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Analytical Method Development and Validation for the Simultaneous Estimation of Sumatriptan and Naproxen by RP-HPLC Method

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Abstract

High performance liquid chromatography is at present one of the most sophisticated tool of the analysis. The estimation of Sumatriptan and Naproxen was done by RP-HPLC. The Phosphate buffer was p^H 3.0 and the mobile phase was optimized with consists of Methanol: Phosphate buffer mixed in the ratio of 70:30 % v/ v. Inertsil C_{18} column (4.6 x 150mm, 5µm) or equivalent chemically bonded to porous silica particles was used as stationary phase. The detection was carried out using UV detector at 260 nm. The solutions were chromatographed at a constant flow rate of 0.8 ml/min. the linearity range of Sumatriptan and Naproxen were found to be from 100-500 µg/ml of Sumatriptan and 1-5µg/ml of Naproxen. Linear regression coefficient was not more than 0.999. The values of % RSD are less than 2% indicating accuracy and precision of the method. The percentage recovery varies from 98-102% of Sumatriptan and Naproxen. LOD and LOQ were found to be within limit. The results obtained on the validation parameters met ICH and USP requirements .it inferred the method found to be simple, accurate, precise and linear. The method was found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision.

Keywords

Methanol: Phosphate buffer, Inertsil C_{18} column, Sumatriptan and Naproxen.

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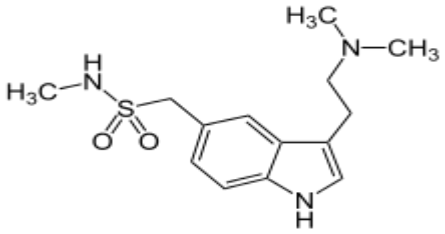
INTRODUCTION:

Sumatriptan is structurally similar to serotonin (5HT), and is a of cases. 5-HT receptor (types 5-HT1D and 5-HT1B) agonist. The specific receptor subtypes it activates are present on the cranial arteries and veins. Acting as an agonist at these receptors, sumatriptan reduces the vascular inflammation associated with migraines. Sumatriptan is also shown to decrease the

activity of the trigeminal nerve, which it is presumed, accounts for sumatriptan's efficacy in treating cluster headaches. The injectable form of the drug has been shown to abort a cluster headache within fifteen minutes in 96%.

Category: H1-antihistamine

Structure:



Naproxen works by reversibly inhibiting both the COX-1 and COX-2 enzymes

Category: NSAID'S

Structure:

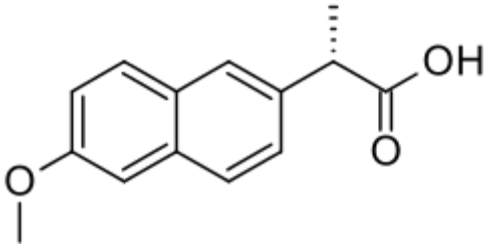


Table 1: List of marketed formulations

Sl.No	Brand name	Drug -1 (mg)	Drug-2(mg)	Manufacturer
1	Treximet	Sumatriptan 85mg	Naproxen 500mg	Glaxosmithkline pharmaceuticals

A variety of methods are available for analyzing pharmaceutical compounds. High Performance/ Pressure Liquid Chromatography (HPLC) is one of the best methods of choice for analyzing a variety of natural and synthetic compounds. It is because it offers high performance over ambient pressure. The phenomenal growth in chromatography is largely due to the introduction of the technique called high-pressure liquid chromatography, which is frequently called high-performance liquid chromatography (both are abbreviated as HPLC).

Reversed-Phase Chromatography, the most widely used chromatographic mode, is used to separate neutral molecules in solution on the basis of their hydrophobicity. As the name suggests, Reversed-Phase Chromatography is the reverse of Normal-Phase Chromatography in the sense that it involves the use of a non-polar stationary phase and a polar mobile phase. As a result, a decrease in the polarity of the mobile phase results in a decrease in solute retention. Modern Reversed-Phase Chromatography typically refers to the use of chemically bonded stationary phases, where a functional group is bonded to silica, for this reason, Reversed-Phase Chromatography is often referred to in the literature as Bonded-Phase Chromatography.

The number of drugs introduced into the market is increasing every year. These Drugs may be either new entities or partial structural modification of the existing one. Very often there is a time lag from the date of introduction of a drug into the market to the date of its inclusion in pharmacopoeias. This happens the possible uncertainty's in the continuous and wider usage of these drugs, reports of new toxicities (Resulting in their withdrawal from the market). Development of Patient resistance and introduction of better drugs by competitors, under these conditions, standards and analytical procedures for these drugs may not be available in the Pharmacopeia, it becomes necessary, therefore to develop newer analytical methods for such drugs.

Analytical Method Validation can be defined as (ICH) "Establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality

characteristics". Method validation study include system suitability, linearity, precision, accuracy, specificity, ruggedness, robustness, limit of detection, limit of quantification and stability of samples, reagents, instruments.

MATERIALS AND METHODS:

1. INSTRUMENTS USED:

Table 2: Instruments used

SL. No	Instrument	Model
1	HPLC	WATER, software: Empower, 2695 separation module, PDA detector.
2	UV/VIS spectrophotometer	LABINDIA UV 3000$^+$
3	pH meter	Adwa – AD 1020
4	Weighing machine	Afcoset ER-200A
5	Pipettes and Burettes	Borosil
6	Beakers	Borosil

2. CHEMICALS USED:

Table 3: Chemicals used

SL. No	Chemical	Brand
1	Sumatriptan	Mylon
2	Naproxen	Cipla
3	KH_2PO_4	FINER chemical LTD
4	Water and Methanol for HPLC	LICHROSOLV (MERCK)
5	Acetonitrile for HPLC	MOLYCHEM
6	Ortho phosphoric Acid	MERCK

Drug samples:

The working standard was received as gift sample from Mylan Laboratories Ltd., Hyderabad, India.

Formulation used:

Treximet tablets containing Sumatriptan 85mg and Naproxen 500mg were procured from local pharmacy.

3. HPLC METHOD DEVELOPMENT:

3.1. Mobile Phase Optimization:

Initially the mobile phase tried was methanol: Ammonium acetate buffer and Methanol: phosphate buffer with various combinations of pH as well as varying proportions. Finally, the mobile phase was optimized to potassium dihydrogen phosphate with buffer (pH 3.0), Methanol in proportion 30: 70 v/v respectively.

3.2. Wave length selection:

UV spectrum of 10 µg / ml Sumatriptan and Naproxen in diluents (mobile phase composition) was recorded by scanning in the range of 200nm to 400nm. From the UV spectrum wavelength selected as 260. At this wavelength both the drugs show good absorbance.

3.3. Optimization of Column:

The method was performed with various columns like C18 column, hypersil column, lichrosorb, and inertsil ODS column. Inertsil ODS (4.6 x 150mm, 5µm) was found to be ideal as it gave good peak shape and resolution at 0.8ml/min flow.

3.4. OPTIMIZED CHROMATOGRAPHIC CONDITIONS:

Instrument used : Waters HPLC with auto sampler and PAD or detector.

Temperature : Ambient

Column : Inertsil ODS (4.6 x 150mm, 5µm)

Buffer : 6.8 grams of potassium dihydrogen ortho phosphate in 1000 ml water pH adjusted with ortho phaosparic acid.

pH : 3.0

Mobile phase : 30% buffer 70% Methanol

Flow rate : 0.8 ml per min

Wavelength : 260 nm

Injection volume : 10 µl

Run time : 10min.

3.5. PREPARATION OF BUFFER AND MOBILE PHASE:

3.5.1. Preparation of Phosphate buffer:

Accurately weighed 6.8 grams of KH_2PO_4 was taken in a 1000ml volumetric flask, dissolved and diluted to 1000ml with HPLC water and the volume was adjusted to pH 3.0 with Orthophosphoric acid.

3.5.2. Preparation of mobile phase:

Accurately measured 300 ml (30%) of above buffer and 700 ml of Methanol HPLC (70%) were mixed and degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

3.5.3. Diluent Preparation:

The Mobile phase was used as the diluent.

3.6. PREPARATION OF THE SUMATRIPTAN AND NAPROXEN STANDARD AND SAMPLE SOLUTION:

3.6.1. Standard Solution Preparation:

Accurately weigh and transfer 10 mg of Sumatriptan and Naproxen 10mg of working standard into a 10mL& 100ml clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 3ml& 0.3ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

3.6.2. Sample Solution Preparation:

Accurately weigh 10 tablets crush in mortar and pestle and transfer equivalent to 10 mg of Sumatriptan and Naproxen (marketed formulation) sample into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further pipette 3 ml of Sumatriptane and Naproxen of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

3.6.3. Procedure:

Inject 20 µL of the standard, sample into the chromatographic system and measure the areas for Sumatriptan and Naproxen peaks and calculate the %Assay by using the formulae.

3.7. SYSTEM SUITABILITY:

Tailing factor for the peaks due to Sumatriptan and Naproxen in Standard Solution Should not be more than 2.0

Theoretical plates for the Sumatriptan and Naproxen peaks in Standard solution should not be less than 2000.

$$\text{Assay \%} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{Avg.}{Label} \times \frac{Wt}{Claim} \times 100$$

Where:

AT = average area counts of sample preparation.

As= average area counts of standard preparation.

WS = Weight of working standard taken in mg.

P = Percentage purity of working standard

LC = LABEL CLAIM OF drug mg/ml

Table 4: Sample and standard details

S. No.	Samples
1	Sumatriptan and Naproxen Tablets 50mg and 0.5mg
2	Sumatriptan and Naproxen working standards

4. METHOD VALIDATION SUMMARY:

4.1. PRECISION:

Preparation of stock solution:

Accurately weigh and transfer 25 mg of Sumatriptan and Naproxen working standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further pipette 3 ml of Sumatriptan &Naproxen of the above stock solution 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.

Acceptance Criteria:

The % RSD for the area of five standard injections results should not be more than 2%**.**

4.2. INTERMEDIATE PRECISION/RUGGEDNESS:

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different day by using different make column of same dimensions.

Preparation of stock solution:

Accurately weigh and transfer 25 mg of Sumatriptan and 10mg of Naproxen working standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further pipette 3ml of Sumatriptan &Naproxen of the above stock solution 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.

Acceptance Criteria:
The % RSD for the area of five standard injections results should not be more than 2%.

4.3. ACCURACY:

Preparation of Standard stock solution:
Accurately weigh and transfer 10 mg of Sumatriptan and Naproxen 10mg of working standard into a 10mL& 100ml clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.
(Stock solution)
Further pipette 3ml& 0.3ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation Sample solutions:

a) . For preparation of 50% solution (With respect to target Assay concentration):
Accurately weigh and transfer 5mg of Sumatriptan and 5.3mg of Naproxen working standard into a 10mL and 100 ml 0f clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock Solution). Further pipette 3 ml of Sumatriptane and 0.3 ml of Naproxen of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

b). For preparation of 100% solution (With respect to target Assay concentration):
Accurately weigh and transfer 10 mg of Sumatriptan and 10 mg of Naproxen working standard into a 10mL and 100 ml 0f clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock Solution). Further pipette 3 ml of Sumatriptan & 0.3 ml of Naproxen of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

c). For preparation of 150% solution (With respect to target Assay concentration):
Accurately weigh and transfer 14.4mg of Sumatriptan and 14.5mg of Naproxen working standards into a 10mL and 100ml of clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further pipette 3 ml of Sumatriptan & 0.3 ml of Naproxen of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure:
Inject the standard solution, Accuracy -50%, Accuracy -100% and Accuracy -150% solutions. Calculate the Amount found and Amount added for Sumatriptan &Naproxen and calculate
the individual recovery and mean recovery values.

Acceptance Criteria:
- The % Recovery for each level should be between 98.0 to 102.0%.

4.4. LINEARITY:

Preparation of stock solution:
Accurately weigh 10 tablets crush in mortar and pestle and transfer equivalent to 10 mg of Sumatriptan and Naproxen (marketed formulation) sample into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).

Preparation of Level – I (100ppm of Sumatriptan &1ppm of Naproxen):
1ml and 0.1 ml of stock solutions has taken in different 10ml of volumetric flasks, dilute up to the mark with diluent.

Preparation of Level – II (200ppm of Sumatriptan &2ppm of Naproxen):
2ml and 0.2 ml of stock solutions has taken in different 10ml of volumetric flasks, dilute up to the mark with diluent.

Preparation of Level – III (300ppm of Sumatriptan &3ppm of Naproxen):
3ml and 0.3 ml of stock solutions has taken in different 10ml of volumetric flasks, dilute up to the mark with diluent.

Preparation of Level – IV (400ppm of Sumatriptan &4ppm of Naproxen):
4ml and 0.4 ml of stock solutions has taken in different 10ml of volumetric flasks, dilute up to the mark with diluent.

Preparation of Level – V (500ppm of Sumatriptan &5ppm of Naproxen)
5ml and 0.5 ml of stock solutions has taken in different 10ml of volumetric flasks, dilute up to the mark with diluent.

Procedure:
Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

Acceptance Criteria:
- Correlation coefficient should be not less than 0.99

4.5. LIMIT OF DETECTION:

4.5.1. Limit of Detection for Sumatriptan:

Preparation of 300µg/ml solution:
Accurately weigh and transfer 10 mg of Sumatriptan working standard into a 10mL clean dry volumetric

flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further pipette 3ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation of 0.12µg/ml solution):

Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Pipette 0.4mL of 1µg/ml solution into a 10 ml of volumetric flask and dilute up to the mark with diluent.

Calculation of S/N Ratio:

Average Baseline Noise obtained from Blank: 52µV
Signal Obtained from LOD solution: 152 µV
S/N = 152/52 = 2.9

Acceptance Criteria:

- S/N Ratio Value Shall be 3 for LOD solution.

4.5.2. Limit of Detection for Naproxen:

Preparation of 3µg/ml solution:

Accurately weigh and transfer 10mg of Naproxen working standard into a 100ml clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further pipette 0.3ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation of 0.015µg/ml solution):

Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Further pipette 0.5ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Calculation of S/N Ratio:

Average Baseline Noise obtained from Blank: 52 µV
Signal Obtained from LOD solution: 156 µV
S/N = 156/52 = 3.0

Acceptance Criteria:

- S/N Ratio value shall be 3 for LOD solution.

4.6. LIMIT OF QUANTIFICATION:

4.6.1 Limit of Quantification for Sumatriptan:

Preparation of 300µg/ml solution:

Accurately weigh and transfer 10 mg of Sumatriptan working standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further pipette 3ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation of 0.42µg/ml solution):

Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Pipette 1.0mL of above solution into a 10 ml of volumetric flask and dilute up to the mark with diluent. Pipette 1.4 mL of above solution into a 10 ml of volumetric flask and dilute up to the mark with diluent.

Calculation of S/N Ratio:

Average Baseline Noise obtained from Blank: 52 µV
Signal Obtained from LOQ solution: 522µV
S/N = 522/52 = 10.03

Acceptance Criteria:

- S/N Ratio value shall be 10 for LOQ solution.

4.6.2 Limit of Quantification for Naproxen:

Preparation of 3µg/ml solution:

Accurately weigh and transfer 10mg of Naproxen working standard into a 100mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further pipette 0.3ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation of 0.05µg/ml solution):

Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Pipette 1.7mL of above solution into a 10 ml of volumetric flask and dilute up to the mark with diluent.

Calculation of S/N Ratio:

Average Baseline Noise obtained from Blank: 52 µV
Signal Obtained from LOQ solution: 524µV
S/N = 524/52 = 10.

4.7. ROBUSTNESS:

As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method.

a). The flow rate was varied at 0.8 ml/min to 1.2ml/min:

Standard solution 300ppm of Sumatriptan &3ppm of Naproxen was prepared and analysed using the varied flow rates along with method flow rate. On evaluation of the above results, it can be concluded that the variation in flow rate affected the method significantly. Hence it indicates that the method is robust even by change in the flow rate ±10%. Results for actual flow (1.0ml/min) have been considered from Assay standard.

b). The Organic composition in the Mobile phase was varied from 50% to 50%:

Standard solution 300 µg/ml of Sumatriptan & 3µg/ml of Naproxen **was** prepared and analysed

using the varied Mobile phase composition along with the actual mobile phase composition in the method.

On evaluation of the above results, it can be concluded that the variation in 10%. Organic composition in the mobile phase affected the method significantly. Hence it. Indicates that the method is robust even by change in the Mobile phase ±10**.** Results for actual Mobile phase composition (55:45 Methanol: Buffer (ph-2.8) has been considered from Accuracy standard.

5. EXPERIMENTAL METHODOLOGY:

5. 1. WAVELENGTH DETECTION:

The detection wavelength was selected by dissolving the drug in mobile phase to get a concentration of 10µg/ml for individual and mixed standards. The resulting solution was scanned in U.V range from 200-400nm. The overlay spectrum of Sumatriptan and naproxen. was obtained and the isobestic point of Sumatriptan and naproxen showed absorbance's maxima at 260 nm. The spectrums are shown in Figs.5.1,5.2&5.3.

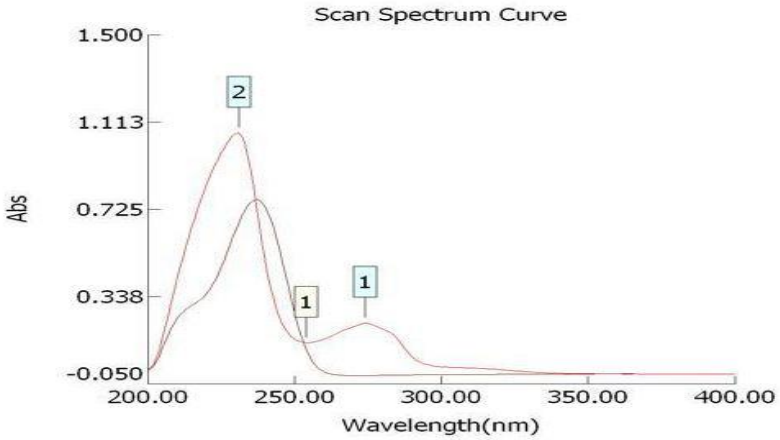


Fig. 5.1. (a). Overlay spectrum of Sumatriptan and naproxen

The UV spectra of individual drugs are as follows:

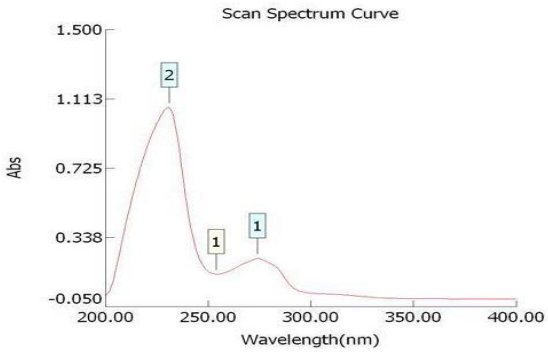


Fig. .5.1. (b). Spectrum of Sumatriptan

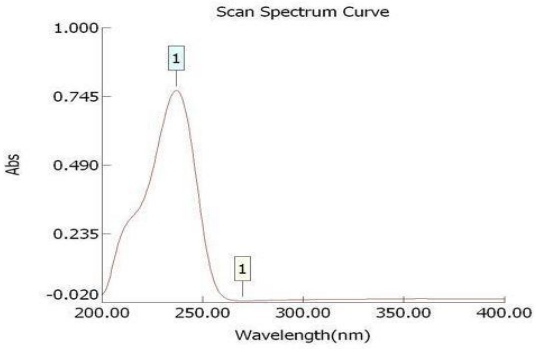


Fig. 5.1. (c). Spectrum of naproxen

5.2. CHROMATOGRAM FOR SUMATRIPTAN AND NAPROXEN:

Column: Inertsil C18 (4.6 x 250mm, 5µm)
Buffer pH:3.0.
Mobile phase: 30% buffer 70% Methanol
Flow rate:1.0ml per min
Wavelength:260 nm
Temperature: ambient
Run time:10min.

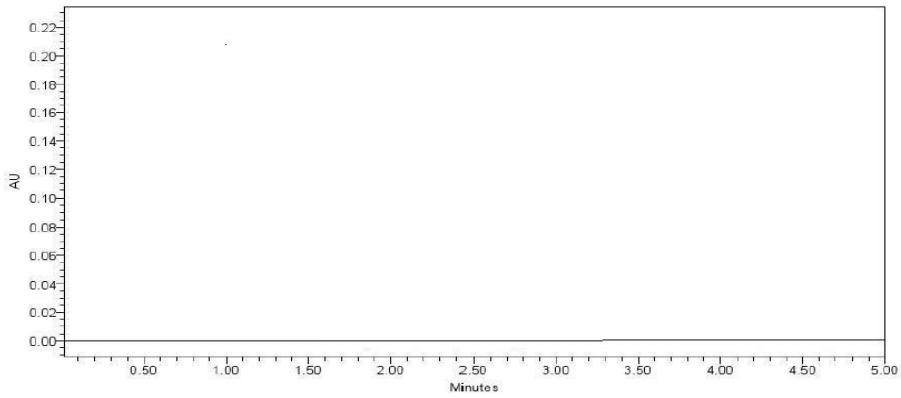
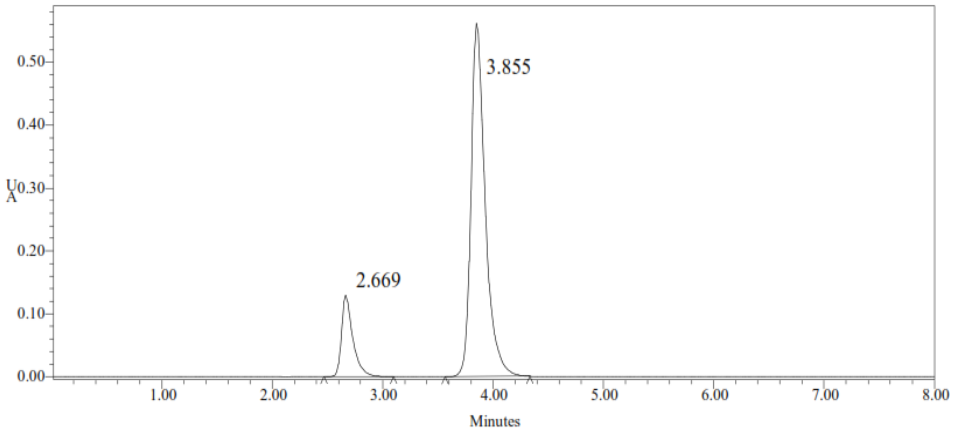


Figure 5.2. (a). chromatogram for blank

Observation:
From the above chromatogram it was observed that there are no interferences.



5.2. (b). Chromatogram for Sumatriptan and naproxen sample Preparation

Observation:
From the above chromatogram it was observed that the Sumatriptan and naproxen peaks are well separated.

Retention time of Sumatriptan – 2.669min
Retention time of naproxen - 3.855 min.

5.3. SYSTEM SUITABILITY:

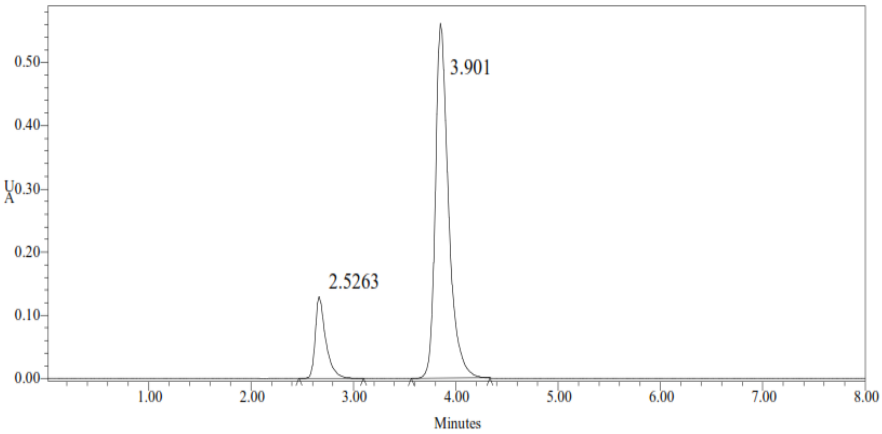


Figure 5.3. Chromatogram for system suitability

5.3. RESULTS:

System Suitability Results for SUMATRIPTAN:

1). Tailing factor Obtained from the standard injection is 1.3

2). Theoretical Plates Obtained from the standard injection is 4668.7

Assay Results:

Weight of 10 tablets: 1.25 grams

Average Weight: 0.125grams

%Assay = 99.95%

System Suitability Results for NAPROXEN:

1). Tailing factor Obtained from the standard injection is 1.3

2). Theoretical Plates Obtained from the standard injection is 6090.3

Assay Results:

Weight of 10 tablets: 1.25 grams

Average Weight: 0.125grams

%Assay = 102%

Table 5: Results of system suitability parameters for Sumatriptan and naproxen

S.No	Name	Retention time(min)	Area (µV sec)	Height (µV)	USP resolution	USP tailing	USP plate count
1	Sumatriptan	2.5	124505	213642		1.2	4673.4
2	naproxen	3.9	1308495	154566	6 0	1.3	6090.3

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.

5.4: VALIDATION PARAMETERS:

5.4.1: PRECISION:

Precision of the method was carried out for standard solutions as described under experimental work. The corresponding chromatograms and results are shown below.

Figure no 5.4.1 (a) chromatogram for standard injection -1

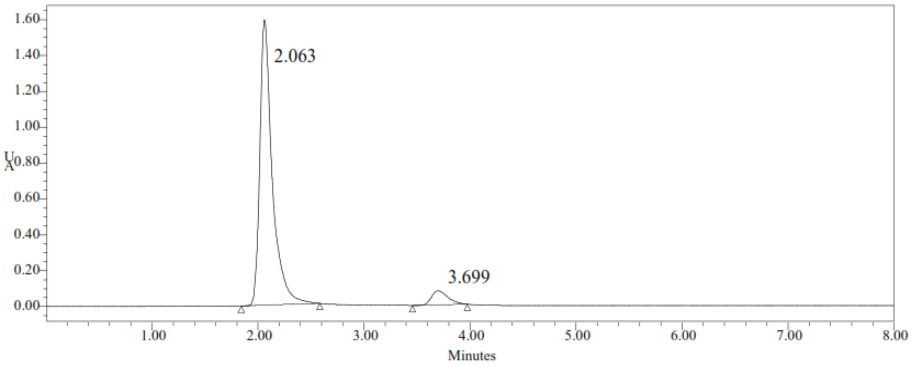


Figure no 5.4.1(b) chromatogram for standard injection-2

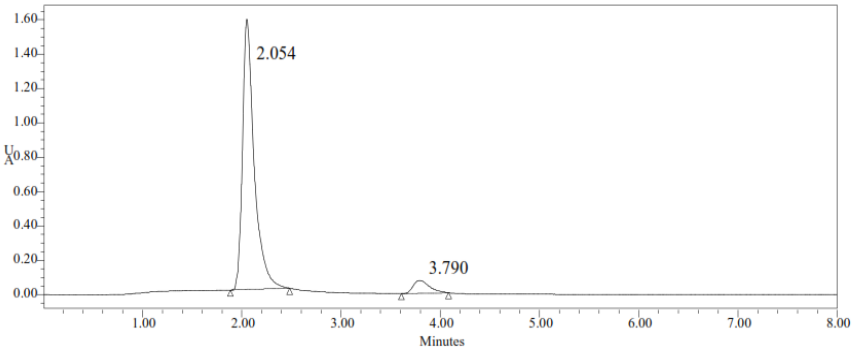


Figure no 5.4.1 (c) chromatogram for standard injection-3

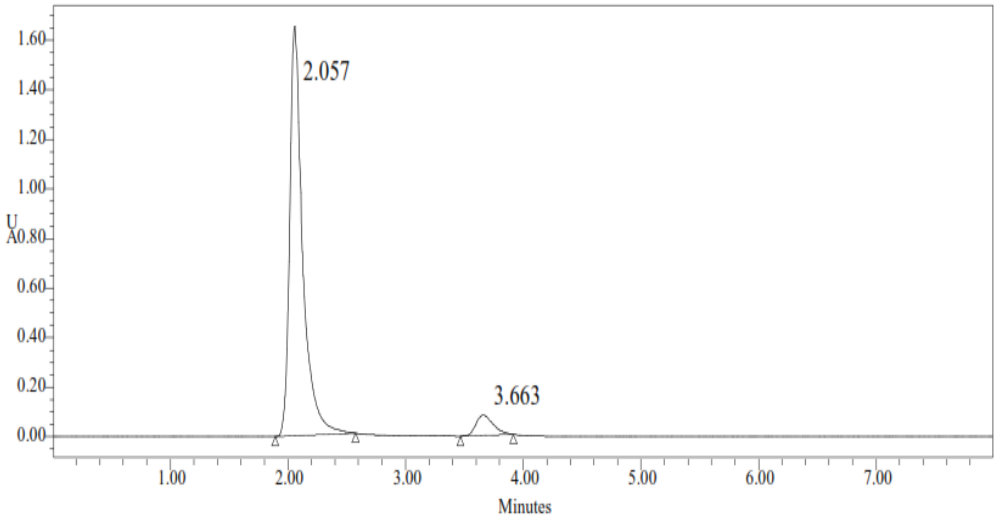


Figure no 5.4.1 (d) chromatogram for standard injection 4

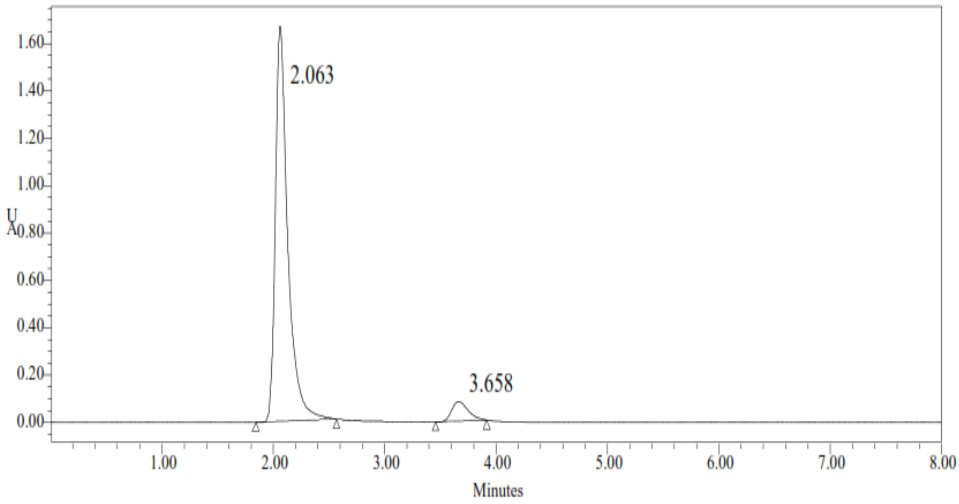


Figure no 5.4.1 (e) chromatogram for standard injection-5

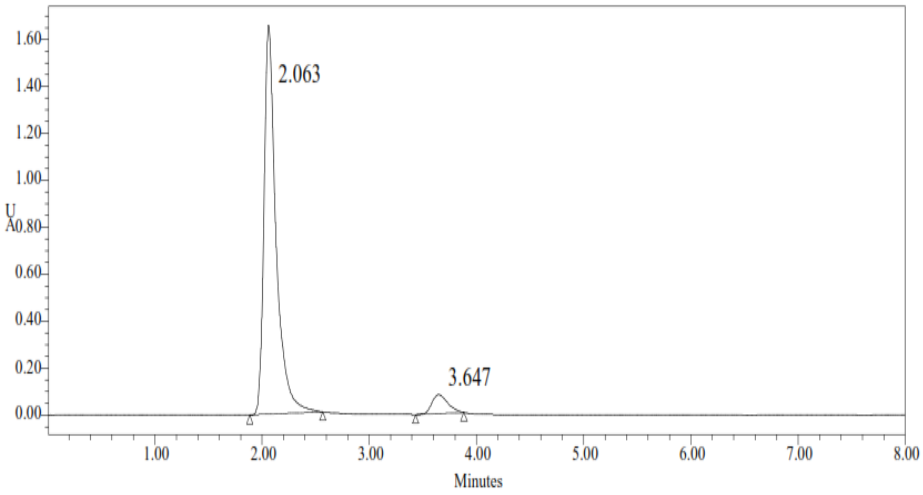


Table 6: Results of method precession for Sumatriptan:

Injection	Area
Injection-1	1302729
Injection-2	1302947
Injection-3	1303236
Injection-4	1303977
Injection-5	1309759
Average	1304529.8
Standard Deviation	2961.1
%RSD	0.2

Table 7: Results of method precession for naproxen:

Injection	Area
Injection-1	123149
Injection-2	123766
Injection-3	124271
Injection-4	124691
Injection-5	124956
Average	124162.7
Standard Deviation	725.6
%RSD	0.6

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.

5.4.2. INTERMEDIATE PRECESSION (RUGGEDNESS):

There was no significant change in assay content and system suitability parameters at different conditions of ruggedness like day to day and system to system variation.

Figure 5.4.2 (a) chromatogram for sample injectiocn-1

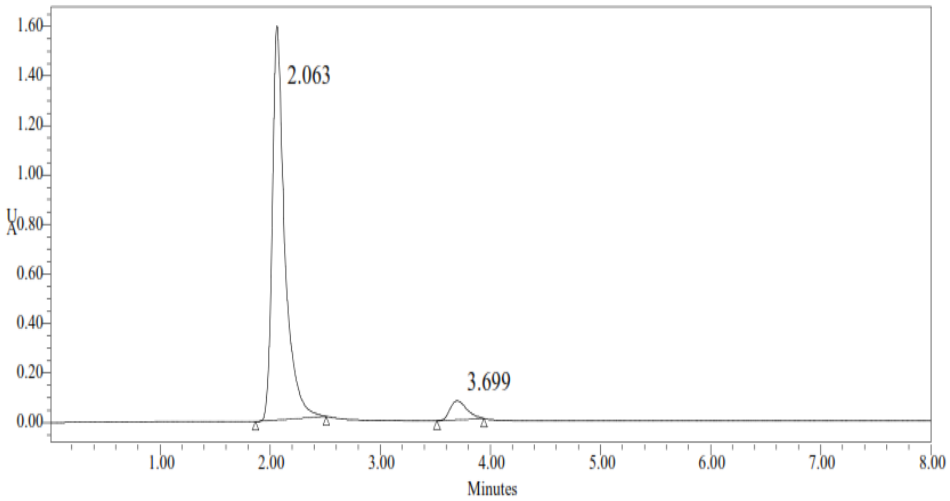


Figure 5.4.2 (b) chromatogram for sample injection-2

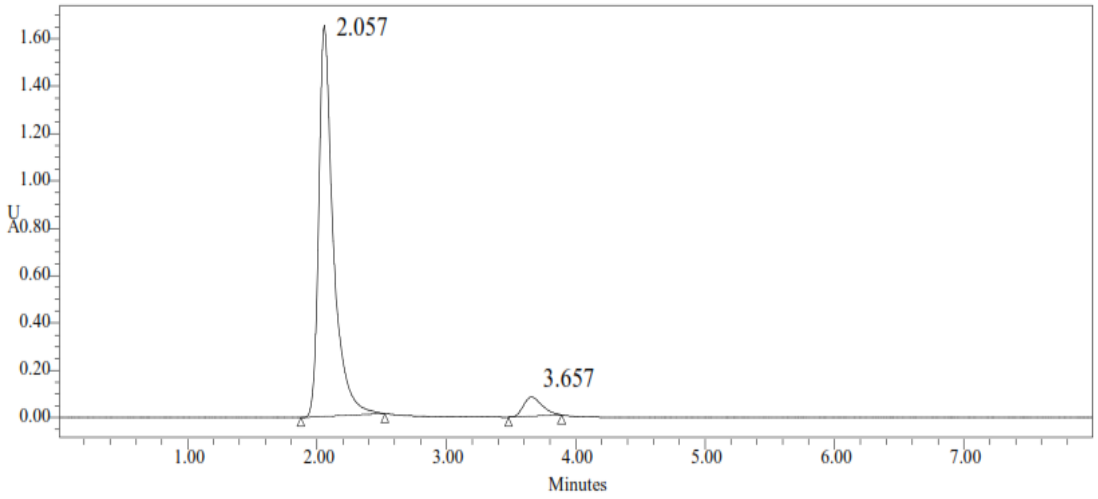


Figure 5.4.2 (c) chromatogram for sample injection-3

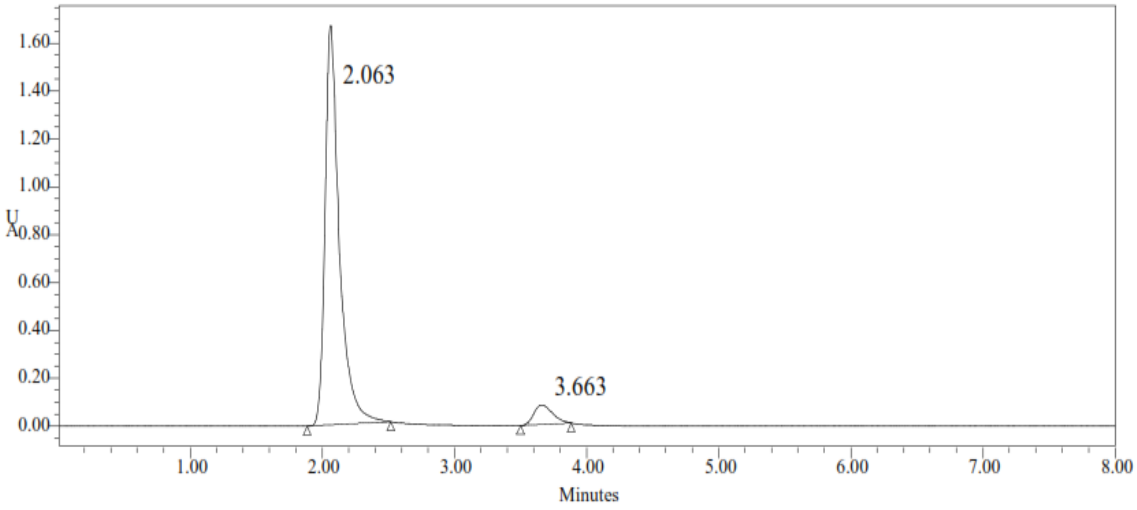


Figure 5.4.2 (d) chromatogram for sample injection-4

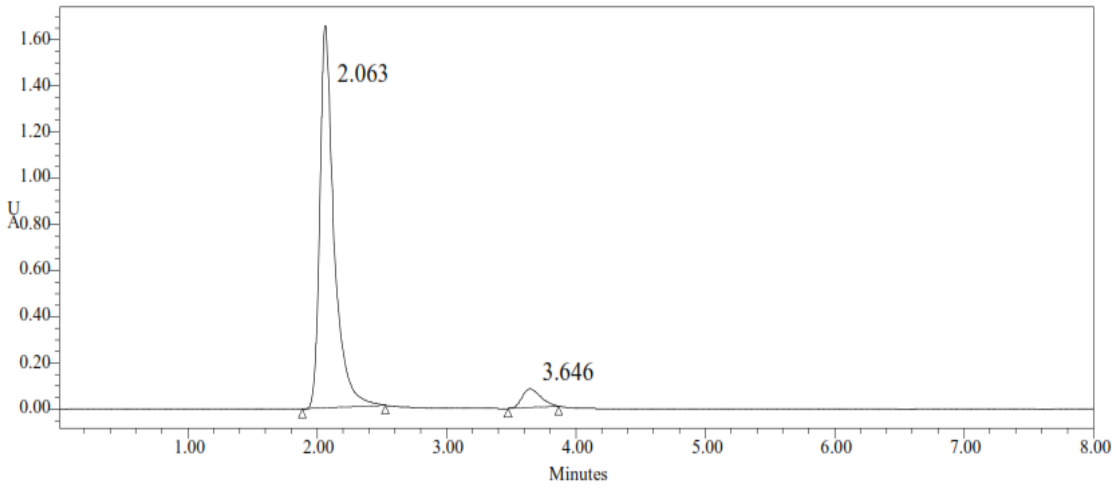


Figure 5.4.2 (e) chromatogram for sample injection-5

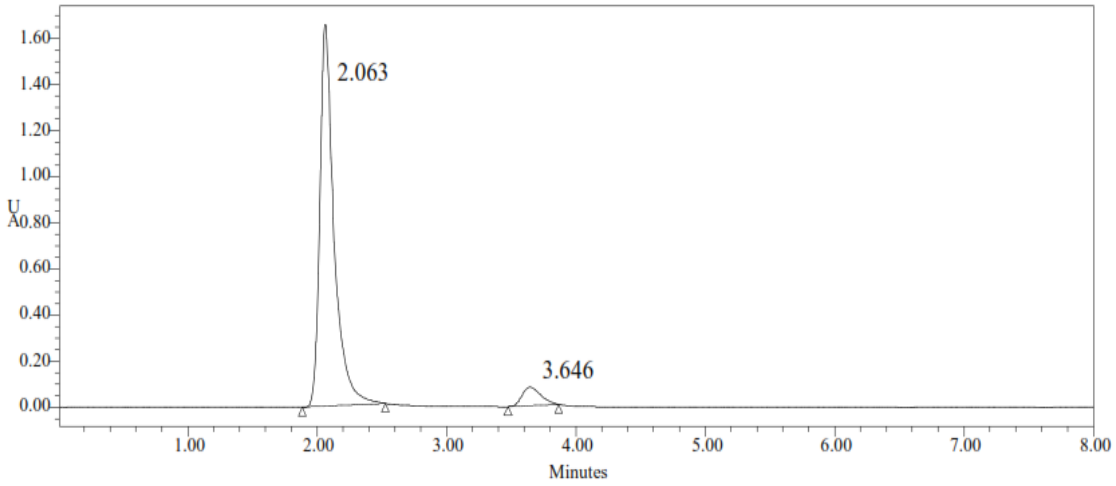


Table 8: Results of Intermediate precision for Sumatriptan

Injection	Area
Injection-1	1300148
Injection-2	1304520
Injection-3	1305937
Injection-4	1306476
Injection-5	130871
Average	1305070.2
Standard Deviation	3061.8
%RSD	0.2

Table 9: Results of Intermediate precision for naproxen

Injection	Area
Injection-1	122487
Injection-2	122626
Injection-3	122632
Injection-4	122702
Injection-5	122962
Average	122681.8
Standard Deviation	174.8
%RSD	0.1

Acceptance criteria:

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

5.4.3: ACCURACY:

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

5.4.3.1. Chromatograms for Sample Concentration-50%:

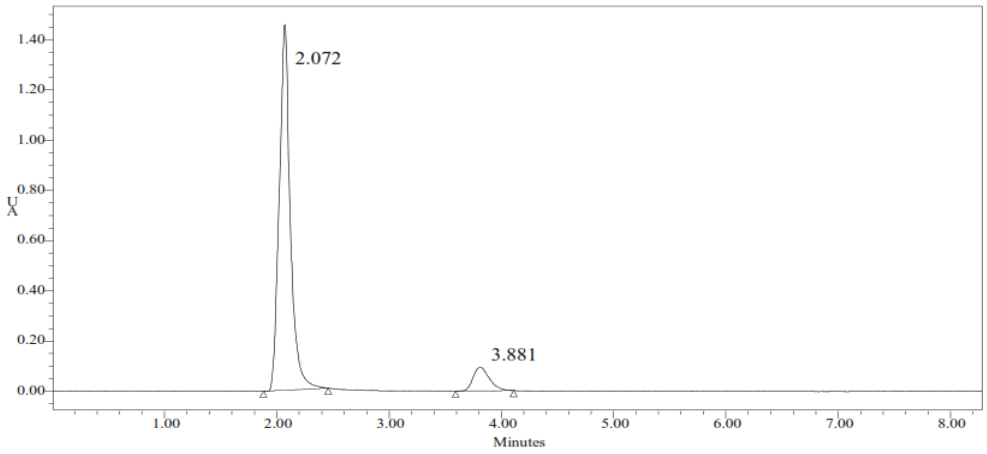


Figure no 5.4.3.1 (a) chromatogram for sample concentration-50%

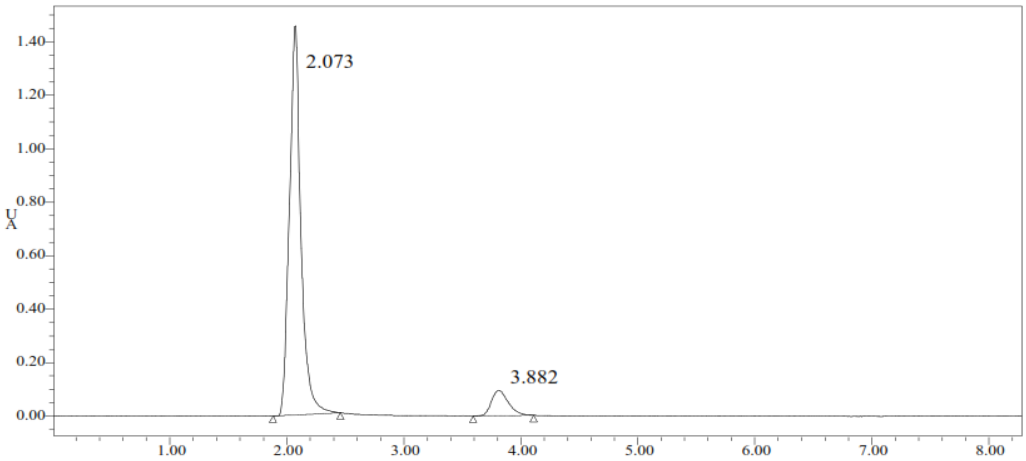


Figure no 5.4.3.1 (b) chromatogram for sample concentration-50%

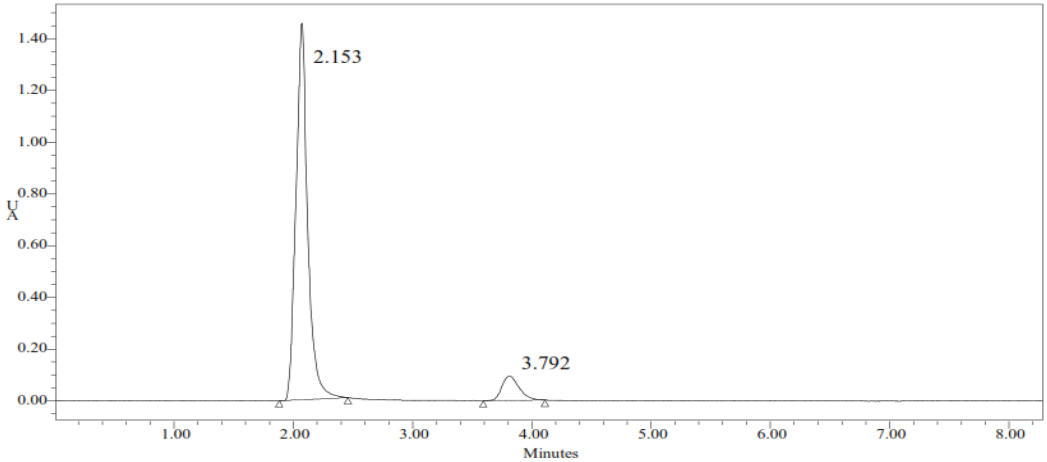


Figure no 5.4.3.1 (c) chromatogram for sample concentration-50%

5.4.3.2. Chromatograms for Sample Concentration-100%:

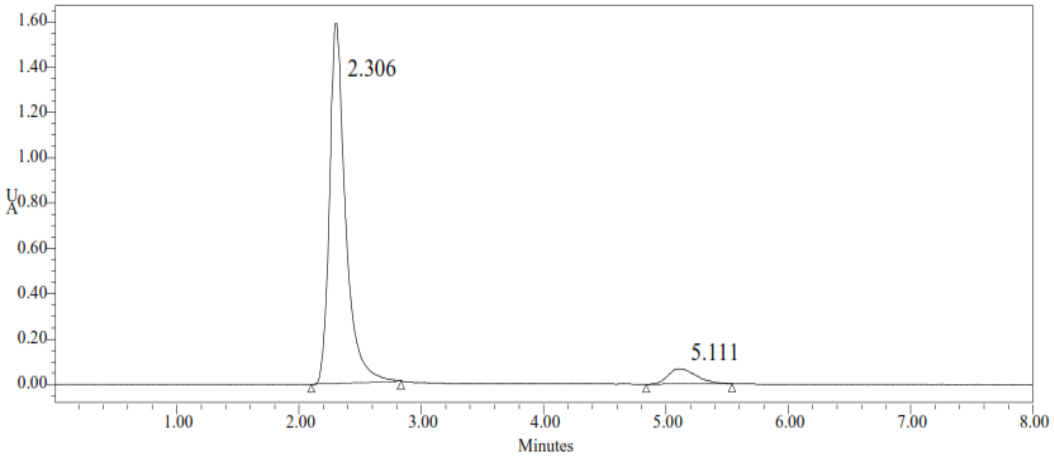


Figure no 5.4.3.2 (a) chromatogram for sample concentration-100%

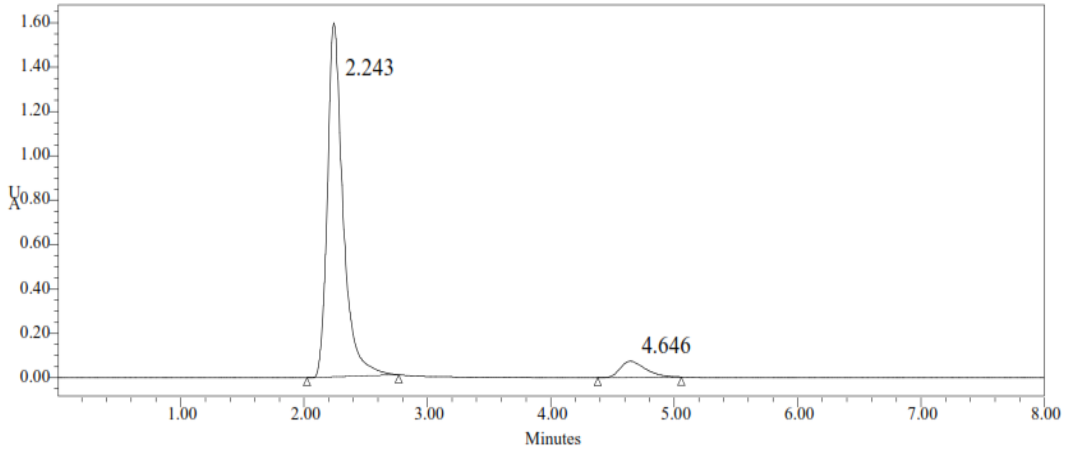


Figure no 5.4.3.2 (b) chromatogram for sample concentration-100%

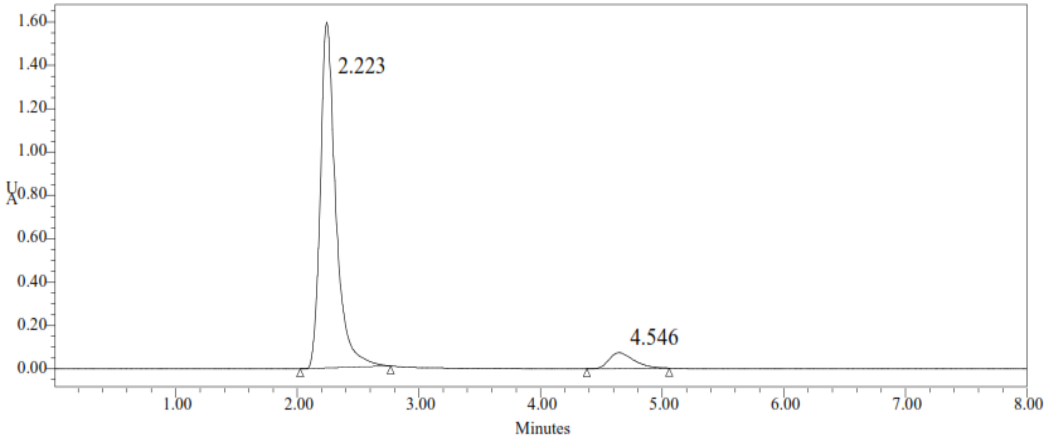


Figure no 5.4.3.2 (c) chromatogram for sample concentration-100%

7.4.3.3. Chromatograms for Sample Concentration-150%:

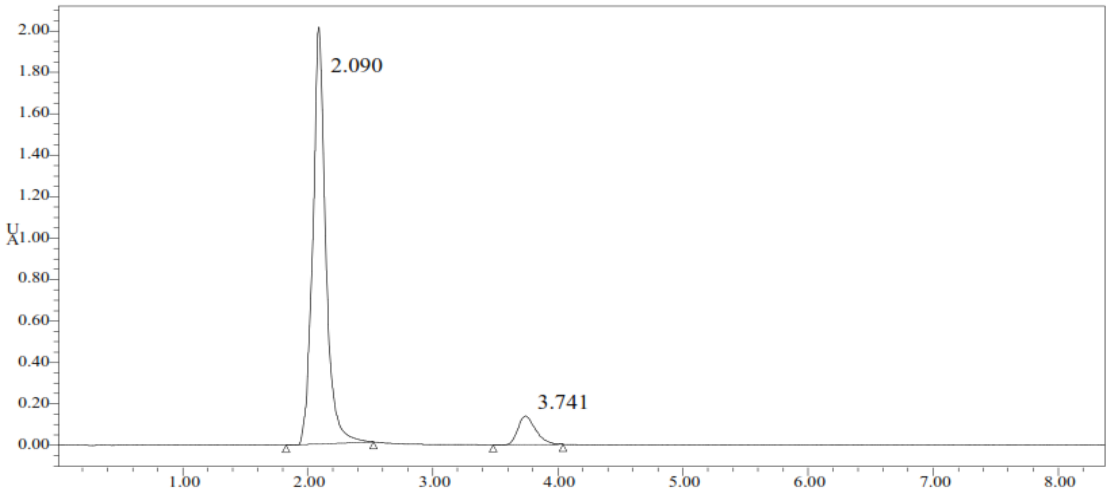


Figure no 5.4.3.3 (a) chromatogram for sample concentration-150%

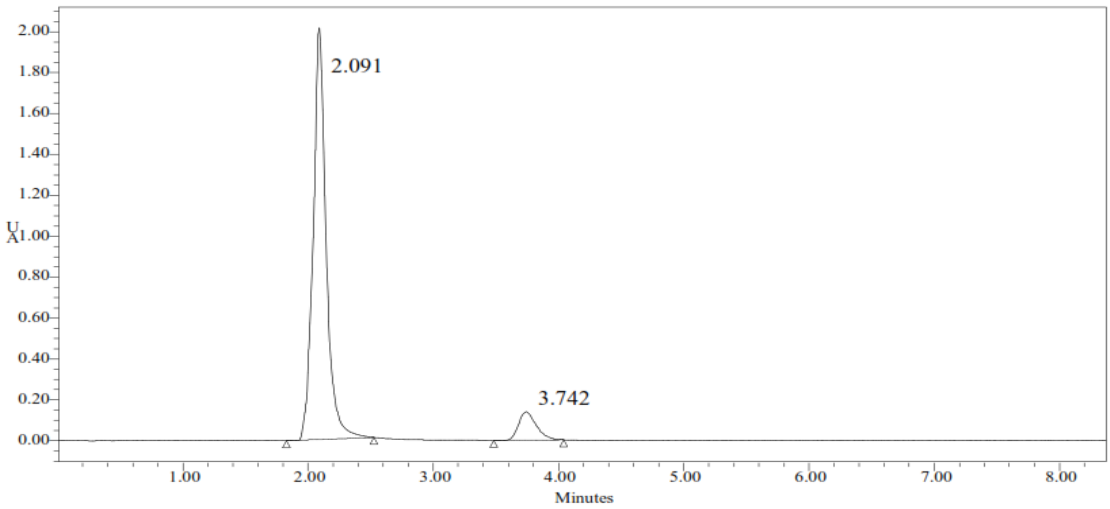


Figure no 5.4.3.3 (b) chromatogram for sample concentration-150%

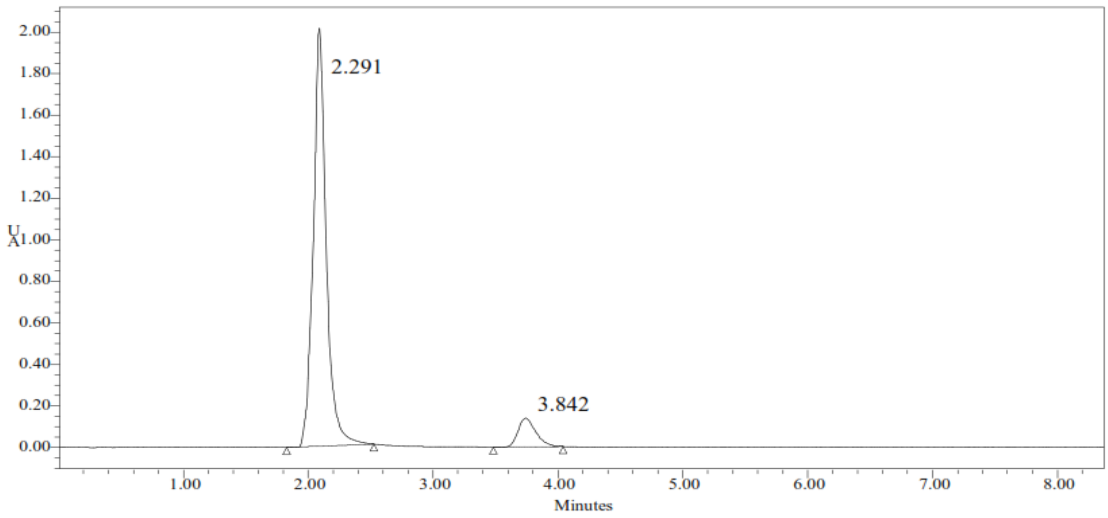


Figure no 5.4.3.3 (c) chromatogram for sample concentration-150%

Table-10: accuracy (recovery) data for Sumatriptan

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	656659.5	5.0	5.036	100.7%	
100%	1304258	10.0	10.003	100.0%	99.84%
150%	1854608	14.4	14.224	98.780%	

Table-11: accuracy (recovery) data for naproxen

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	65800	5.3	5.34	100.8%	
100%	124353	10	10.10	100.01%	100.51%
150%	177940	14.2	14.45	99.68%	

Acceptance Criteria:

- The % Recovery for each level should be between 98.0 to 102.0%.
- The percentage recovery was found to be within the limit (97-103%).

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

5.4.4. LINEARITY:

The linearity range was found to lie from 100µg/ml to 500µg/ml of Sumatriptan, 5µg/ml to 25µg/ml 0f naproxen and chromatograms are shown below.

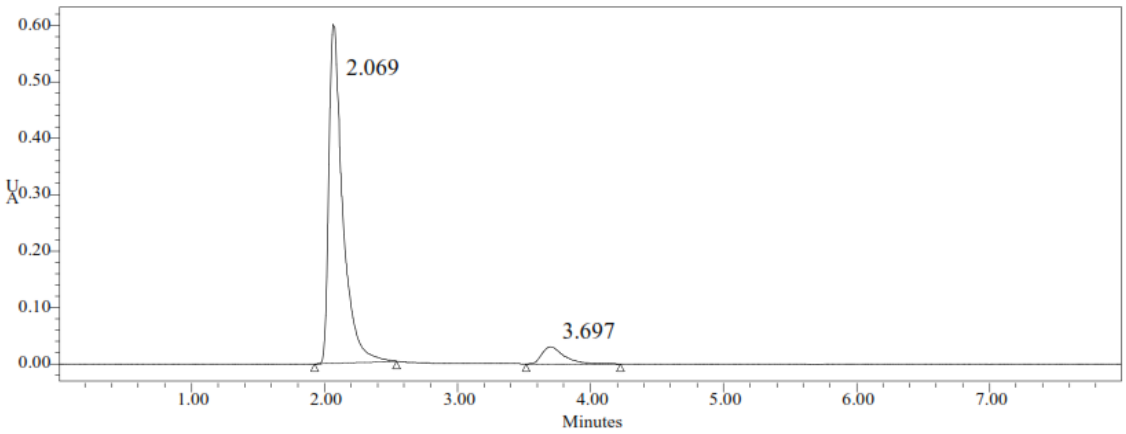


Figure no 5.4.4 (a) chromatogram for linearity concentration-100µg/ml of Sumatriptan & 5 µg/ml of naproxen

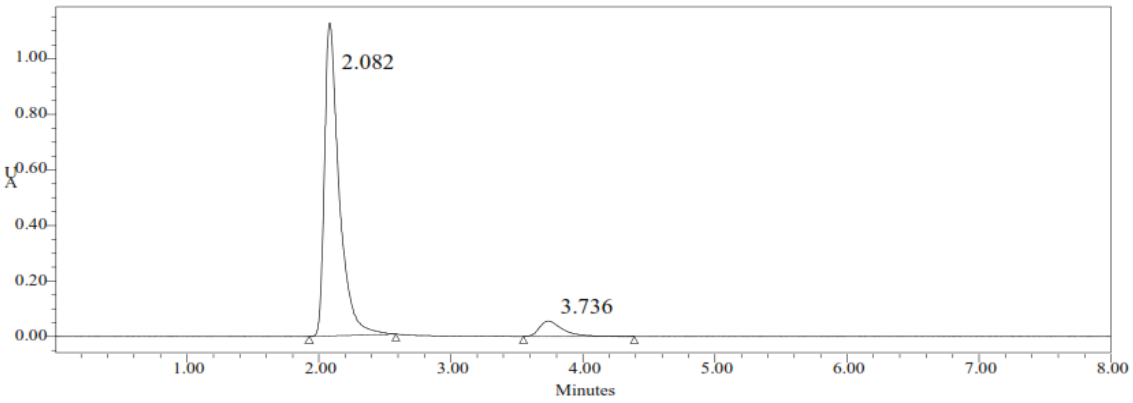


Figure no 5.4.4 (b) chromatogram for linearity concentration-200 µg/ml of Sumatriptan & 10 µg/ml of naproxen

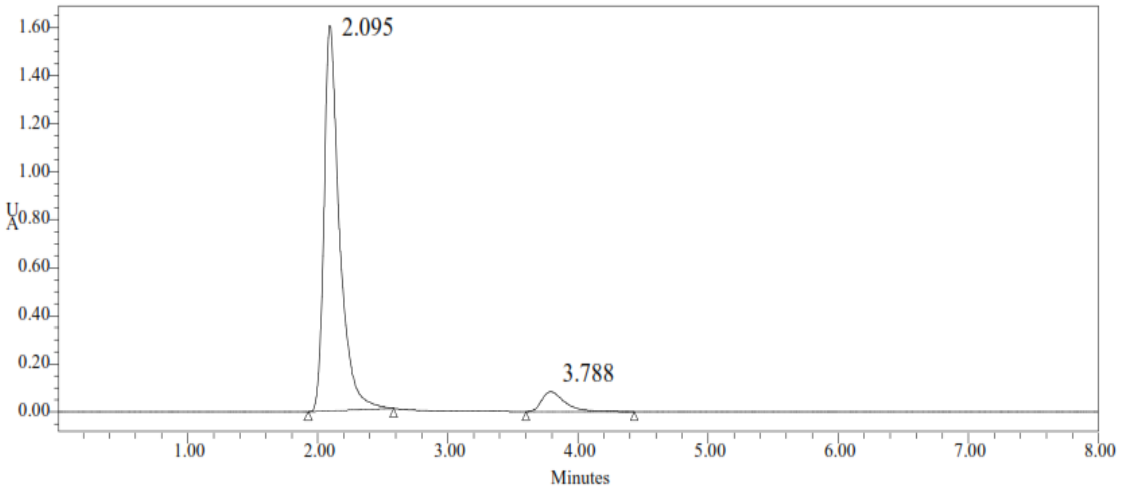


Figure no 5.4.4 (c) chromatogram for linearity concentration--300 µg/ml of Sumatriptan & 15 µg/ml of naproxen

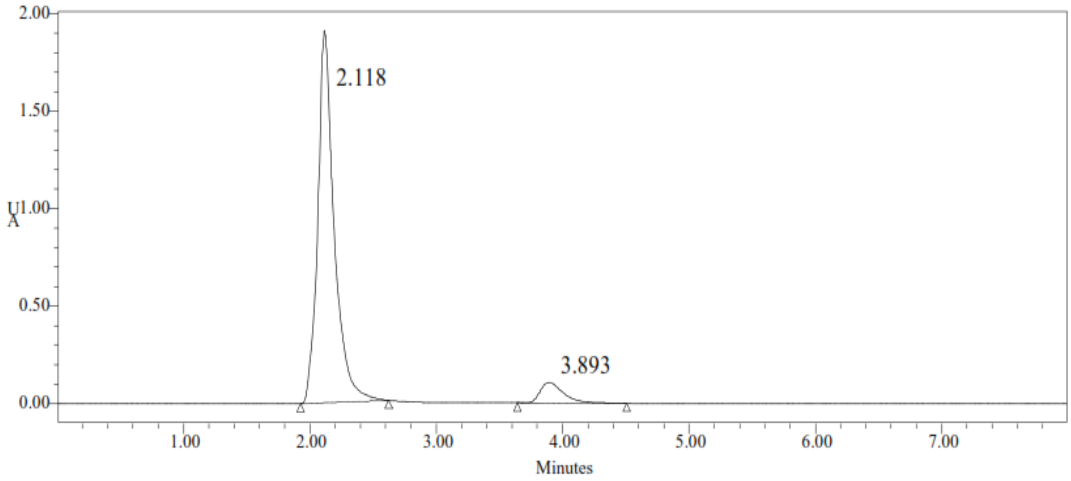


Figure no 5.4.4 (d) chromatogram for linearity concentration-400ppm of Sumatriptan & 20ppm of naproxen

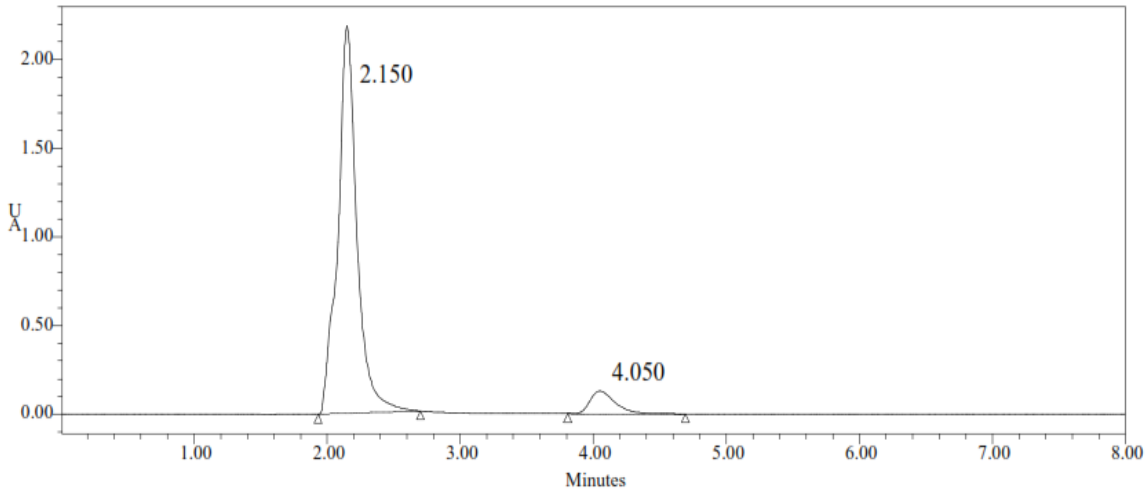


Figure no 5.4.4 (e) chromatogram for linearity concentration-500 µg/ml of Sumatriptan & 25 µg/ml of naproxen

Table-12: Area of different concentration of Sumatriptan

S.No.	Linearity Level	Concentration	Area
1	I	100ppm	668934
2	II	200ppm	956781
3	III	300ppm	1313873
4	IV	400ppm	1563458
5	V	500ppm	1867084
Correlation Coefficient			0.999

Table-13: Area of different concentration of naproxen

S.No	Linearity Level	Concentration	Area
1	I	1ppm	66510
2	II	2ppm	94701
3	III	3ppm	124802
4	IV	4ppm	152731
5	V	5ppm	179732
Correlation Coefficient			0.999

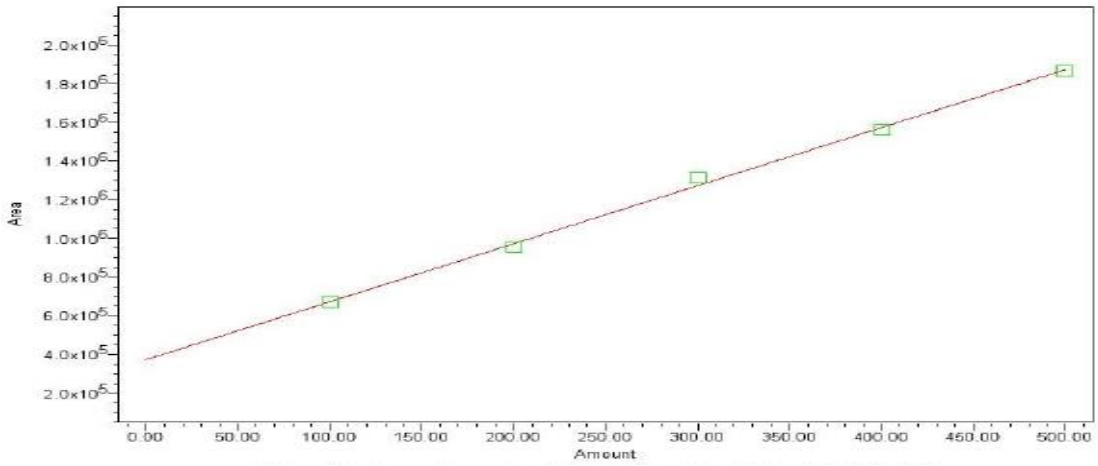


Figure 5.4.4 (f) calibration graph for Sumatriptan at 260 nm

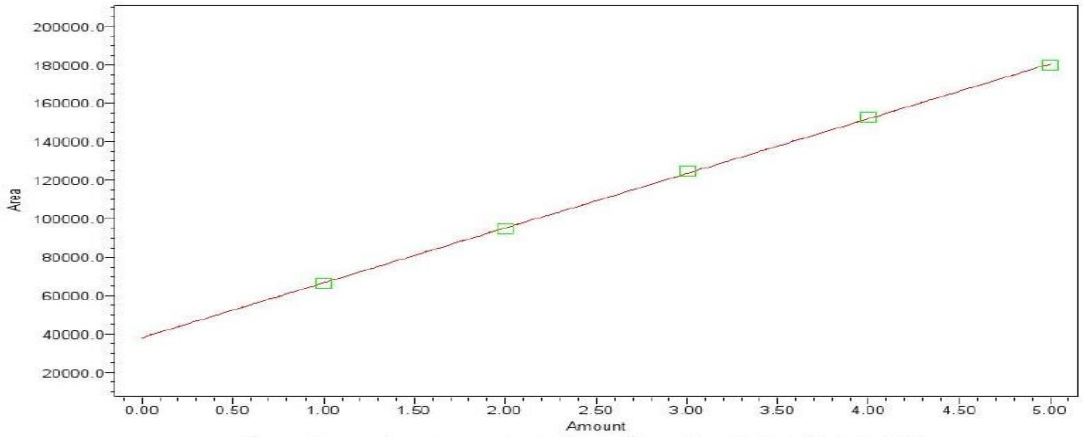


Figure 5.4.4 (f) calibration graph for naproxen at 260 nm

Table-14: Analytical performance parameters of Sumatriptan and naproxen

Parameters	Sumatriptan	Naloxone
Slope (m)	66574	12529
Intercept (c)	53592	50245
Correlation coefficient (R^2)	0.999	0.999

Acceptance criteria:

Correlation coefficient (R^2) should not be less than 0.999

- The correlation coefficient obtained was 0.999 which is in the acceptance limit. The linearity was established in the range of 10% to 50% of Sumatriptan and 5% to 25% of naproxen.

5.4.5: LIMIT OF DETECTION (LOD):

The lowest concentration of the sample was prepared with respect to the base line noise and measured the signal to noise ratio

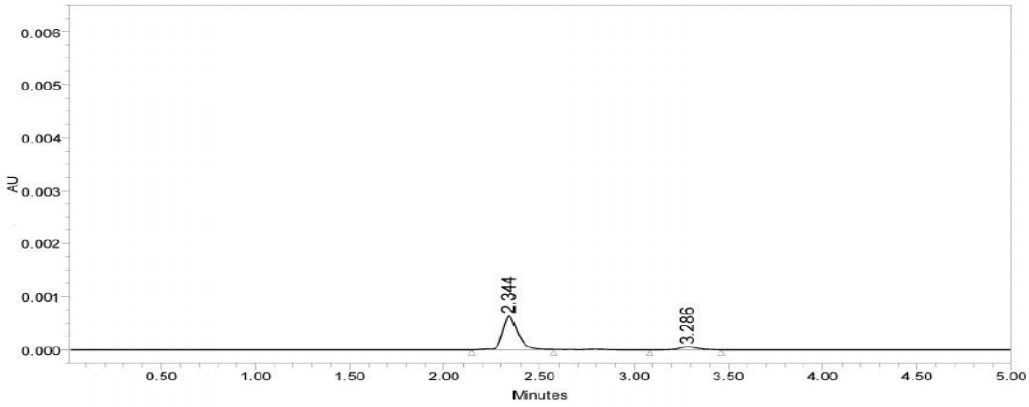


Figure no 5.4.5 chromatogram of Sumatriptan & naproxen showing LOD

Table-15: Results of LOD

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Sumatriptan	52	152	2.9
naproxen	52	156	3

- Signal to noise ratio shall be 3 for LOD solution
- The result obtained is within the limit

5.4.6: LIMIT OF QUANTIFICATION (LOQ):

The lowest concentration of the sample was prepared with respect to the base line noise and measured the signal to noise ratio.

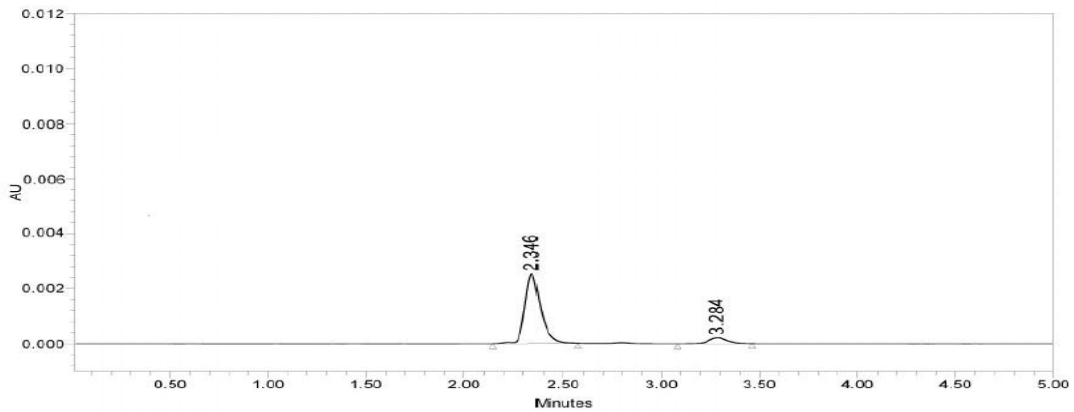


Figure no 5.4.6 (a) chromatogram of Sumatriptan & naproxen showing LOQ

Table 16: Results of LOQ

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Sumatriptan	52	522	10.03
naproxen	52	524	10.1

- Signal to noise ratio shall be 10 for LOQ solution
- The result obtained is within the limit.

5.4.7: ROBUSTNESS:

The standard and samples of Sumatriptan and naproxen 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.

5.4.7.1: Variation in Flow

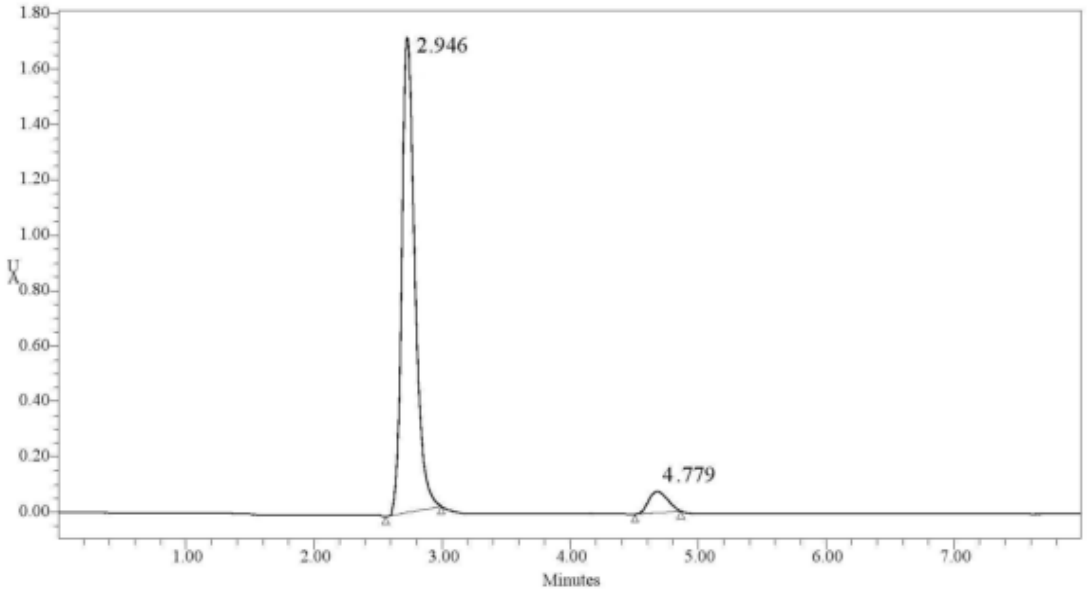


Figure no 5.4.7.1 (a) chromatogram showing less flow of 0.6ml/min

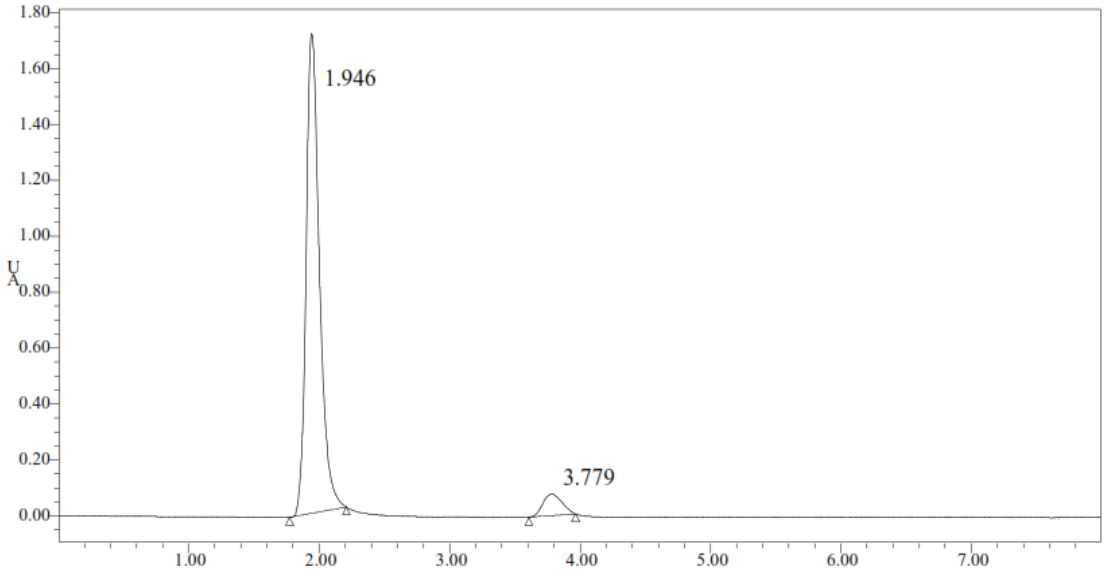


Figure no 5.4.7.1 (b) chromatogram snowing more flow of 1.0ml/min

Table-17: Flow Rate (ml/min) data for Sumatriptan

		System Suitability Results	
S. No	**Flow Rate (ml/min)**	**USP Plate Count**	**USP Tailing**
1	0.6	5339.9	1.4
2	0.8	4673.4	1.3
3	1.0	5216.0	1.4

Table-18: flow rate (ml/min) data for naproxen

		System Suitability Results	
S. No	**Flow Rate (ml/min)**	**USP Plate Count**	**USP Tailing**
1	0.8	7063.3	1.3
2	1.0	6090.3	1.2
3	1.2	6998.0	1.3

5.4.7.2 Variation of Mobile Phase Organic Composition:

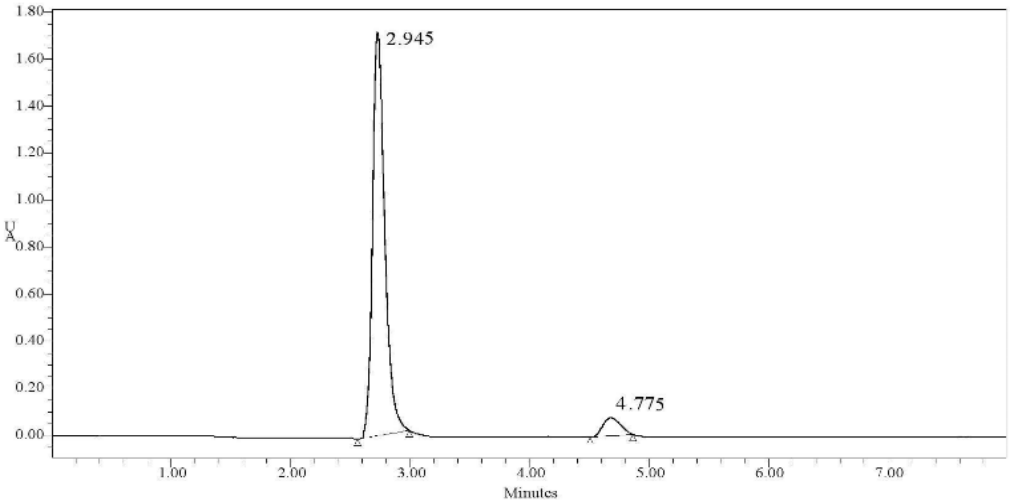


Figure no 5.4.7.2 (a) chromatogram showing less organic composition

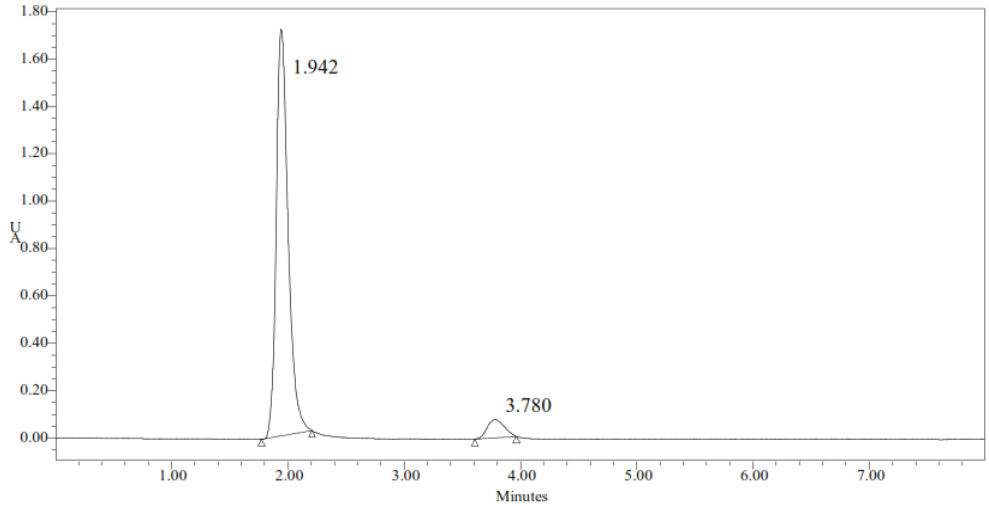


Figure no 5.4.7.2 (b) chromatogram showing more organic composition

Table -19: Change in Organic Composition in the Mobile Phase for Sumatriptan

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	4508.4	1.3
2	*Actual	4673.4	1.4
3	10% more	4318.1	1.3

Table -20: Change in Organic Composition in the Mobile Phase for naproxen

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	6387.7	1.2
2	Actual	6090.3	1.2
3	10% more	6232.5	1.2

Acceptance criteria:

Percentage RSD should be below 2.

- The %RSD obtained for change of flow rate, variation in mobile phase was found to be below 1, which is within the acceptance criteria. Hence the method is robust.

CONCLUSION:

High performance liquid chromatography is at present one of the most sophisticated tool of the analysis. The estimation of Sumatriptan and Naproxen was done by RP-HPLC. The Phosphate buffer was p^H 3.0 and the mobile phase was optimized with consists of Methanol: Phosphate buffer mixed in the ratio of 70:30 % v/ v. Inertsil C_{18} column C18 (4.6 x 150mm, 5µm) or equivalent chemically bonded to porous silica particles was used as stationary phase. The detection was carried out using UV detector at 260 nm. The solutions were chromatographed at a constant flow rate of 0.8 ml/min. the linearity range of Sumatriptan and Naproxen were found to be from 100-500 µg/ml of Sumatriptan and 1-5µg/ml of Naproxen . Linear regression coefficient was not more than 0.999.

The values of % RSD are less than 2% indicating accuracy and precision of the method. The percentage recovery varies from 98-102% of Sumatriptan and naproxen. LOD and LOQ were found to be within limit.

The results obtained on the validation parameters met ICH and USP requirements .it inferred the method found to be simple, accurate, precise and linear. The method was found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision.

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