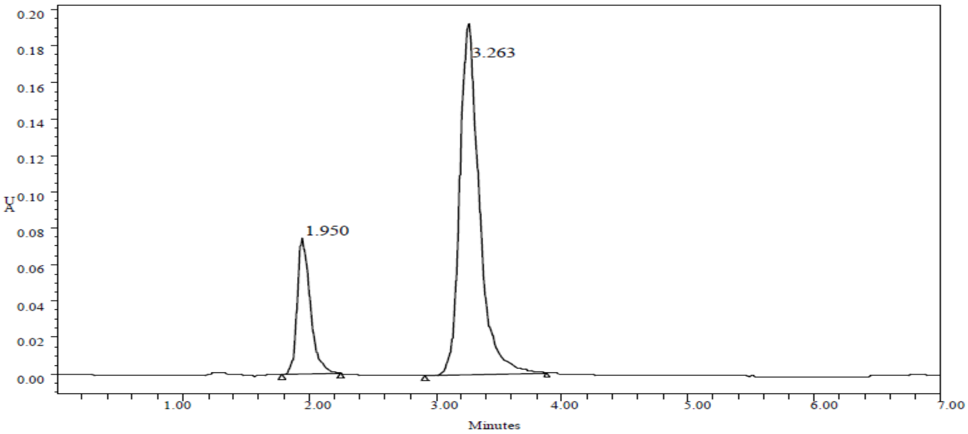

Figure 53: Chromatogram showing more flow of 1.1 ml/min

Variation of mobile phase organic composition

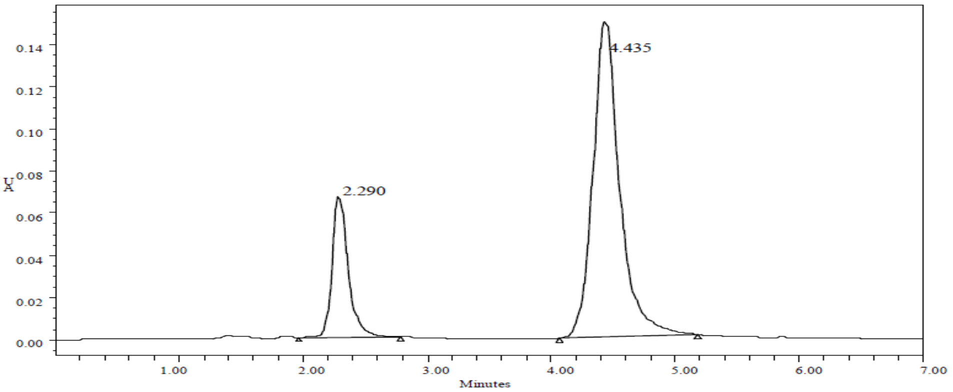

Figure 54: Chromatogram showing less organic composition

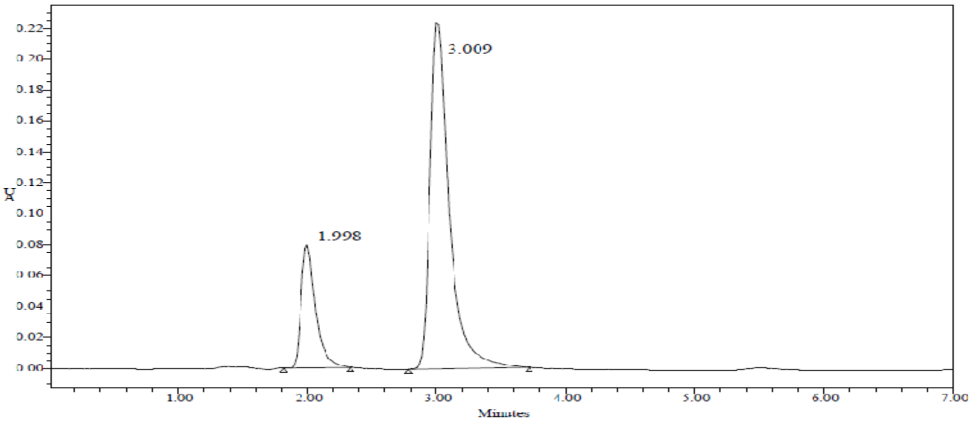

Figure 55: Chromatogram showing more organic composition

Dapagliflozin

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	765789	2.102	5587	1.7
Less Flow rate of 0.9 mL/min	758698	2.330	5458	1.7
More Flow rate of 1.1 mL/min	7689584	1.950	5696	1.7
Less organic phase	758412	2.290	5586	1.4
More organic phase	769852	1.998	5355	1.5

Acceptance criteria

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

Saxagliptin

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	2532158	3.537	5398	1.6
Less Flow rate of 0.9 mL/min	2458692	3.885	5329	1.7
More Flow rate of 1.1 mL/min	2658642	3.263	5256	1.7
Less organic phase	2452148	4.435	5214	1.2
More organic phase	2653894	3.009	5524	1.0

Acceptance criteria

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

SUMMARY AND CONCLUSION

A new method was established for simultaneous estimation of Dapagliflozin and Saxagliptin by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Dapagliflozin and Saxagliptin by using Phenomenex Luna C18 (4.6×250mm, 5µm) particle size, flow rate was 1ml/min, mobile phase ratio was (45:55 v/v) Acetonitrile: Phosphate Buffer (pH-4.6 was adjusted with orthophosphoric acid),detection wave length was 245nm. The instrument used was WATERS HPLC Auto Sampler, Separation module 2695, photo diode array detector 996, Empower-software version-2. The retention times were found to be 2.102mins and 3.537mins. The % purity of Dapagliflozin and Saxagliptin was found to be 99.8%.The system suitability parameters for Dapagliflozin and Saxagliptin such as theoretical plates and tailing factor were found to be within limits. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study n Dapagliflozin and Saxagliptin was found in concentration range of 6µg-14µg and 18µg-42µg and correlation coefficient (r^2) was found to be 0.999 and 0.999, % recovery was found to be 100.351% and 100.93%, %RSD for repeatability was 0.177 and 0.595. The precision study was precise, robust, and repeatable. LOD value was 0.6 and 0.8, and LOQ value was 1.8 and 2.4 respectively.

Hence the suggested RP-HPLC method can be used for routine analysis of Dapagliflozin and Saxagliptin in API and Pharmaceutical dosage form.

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