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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF SUMATRIPTAN AND NAPROXEN BY RP-HPLC

Kantlam Chamakuri *[1], Sunitha SarillA[2]

[1]Associate Professor, Department of Pharmaceutical Analysis & Quality Assurance, Netaji Institute of Pharmaceutical Sciences, Toopranpet, Choutuppal, Nalgonda-508252, Telangana state, India

[2]M.Pharmacy student, Department of Pharmaceutical Analysis & Quality Assurance, Netaji Institute of Pharmaceutical Sciences, Toopranpet, Choutuppal, Nalgonda-508252, Telangana state, India.

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Abstract:

For the simultaneous estimation of Sumatriptan and Naproxen in Tablet dosage form, a simple, rapid, specific, accurate and precise reverse phase high performance liquid chromatographic method was developed. A Column Symmetry C8 (4.6 x 150mm, 3.5µm, Make: XTerra) in Isocratic mode with mobile phase containing Methanol: Acetonitrile (70:30) was used. The flow rate was 0.7 ml/min and The optimum wavelength for detection was 285 nm at which better detector response for drug was obtained. The method was validated for Accuracy, Precision, Specificity, Linearity and Sensitivity. The average retention time for Sumatriptan and Naproxen were found to be 5.87 and 2.24 min. The calibration was linear in concentration range of 60 – 100 µg/ml. The low values of % R.S.D. indicate the method is precise and accurate. the % R.S.D. reported was found to be less than 2 %. The LOD for Sumatriptan and Naproxen was found to be 3.36 and 3.20. The LOQ for Sumatriptan and Naproxen was found to be 9.90 and 9.86. The proposed method was validated in accordance with ICH parameters and the results of all methods were very close to each other as well as to the label value of commercial pharmaceutical formulation. Therefore, there is no significant difference in the results achieved by the proposed method.

Keywords: *Sumatriptan, Naproxen, Methanol, Acetonitrile, Potassium di hydrogen phosphate, Sodium di hydrogen phosphate, Orthophosphoric acid and Trimethyl amine.*

Corresponding author:

Kantlam Chamakuri,

Associate Professor, Dept. of Pharmaceutical Analysis & Quality Assurance,
Netaji Institute of Pharmaceutical Sciences,
Toopranpet, Choutuppal, Nalgonda-508252.
Telangana state, India.
E Mail: kantlam3@gmail.com.

QR code

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INTRODUCTION:

A good method development strategy should require only as many experimental runs as are necessary to achieve the desired final result. Finally method development should be as simple as possible, and it should allow the use of sophisticated tools such as computer modeling. The final procedure should meet all the goals that were defined at the beginning of HPLC method development. The method should also be robust in routine operation and usable by all laboratories and personnel for which it is intended.

THE DEVELOPMENT OF NEW HPLC METHODS

Parameters of HPLC method

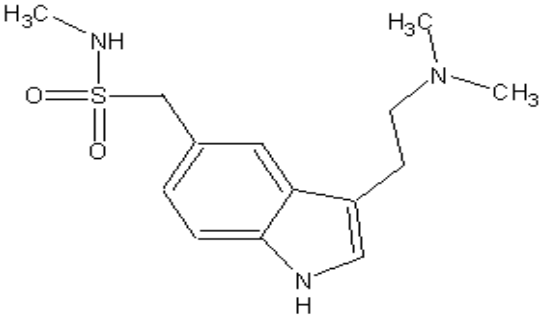
System suitability experiments can be defined as tests to ensure that the method can generate results of acceptable accuracy and precision. The requirements for system suitability are usually developed after method development and validations have been completed. The criteria selected will be based on the actual performance of the method as determined during its validation. For example, if sample retention times form part of the system suitability criteria, their variation (SD) can be determined during validation. System suitability might then require that retention times fall within a ± 3 SD range during routine performance of the method. The USP (2000) defines parameters that can be used to determine system suitability prior to analysis. These parameters include plate number (N), tailing factor, k and or α, resolution (Rs) and relative standard deviation (RSD) of peak height or peak area for respective injections.

METHOD VALIDATION PARAMETERS

Method Validation Parameters, used in Analytical Method Development And Validation Of Sumatriptan And Naproxen By RP-HPLC, are Selectivity/Specificity, Precision and Reproducibility, Accuracy and Recovery, Linearity and Calibration Curve, Range, Limit of Detection, Limit of Quantitation, Ruggedness, and Robustness.

SUMATRIPTAN

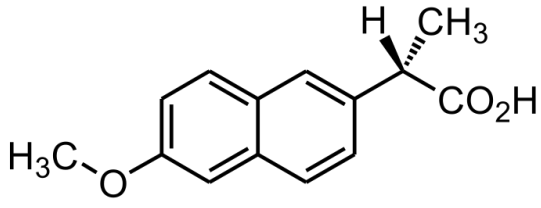
Sumatriptan is a serotonin agonist that acts selectively at 5HT1 receptors. It is used in the treatment of migraine disorder. Chemical Formula is $C_{14}H_{21}N_3O_2S$. Sumatriptan acts as Vasoconstrictor Agents, Selective Serotonin Agonists and Serotonin Agonists. The 5-HT_{1B} and 5-HT_{1D} receptors function as autoreceptors, which inhibit the firing of serotonin neurons and a reduction in the synthesis and release of serotonin upon activation. After sumatriptan binds to these receptors, adenylate cyclase activity is inhibited via regulatory G proteins, incrases intracellular calcium, and affects other intracellular events. This results in vasoconstriction and inhibtion of sensory nociceptive (trigeminal) nerve firing and vasoactive neuropeptide release.



NAPROXEN

Naproxen is an anti-inflammatory agent with analgesic and antipyretic properties. Both the acid and its sodium salt are used in the treatment of rheumatoid arthritis and other rheumatic or musculoskeletal disorders, dysmenorrhea, and acute gout.

Formula of the Naproxen is $C_{14}H_{14}O_3$, it is freely soluble in water, practically insoluble at low pH and soluble at high pH. The mechanism of action of naproxen, like that of other NSAIDs, is believed to be associated with the inhibition of cyclooxygenase activity. Two unique cyclooxygenases have been described in mammals. The constitutive cyclooxygenase, COX-1, synthesizes prostaglandins necessary for normal gastrointestinal and renal function. The inducible cyclooxygenase, COX-2, generates prostaglandins involved in inflammation. Inhibition of COX-1 is thought to be associated with gastrointestinal and renal toxicity while inhibition of COX-2 provides anti-inflammatory activity. Naproxen is used For the treatment of rheumatoid arthritis, osteoarthritis, ankylosing spondylitis, tendinitis, bursitis, and acute gout. Also for the relief of mild to moderate pain and the treatment of primary dysmenorrhea.



EXPERIMENTAL DETAILS

Equipments and apparatus:

Different kinds of equipments viz Analytical weighing balance (shimadzu AUX 200), High performance liquid chromatography(waters, separation module 2695) equipped with Auto Sampler and UV detector. Column Symmetry C8 (4.6 x 150mm, 3.5µm, Make: XTerra), pH meter, Vacuum

filter pump (model XI 5522050 of Millipore), Millipore filtration kit, mobile phase reservoir, Water bath, Sample filtration assembly and glassware's were used throughout the experiment.

Recording the Chromatogram:

The **Empower-2** software was used for acquisition, evaluation and storage of chromatographic data with the following Details.

Retention time (RT), peak area, peak height, percentage area, assigning the name to the Peaks.

Reagents used for the Study:

1. Methanol **:** HPLC grade
2. Acetonitrile **:** HPLC grade
3. water **:** Milli-Q grade
4. Potassium di hydrogen phosphate**:** GR grade
5. Sodium di hydrogen phosphate **:** GR grade
6. Orthophosphoric acid **:** GR grade
7. Trimethyl amine **:** GR grade

METHOD DEVELOPMENT:

The objective of this experiment was to optimize the assay method for estimation of Sumatriptan and Naproxen based on the literature survey . So here the trials mentioned describes how the optimization was done.

TRAIL-1:

Preparationof mobile phase.

Mix a mixtue of above Methanol 700 mL (70%) and 300 mL(30%) of Acetonitrile and degas in ultrasonic water bath for 5 minutes. Filter through 0.45 µ filter under vacuum filtration.

Mobile phase Methanol : Acetonitrile (70:30)

The Chromtograms were shown in Fig 1.

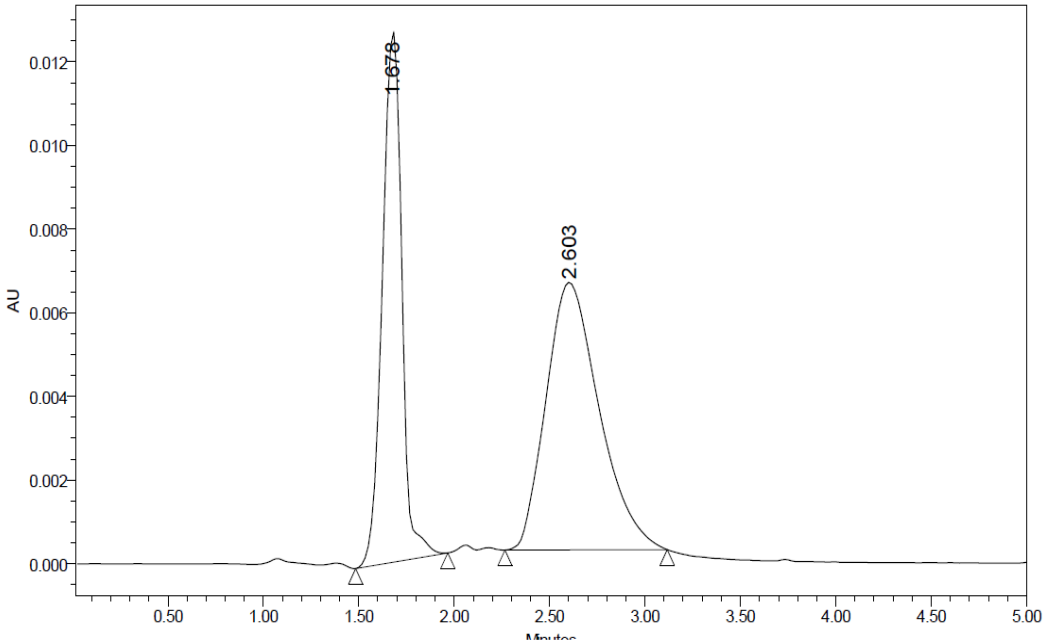


Figure 1: chromatogram of Sumatriptan and Naproxen

Chromatographic Parameters

Equipment : High performance liquid chromatography equipped with Auto Sampler and UV detector
Column : Symmetry C8 (4.6 x 150mm, 3.5µm, Make: XTerra)
Flow rate : 1 mL per min
Wavelength : 285 nm
Injection volume : 20 µl
Column oven : Ambient
Run time : 3min

Isocratic programme:

Name of the peak	Retention time(min)
NAPROXEN	1.678
SUMATRIPTAN	2.603

Conclusion: The retention time was low and the peak was asymmetric.

TRIAL-2:
Preparation of Phosphate buffer:
Weighed 7.0 grams of KH_2PO_4 into a 1000ml beaker, dissolved and diluted to 1000ml with HPLC water. Adjusted the pH to 3with Orthophosporic acid. filter through 0.45µm nylon membrane filter and degas.
Mobile phase: pH 3 buffer : Acetonitrile (55:45)
The Chromatograms were shown in Fig 2.

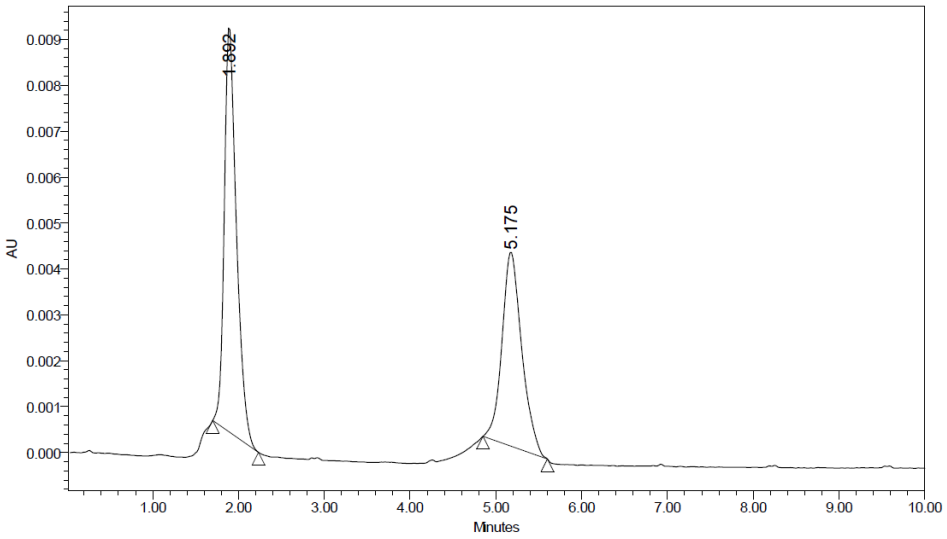


Figure 2: chromatogram of Sumatriptan and Naproxen

Chromatographic Parameters

Equipment : High performance liquid chromatography equipped with Auto Sampler and UV detector
Column : Symmetry C8 (4.6 x 150mm, 3.5µm, Make: XTerra)
Flow rate : 0.8 mL per min
Wavelength : 285 nm
Injection volume : 20 µl
Column oven : Ambient
Run time : 10min

Isocratic programme:

Name of the peak	Retention time(min)
NAPROXEN	1.892
SUMATRIPTAN	5.175

Conclusion: The retention time was normal and the peak was a symmetric but possess fronting.

TRIAL-3:

Preparation of Phosphate buffer:

Weighed 7.0 grams of KH_2PO_4 into a 1000ml beaker, dissolved and diluted to 1000ml with HPLC water. Adjusted the pH to 2.5with Orthophosporic acid. filter through 0.45µm nylon membrane filter and degas.

Mobile phase: pH 2.5 buffer : Acetonitrile (60:40)

The Chromatograms were shown in Fig 3.

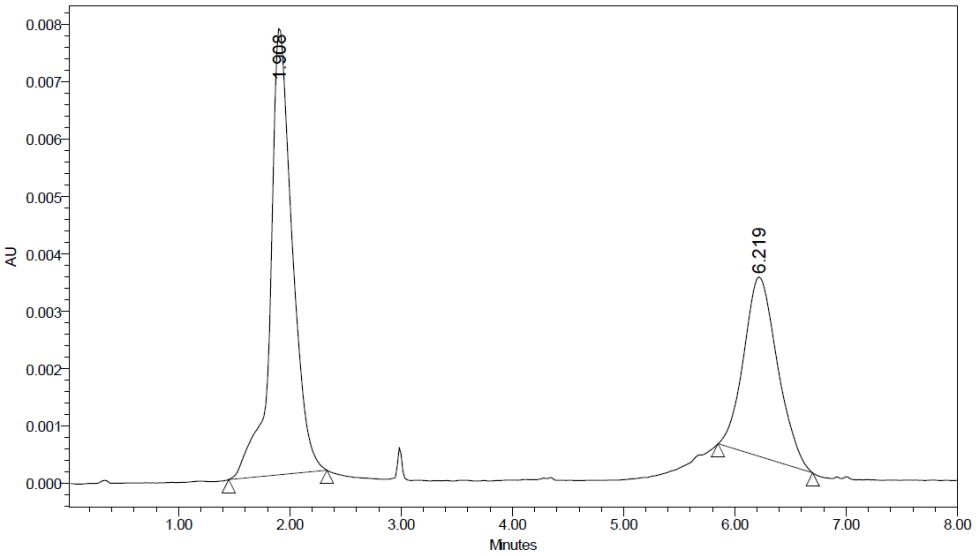


Figure 3: chromatogram of Sumatriptan and Naproxen

Chromatographic Parameters

Equipment : High performance liquid chromatography equipped with Auto Sampler and UV detector
Column : Symmetry C18 (4.6 x 150mm, 3.5µm, Make: XTerra)
Flow rate : 0.8 mL per min
Wavelength : 285 nm
Injection volume : 20 µl
Column oven : Ambient
Run time : 8min

Isocratic programme:

S.NO	Name of the peak	Retention time(min)
1	NAPROXEN	1.908
2	SUMATRIPTAN	6.219

Conclusion: The retention time and resolution was too long and the peak was symmetric.

OPTIMIZED METHOD:

Preparation of Phosphate buffer:

Weighed 7.0 grams of KH_2PO_4 into a 1000ml beaker, dissolved and diluted to 1000ml with HPLC water. Adjusted the pH to 3 with Orthophosporic acid. filter through 0.45µm nylon membrane filter and degas.

Mobile phase: pH 3 buffer : Acetonitrile (50:50)

The Chromatograms were shown in Fig 4.

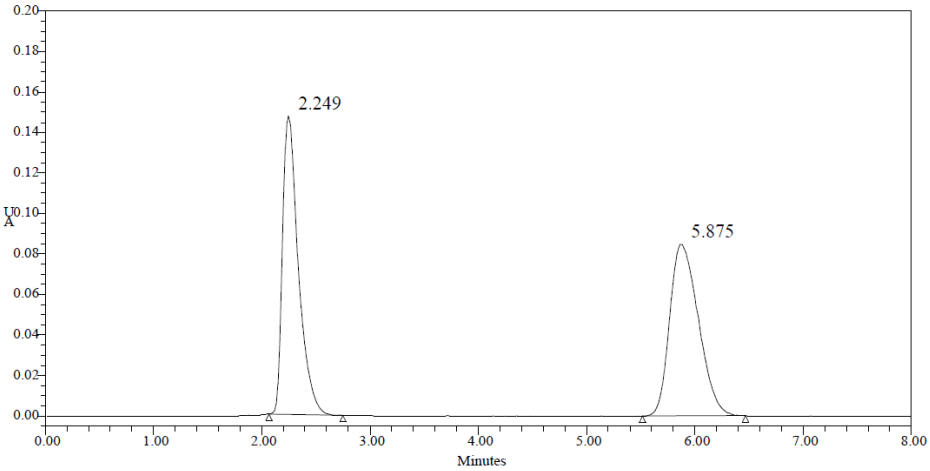


Figure 4: chromatogram of Sumatriptan and Naproxen

Chromatographic Parameters

Equipment : High performance liquid chromatography equipped with Auto Sampler and UV detector
Column : Symmetry C8 (4.6 x 150mm, 3.5µm, Make: XTerra)
Flow rate : 0.7 mL per min
Wavelength : 285 nm
Injection volume : 20 µl
Column oven : Ambient
Run time : 8min

Isocratic programme:

S.NO	Name of the peak	Retention time(min)
1	NAPROXEN	2.249
2	SUMATRIPTAN	5.875

Conclusion: The retention time and shape was good, hence this method was finalized for the estimation of Sumtriptan and Naproxen

METHODOLOGY
METHOD DEVELOPMENT
Chromatographic Parameters

Equipment : High performance liquid chromatography equipped with Auto Sampler and UV detector
Column : Symmetry C8 (4.6 x 150mm, 3.5µm, Make: XTerra)
Flow rate : 0.7 mL per min
Wavelength : 285 nm
Injection volume : 20 µl
Column oven : Ambient
Run time : 8min

Preparation of Phosphate buffer:
Weighed 7.0 grams of KH_2PO_4 into a 1000ml beaker, dissolved and diluted to 1000ml with HPLC water. Adjusted the pH to 3 with Orthophosporic acid.

Preparation of mobile phase.
Mix a mixture of above buffer 500 mL (50%) and 500 mL of Acetonitrile HPLC (50%) and degas in ultrasonic water bath for 5 minutes. Filter through 0.45 µ filter under vacuum filtration.

Preparation of standard solution: (Stock solution)
Accurately weigh and transfer 10 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. Further pipette 5ml of Sumatriptan&Naproxen the above stock solution into a 50ml volumetric flask and dilute up to the mark with diluent. Further pipette 8ml of Sumatriptan & Naproxen the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation of sample solution: (Stock solution)
Accurately weigh and transfer equivalent to 10 mg of Sumatriptan and Naproxen 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 diluent. Further pipette 5ml of Sumatriptan & Naproxen of the above stock solution into a 50ml volumetric flask and dilute up to the mark with diluent. Further pipette 8ml of Sumatriptan & Naproxen the above stock solution into a10ml volumetric flask and dilute up to the mark with diluents.

METHOD VALIDATION
Linearity:
Preparation of stock solution: Accurately weigh and transfer 10 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 5ml of Sumatriptan&Naproxen of the above stock solution into a 50ml volumetric flask and dilute up to the mark with diluent.

Preparation of Level – I (60ppm of Sumatriptan & Naproxen):
6ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with diluent.

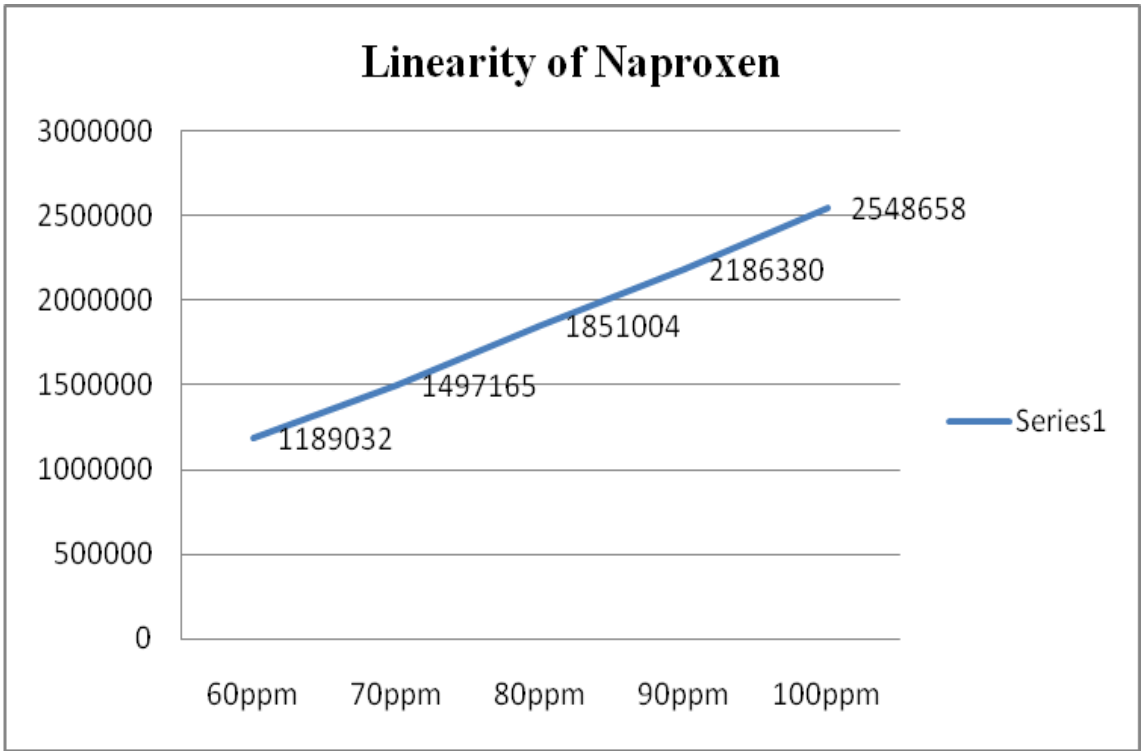
Preparation of Level – II (70ppm of Sumatriptan & Naproxen):
7ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – III (80ppm of Sumatriptan & Naproxen):
8ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – IV (90ppm of Sumatriptan & Naproxen):
9ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – V (100ppm of Sumatriptan & Naproxen)
10ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with diluent.
Procedure:
Inject each level into the chromatographic system and measure the peak area. A calibration curve was plotted for concentration v/s peak area and calculate the correlation coefficient it was given in the Fig 5, 6. The results were discussed in Table 1.

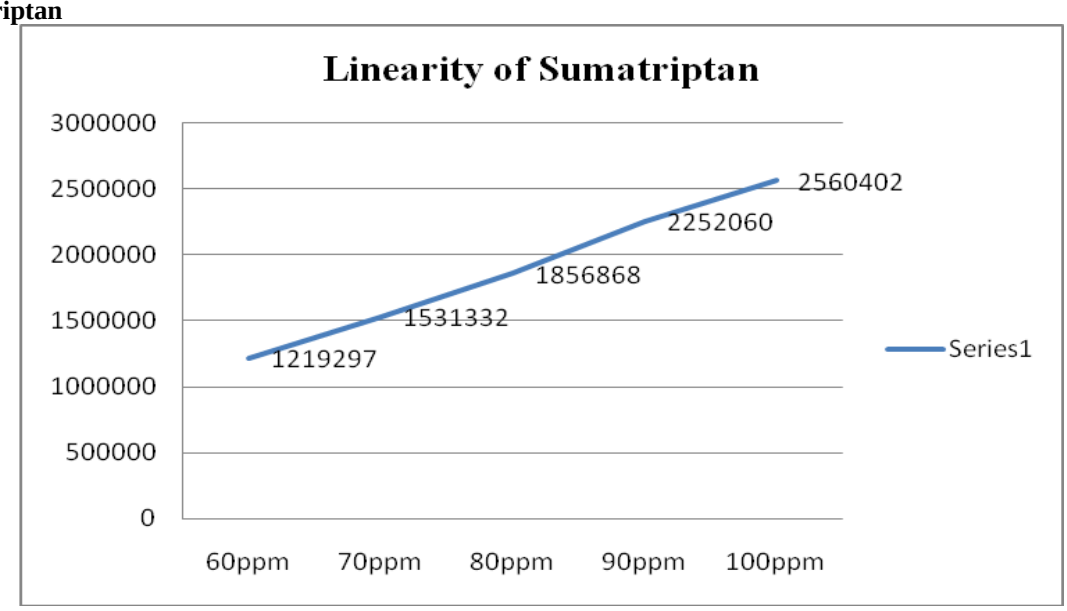
Naproxen



	Conc. in µg/ml	Response
1	60	1189032
2	70	1497165
3	80	1851004
4	90	2186380
5	100	2548658

Figure 5: Linearity of Naproxen

Sumatriptan



	Conc. in µg/ml	Response
1	60	1219297
2	70	1531332
3	80	1856868
4	90	2252060
5	100	2560402

Figure 6: Linearity of Sumatriptan

ASSAY

Assay of different formulations available in the market was carried by injecting sample corresponding to equivalent weight into HPLC system. And percent purity was found out by following formulae. Recovery studies were carried out. The results were discussed in the Table 2,3. The Chromatograms were shown in Fig 7, 8, 9.

Calculate the percentage purity of Sumatriptan and Naproxen present in tablet using the formula:

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

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 Naproxen/Sumatriptan

Blank

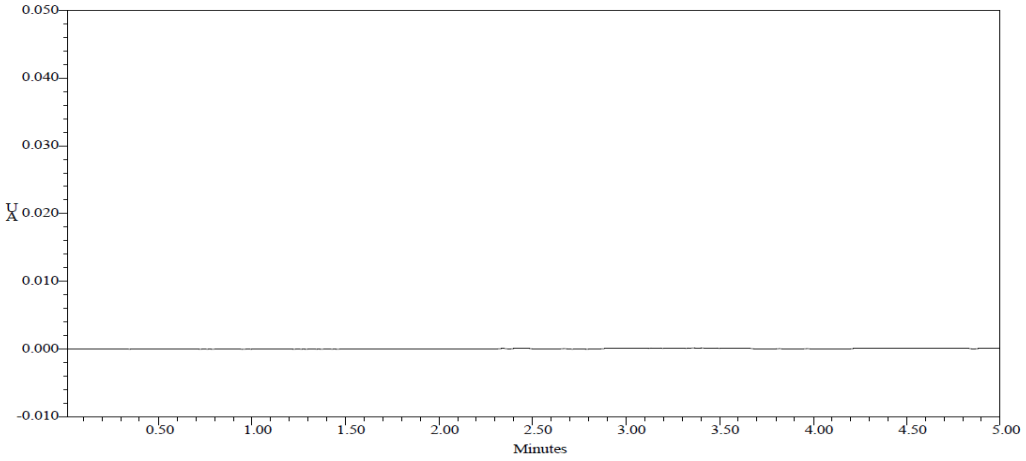
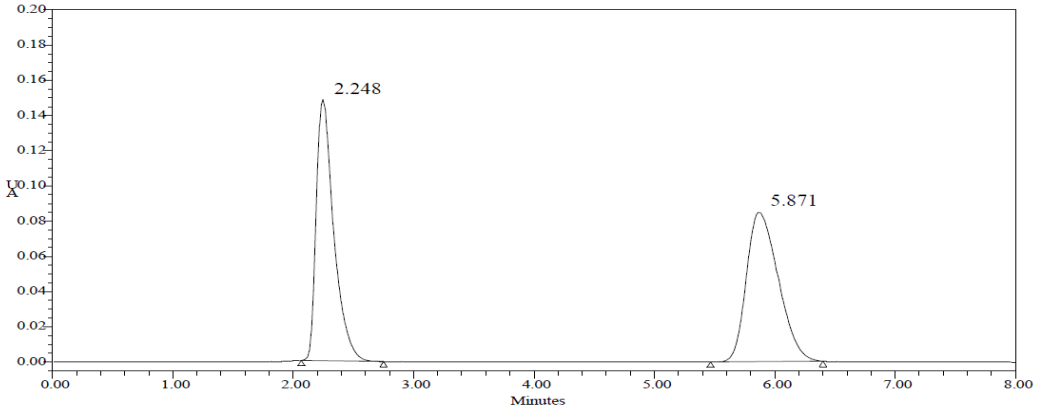


Figure 7: chromatogram of blank

Standard
Injection-1



Injection-2

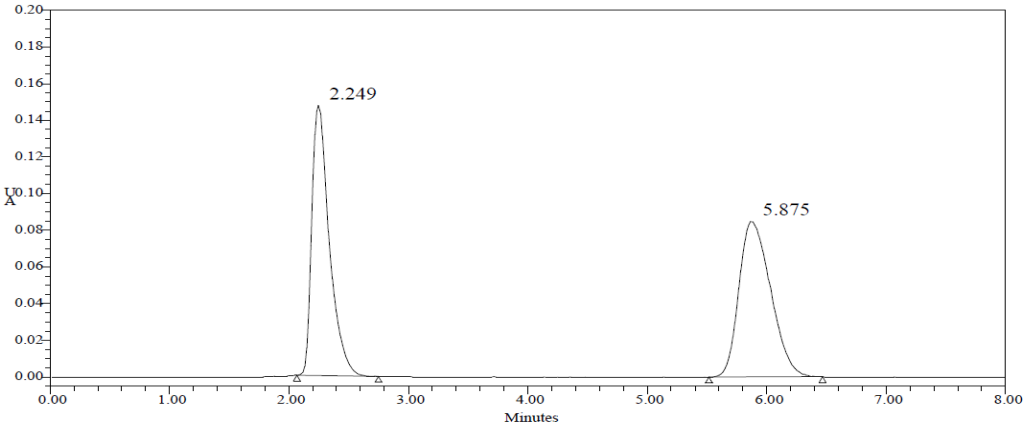
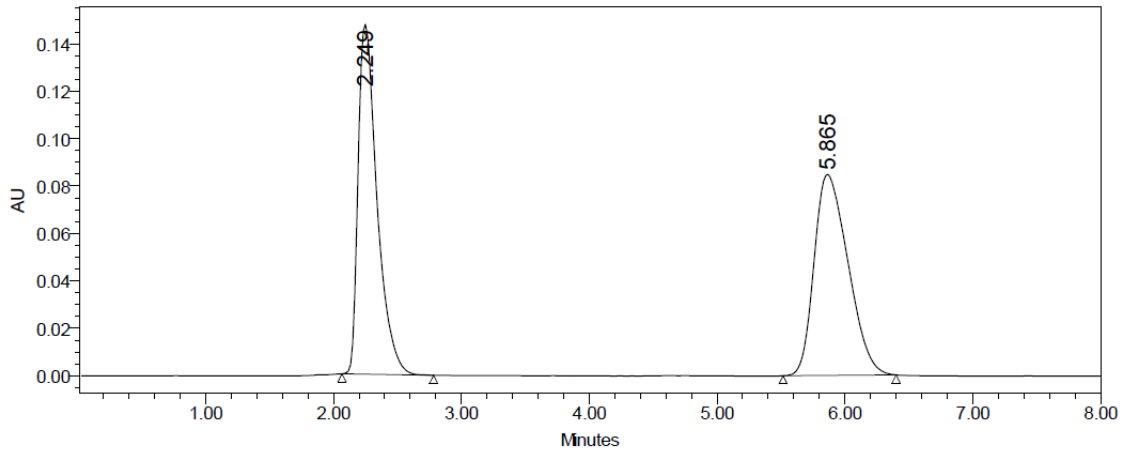


Figure 8:chromatogram of Standard

Sample

Injection-1



Injection-2

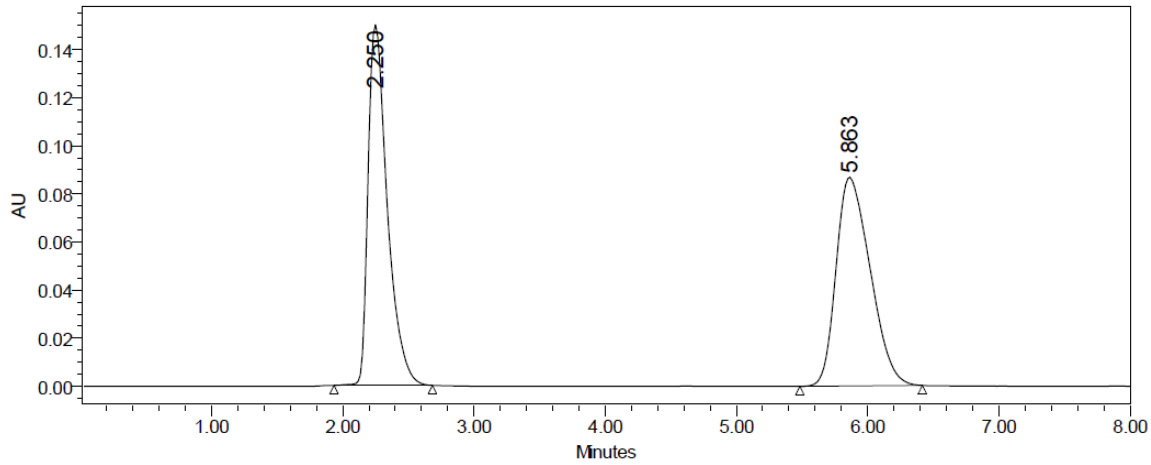


Figure 9:chromatogram of Sample

Accuracy:

Preparation of Standard stock solution:

Accurately weigh and transfer 10 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 5ml of Sumatriptan & Naproxen of the above stock solution into a 50ml volumetric flask and dilute up to the mark with diluent. Further pipette 8ml of Sumatriptan & Naproxen the above stock solution into a 10 ml volumetric flask and dilute up to the mark with diluent.

Preparation Sample solutions:

For preparation of 50% solution (With respect to target Assay concentration):

Accurately weigh and transfer 6.15mg of Sumatriptan and 6.15 mg 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 5ml of Sumatriptan & Naproxen of the above stock solution into a 50ml volumetric flask and dilute up to the mark with diluent. Further pipette 8ml of Sumatriptan & Naproxen the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

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 standards into a 10mL clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further pipette 5ml of Sumatriptan & Naproxen of the above stock solution into a 50 ml volumetric flask and dilute up to the mark with diluent. Further pipette 8 ml of Sumatriptan & Naproxen the above stock solution into a 10ml volumetric up to the mark with diluent

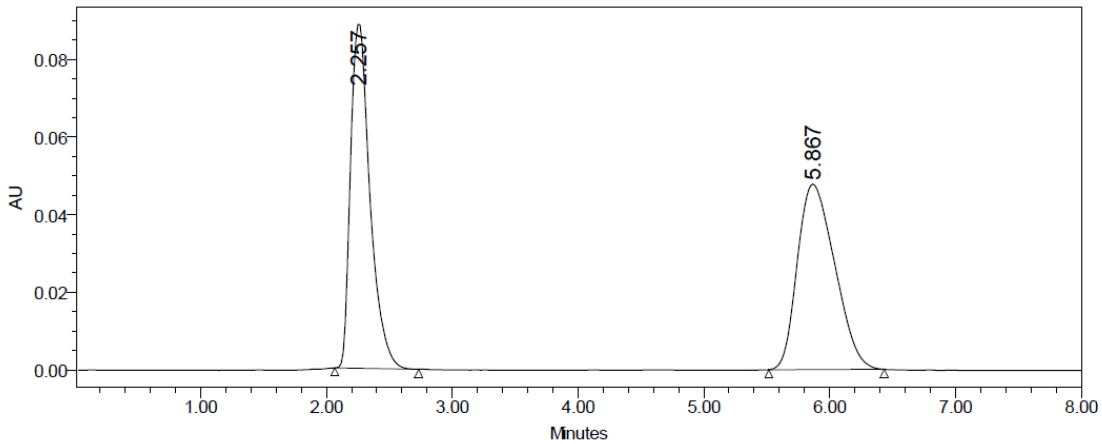
For preparation of 150% solution (With respect to target Assay concentration):

Accurately weigh and transfer15.5mg of Sumatriptan and 15.6mg of Naproxen working standards 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 5ml of Sumatriptan & Naproxen of the above stock solution into a 50 ml volumetric flask and dilute up to the mark with diluent. Further pipette 8 ml of Sumatriptan & Naproxen the above stock solution into a 10 ml volumetric up to the mark with diluent

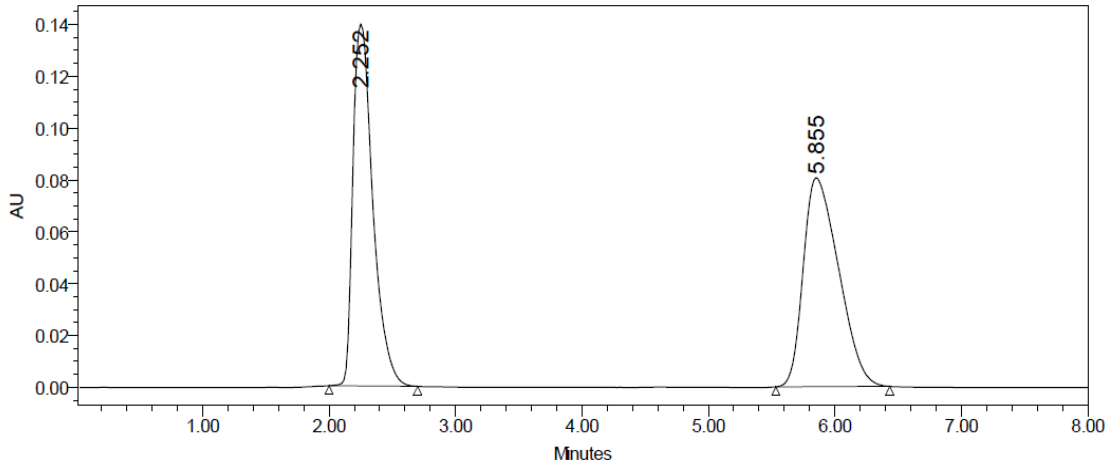
Procedure:

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. The Chromatograms were shown in Fig 10. The results were discussed in the Table 4, 5.

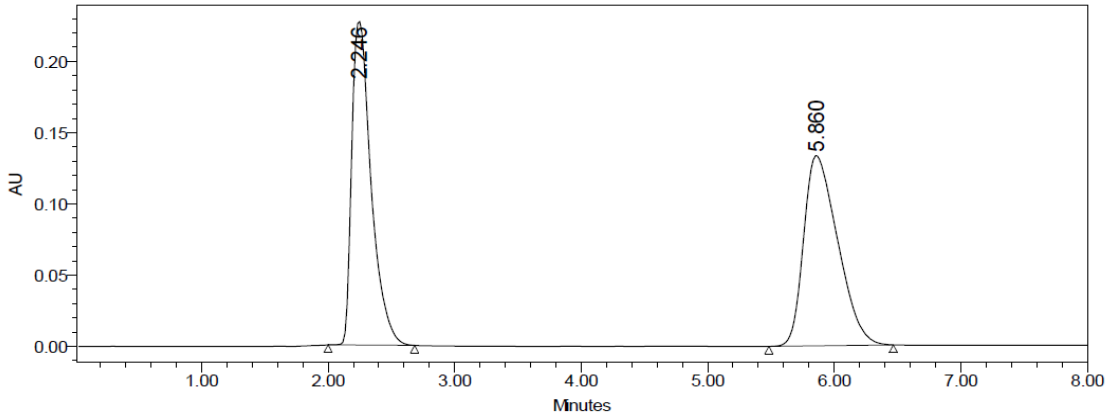
Accuracy:-



Accuracy 50%



Accuracy 100%



Accuracy 150%

Figure 10: chromatogram of Accracy

Precision:

Preparation of stock solution:

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

Further pipette 5ml of Sumatriptan & Naproxen of the above stock solution into a 50 ml volumetric flask and dilute up to the mark with diluent. Further pipette 8ml of Sumatriptan & Naproxen the above stock solution into a 10 ml 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. The Chromatograms were shown in Fig 11. The results were discussed in the Table 6.

Standard Deviation

$$\text{S.D } \sigma = \sqrt{\frac{\sum (x - x_i)^2}{n-1}}$$

Where, x=Sample,

x_i=Mean value of samples.

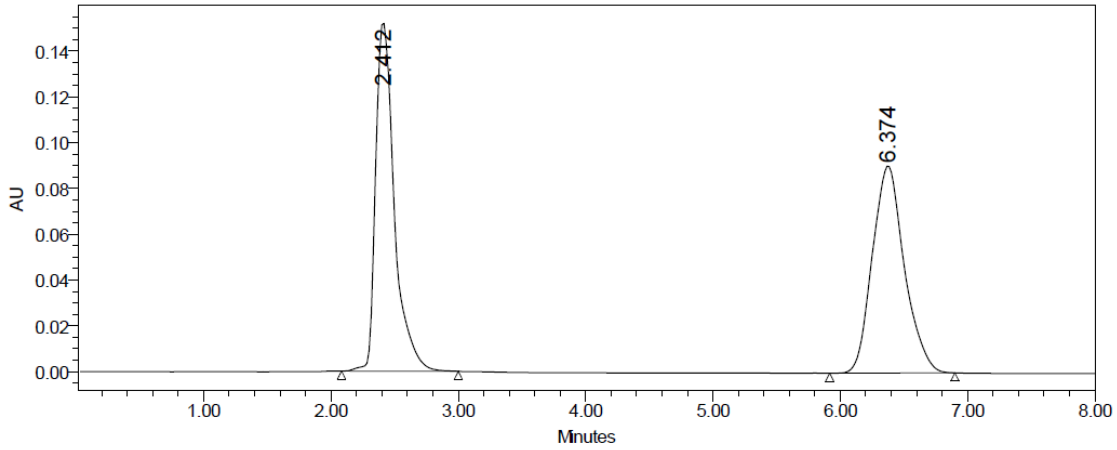
n=number of samples.

Coefficient of variance / Relative standard deviation

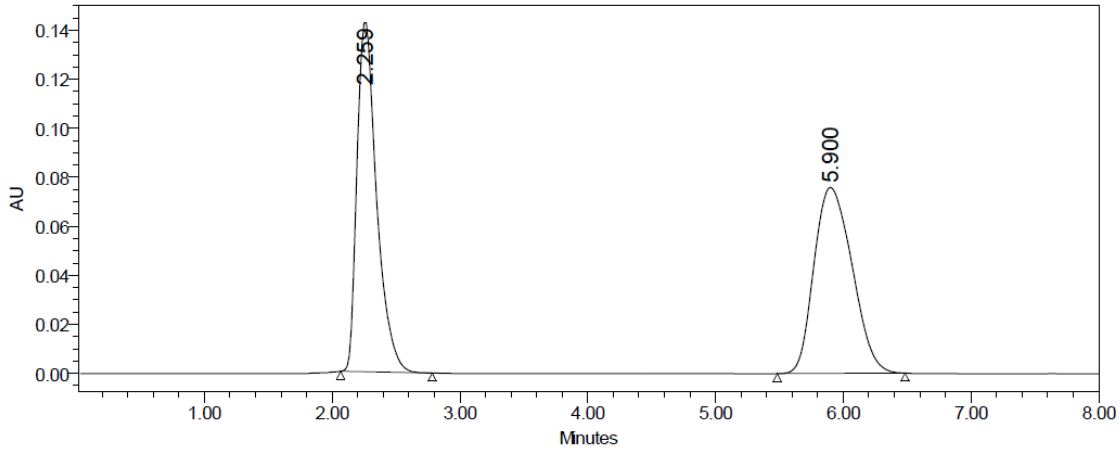
$$C.V = \frac{\text{Standard Deviation}}{\text{Mean}} \times 100$$

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

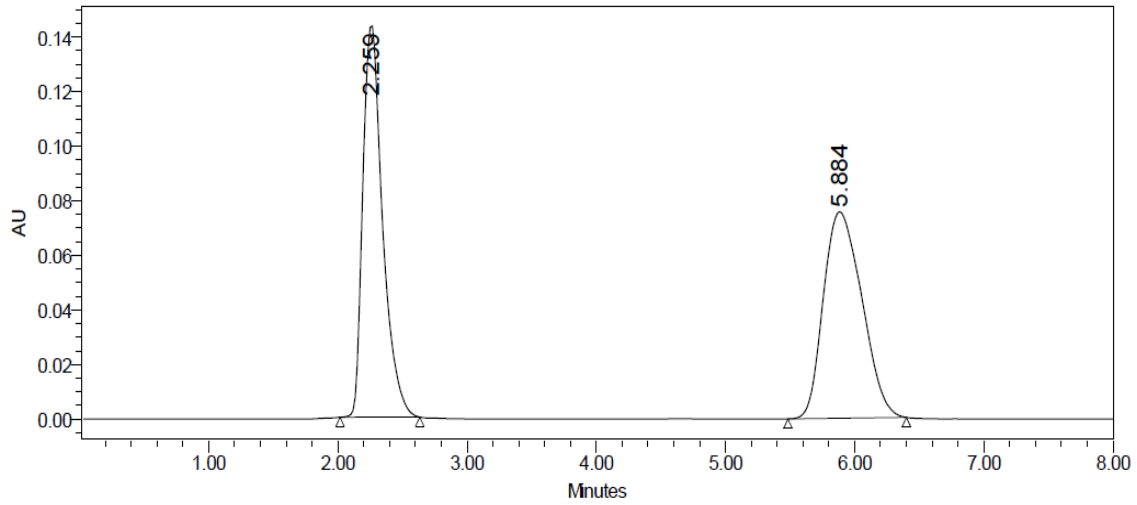
Injection 1



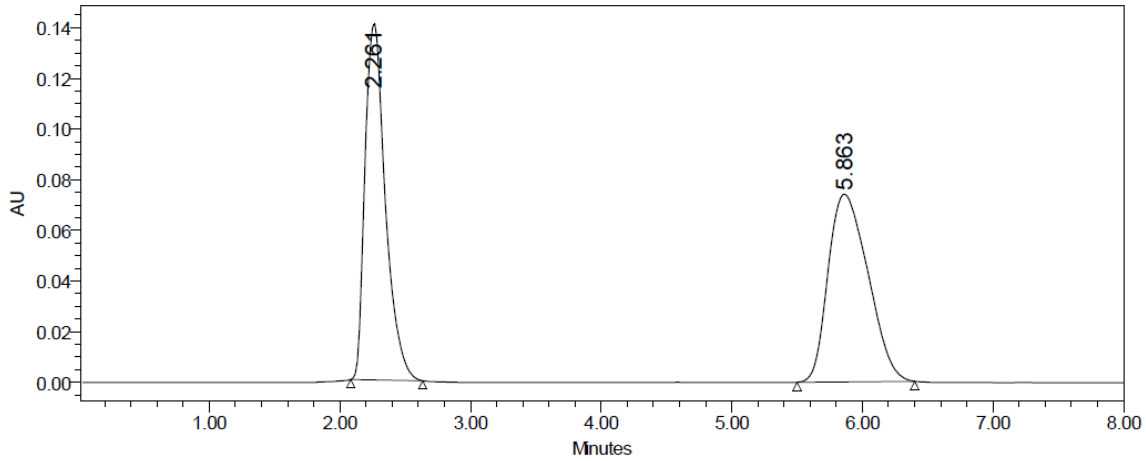
Injection 2



Injection 3



Injection 4



Injection 5

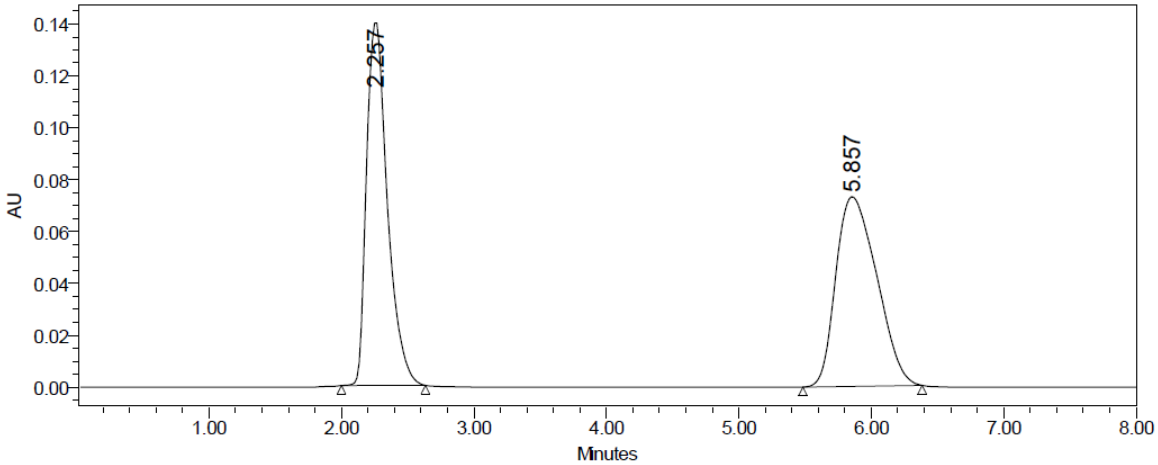


Figure 11: chromatograms indicating Precision

SYSTEM SUITABILITY

A Standard solution of Sumatriptan and Naproxen working standard was prepared as per procedure and was injected six times into the HPLC system. The system suitability parameters were evaluated from standard Chromatograms obtained by calculating the % RSD of retention times, tailing factor, theoretical plates and peak areas from six replicate injections.

Acceptance criteria

1. The % RSD for the retention times of principal peak from 6 replicate injections of each Standard solution should be not more than 2.0 %.
2. The number of theoretical plates (N) for the Sumatriptan and Naproxen peaks should be not less than 2000.
3. The Tailing factor (T) for the Sumatriptan and Naproxen peaks should be not more than 2.0.

From the system suitability studies it was observed that all the parameters were within limit. Hence it was concluded that the Instrument, Reagents and Column were suitable to perform the assay. The results were discussed in Table below 7 .the chromatogram was shown in fig. 12.

System suitability:-

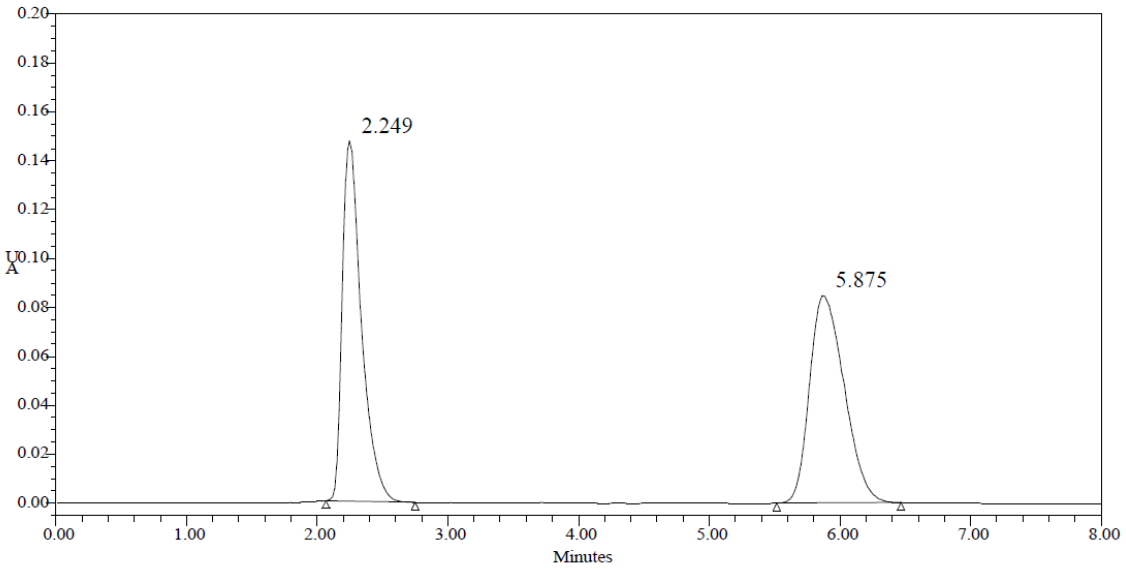


Figure 12: chromatogram indicating system suitability

	Name	Retenction Time (min)	Area (µv*sec)	Height (µv)	USP Plate count	USP Tailing
1	Naproxen	2.249	1531670	147652	2187.1	1.6
2	Sumatriptan	5.875	1573899	84946	2196.7	1.3

ROBUSTNESS:

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

a). The flow rate was varied at 0.6 ml/min to 1.0ml/min.

Standard solution 80 ppm of Sumatriptan &Naproxen was prepared and analysed using the varied flow rates along with method flow rate.

The results are summarized

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 not robust even by change in the flow rate ±10%.

The method is robust only in less flow condition.

b). The Organic composition in the Mobile phase was varied from 45% to 55%.

Standard solution 80 µg/ml of Sumatriptan & Naproxen was prepared and analysed using the varied Mobile phase composition along with the actual mobile phase composition in the method.

The results are summarized

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 not robust even by change in the Mobile phase ±10

The Chromatograms were shown in Fig 13, 14, 15, 16. The results were discussed in Table no 8, 9.

More organic solvent (Organic Plus)

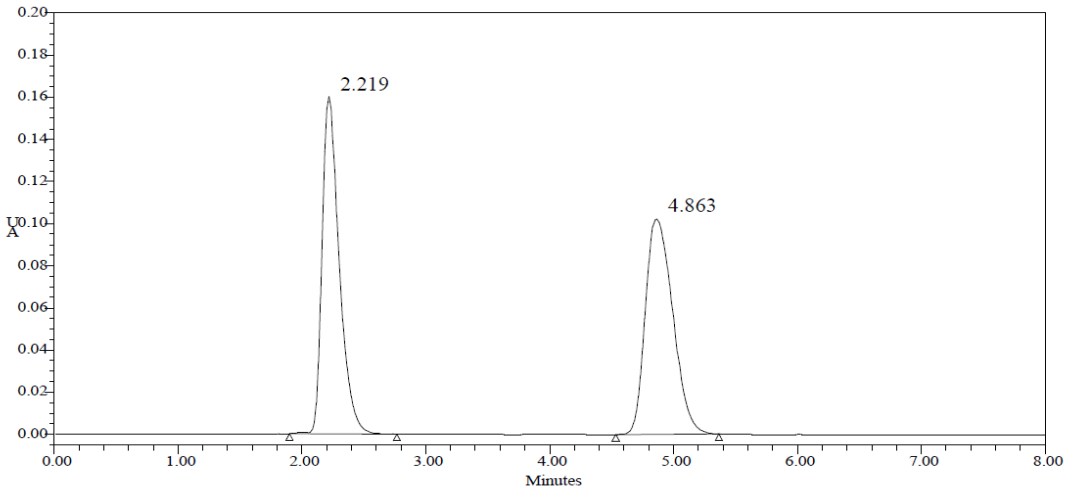


Figure 13: chromatogram indicating robustness (more organic solvent)

Less organic solvent (Organic Minus)

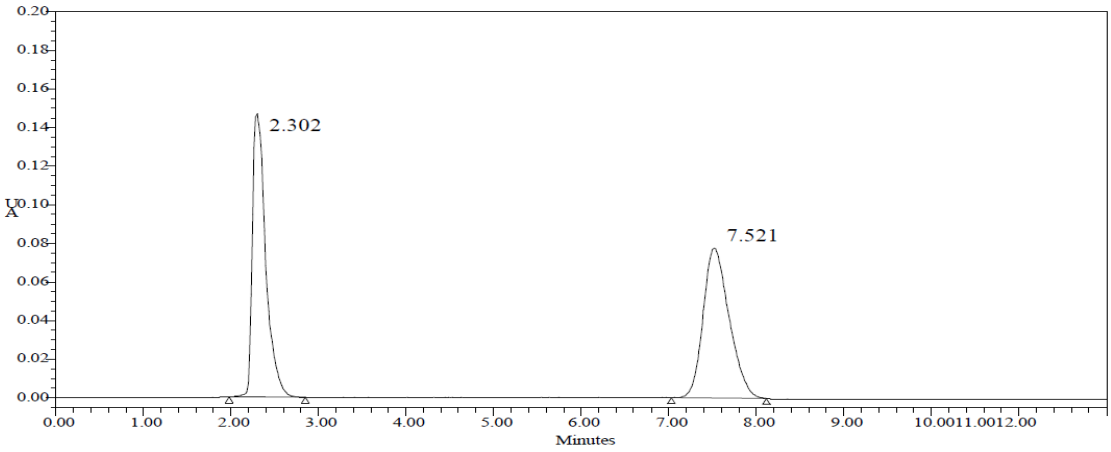


Figure 14: chromatogram indicating robustness (less organic solvent)

More flow (Flow Plus)

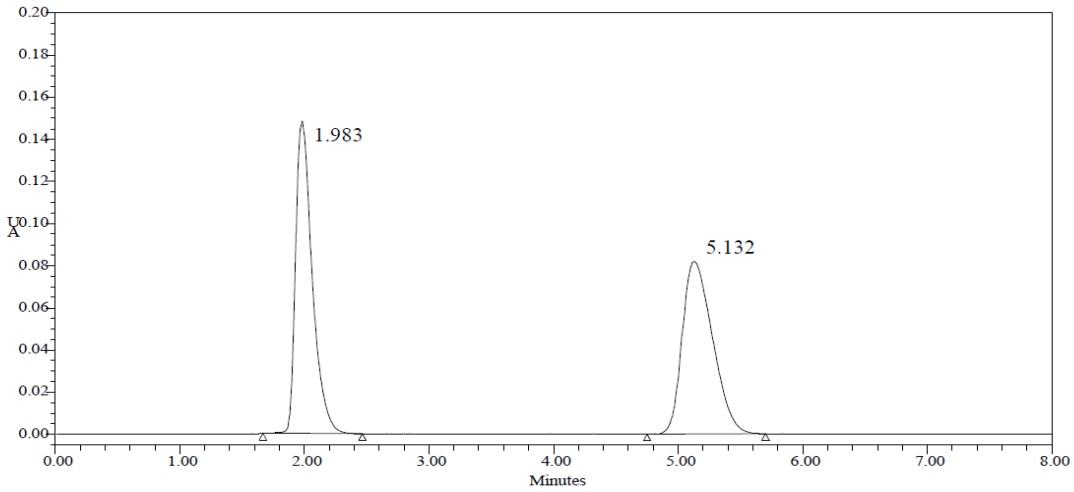


Figure 15: chromatogram indicating robustness (More flow)

Less flow (Flow Minus)

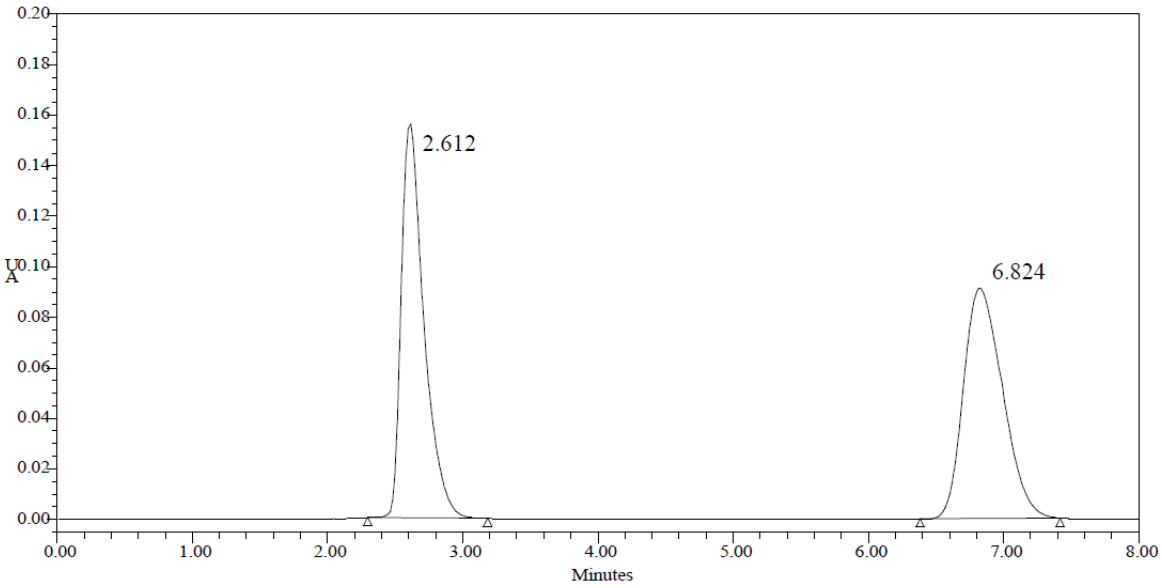


Figure 16: chromatogram indicating robustness (less flow)

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 10 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 5ml of Sumatriptan & Naproxenof the above stock solution into a 50ml volumetric flask and dilute up to the mark with diluent. Further pipette 8ml of Sumatriptan & Naproxen the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluents

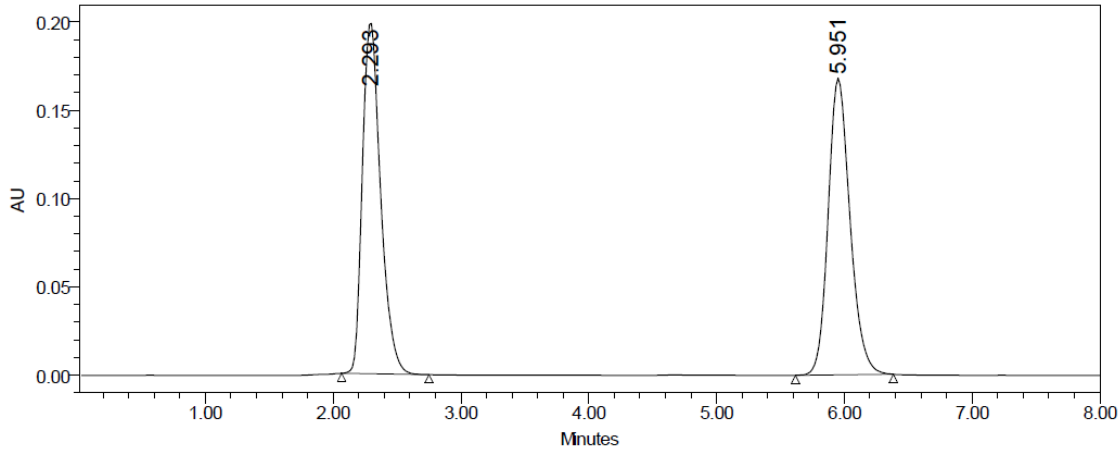
Procedure:

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:**

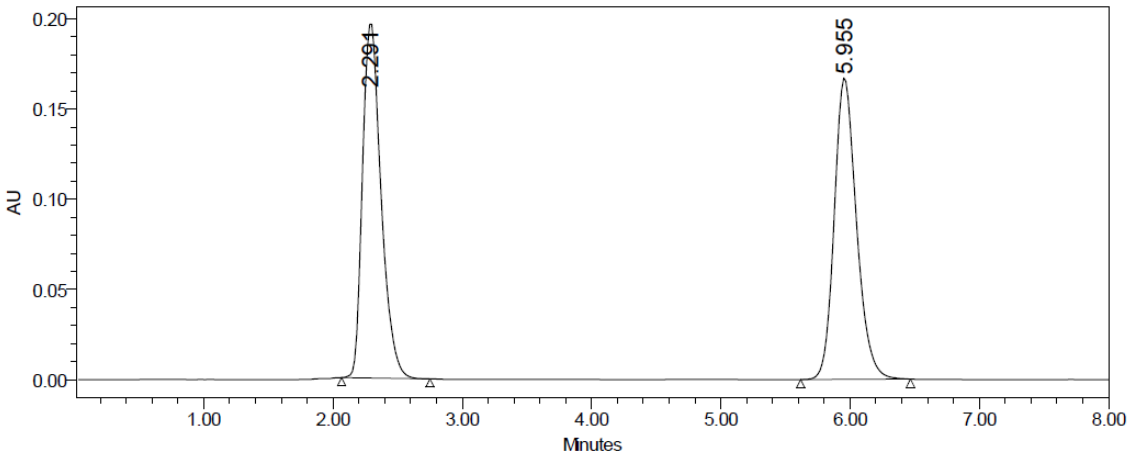
The % RSD for the area of five standard injections results should not be more than 2%.

The Chromatograms were shown in Fig 17. The results were discussed in Table no 10.

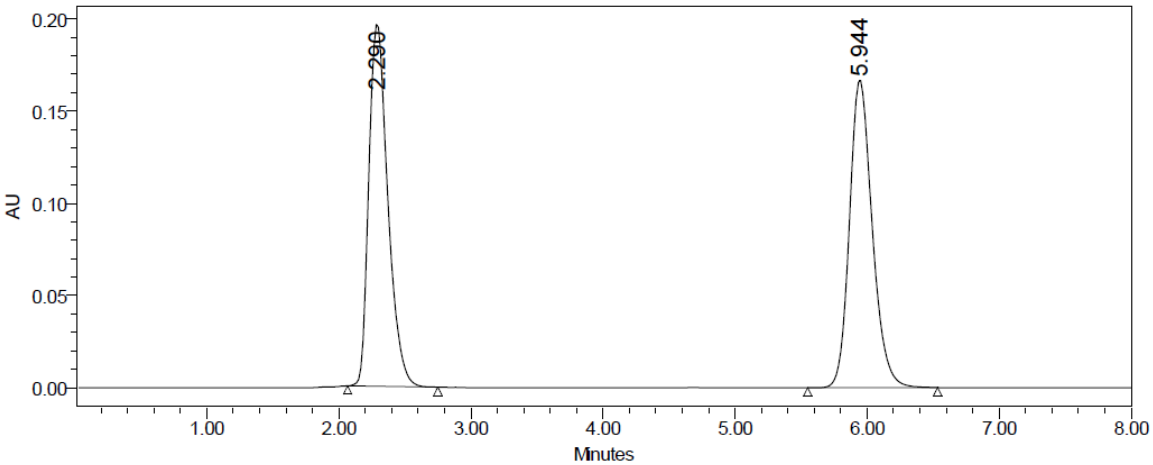
Injection 1



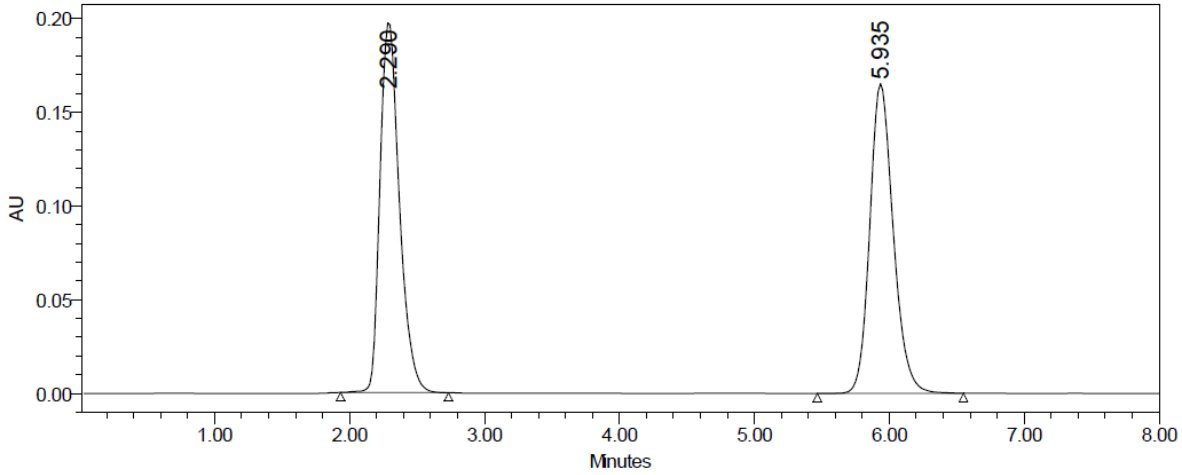
Injection 2



Injection 3



Injection 4



Injection 5

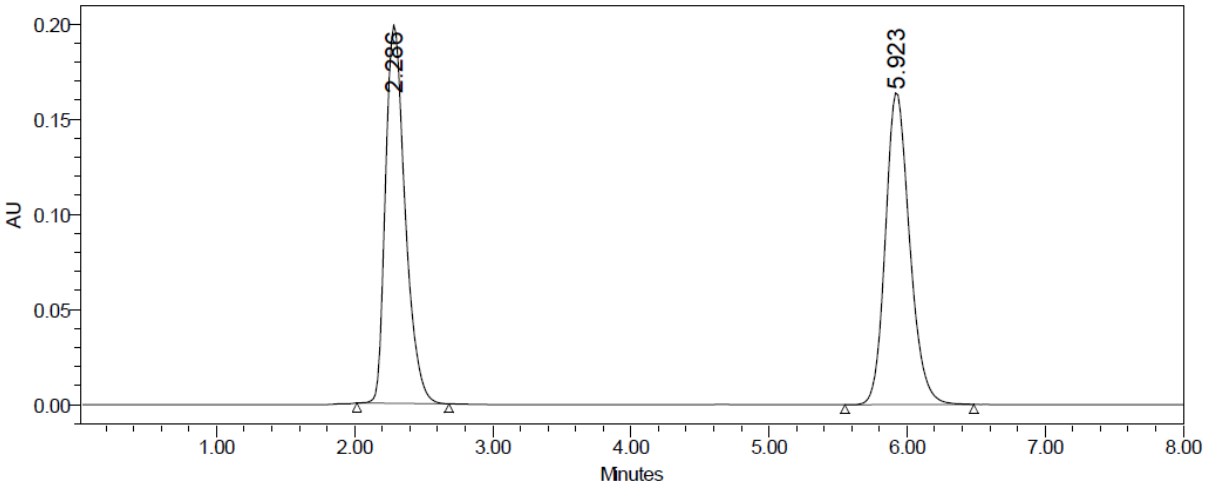


Table (17): Ruggedness of Sumatriptan and Naproxen

LIMIT OF DETECTION:

Sumatriptan:

Preparation of 80µg/ml solution:

Accurately weigh and transfer 10 mg of Sumatriptan working standard into a 10 mL 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 5ml of the above stock solution into a 50 ml volumetric flask and dilute up to the mark with diluent.

Further pipette 8ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation of 0.9% solution At Specification level (0.72µg/ml solution):

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

Pipette 0.9 mL of 1µg/ml solution into a 10 ml of volumetric flask and dilute up to the mark with diluent.

Naproxen:

Preparation of 80µg/ml solution: Accurately weigh and transfer 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 5ml of the above stock solution into a 50ml volumetric flask and dilute up to the mark with diluent.

Further pipette 8ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation of 0.16% solution At Specification level (0.13µg/ml solution):

Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluents. Pipette 0.16mL of 1µg/ml solution into a 10 ml of volumetric flask and dilute up to the mark with diluent.
The Chromatograms were shown in Fig 18, 19.

Sumatriptan

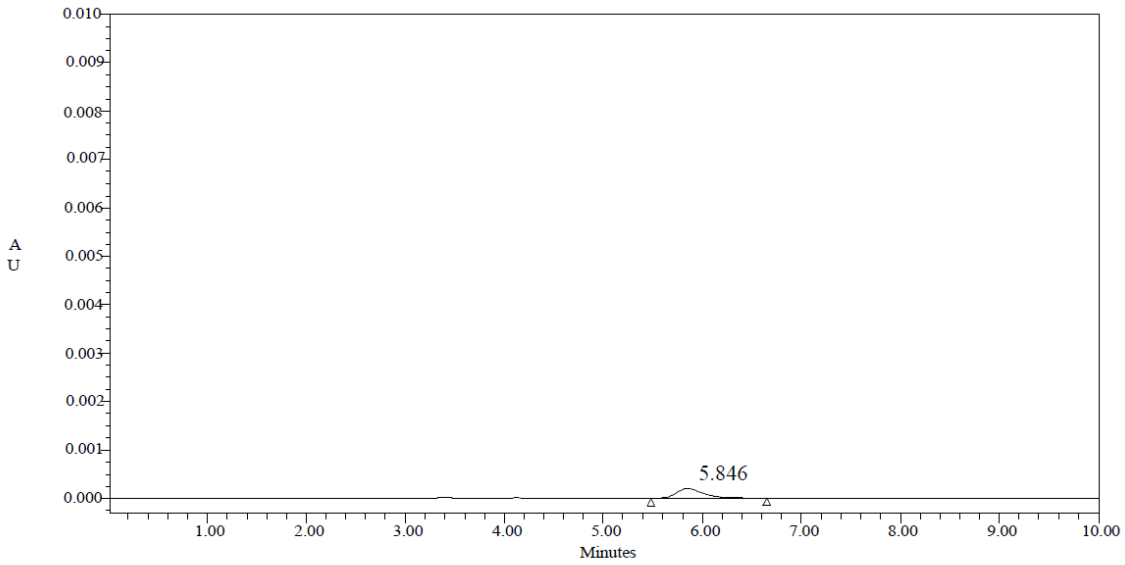


Figure 18: chromatogram indicating LOD of Sumatriptan

Naproxen

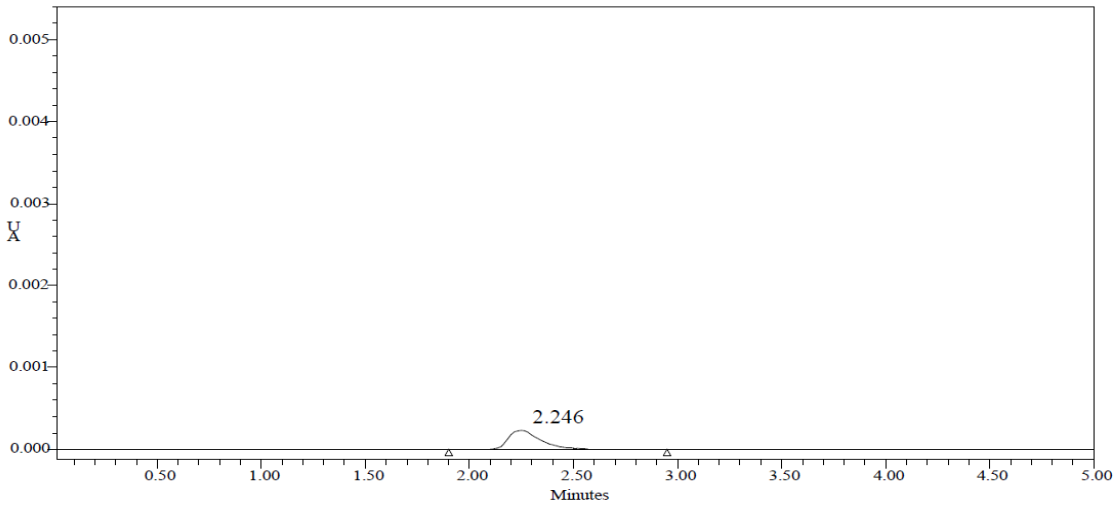


Figure 19: chromatogram indicating LOD of Naproxen

LIMIT OF QUANTIFICATION:

Sumatriptan:

Preparation of 80µg/ml solution:

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

Further pipette 5ml of the above stock solution into a 50ml volumetric flask and dilute up to the mark with diluent. Further pipette 8ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation of 0.29% solution At Specification level (0.232µg/ml solution):

Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Pipette 0.29mL of 1µg/ml solution into a 10 ml of volumetric flask and dilute up to the mark with diluents.

Naproxen:

Preparation of 80µg/ml solution:

Accurately weigh and transfer 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 5ml of the above stock solution into a 50ml volumetric flask and dilute up to the mark with diluent.

Further pipette 8ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation of 0.49% solution At Specification level (0.39 µg/ml solution):

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

Pipette 0.49mL of 1µg/ml solution into a 10 ml of volumetric flask and dilute up to the mark with diluent.

The Chromatograms were shown in Fig 20, 21.

Sumatriptan

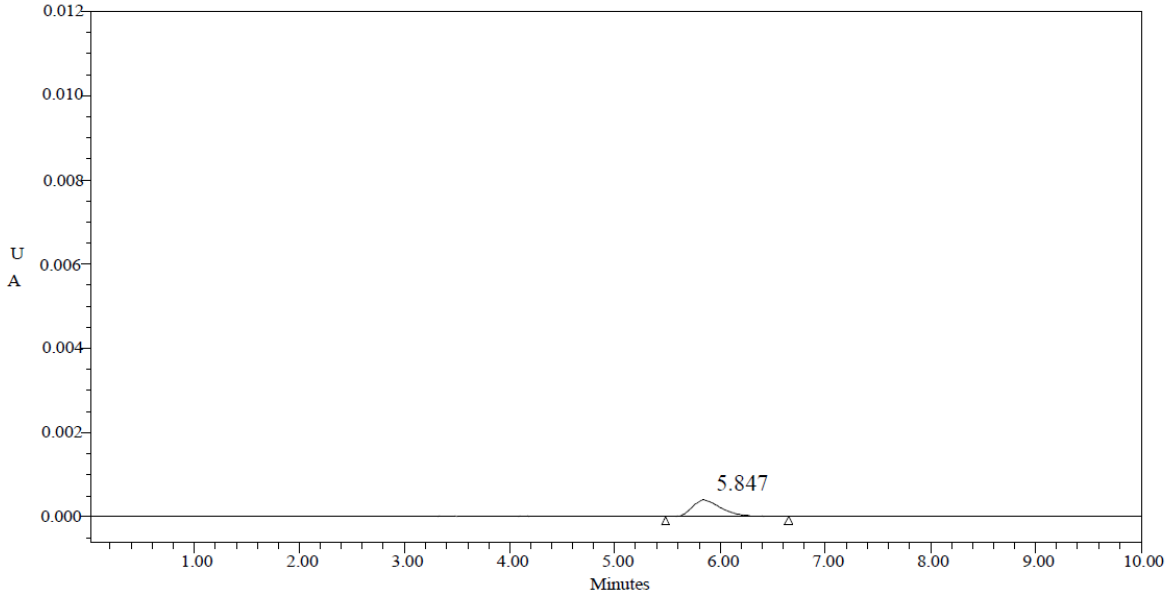


Figure 20: chromatogram indicating LOQ of Sumatriptan

Naproxen

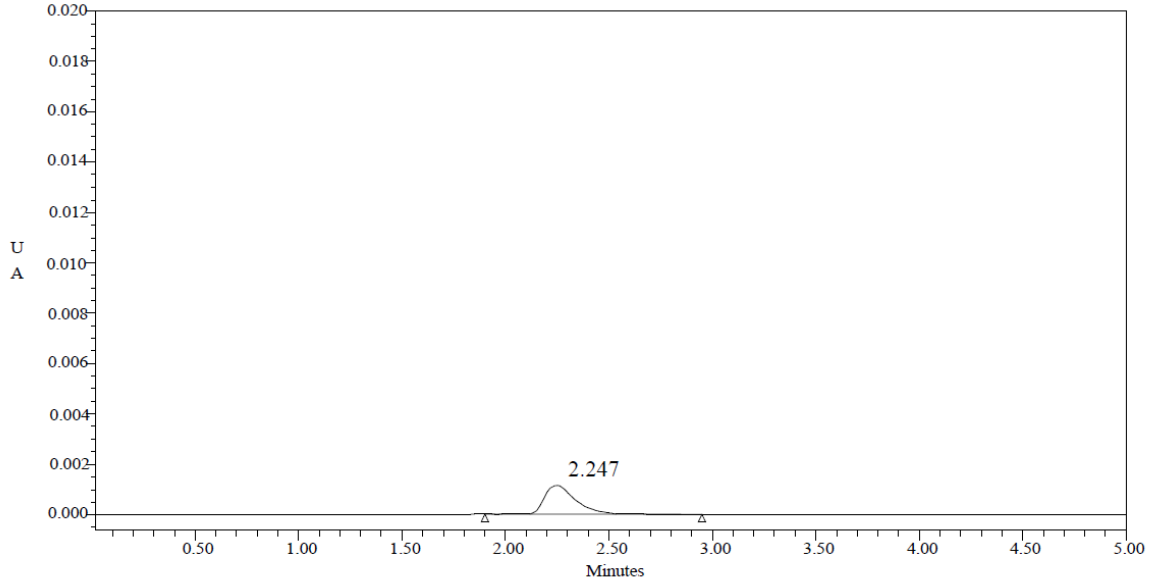


Figure 21: chromatogram indicating LOQ of Naproxen

RESULTS AND DISCUSSIONS:

Sumatriptan is an serotonin agonist that acts selectively at 5HT1 receptors and Naproxen An anti-inflammatory agent with analgesic and antipyretic properties. These both are combindly used mainly to treat acute migraine. A simple reverse phase HPLC method was developed for the determination of Sumatriptan and Naproxen. Symmetry C8 (4.6 x 150mm, 3.5 µm, Make: XTerra) in an isocratic mode with mobile phase Acetonitrile:Phosphate buffer PH3 (50:50) was used. The flow rate was 0.7 ml/ min and effluent was monitored at 285 nm. The retention time for Sumatriptan is 2.2 min and Naproxen 5.8 min.

S.no	Naproxen				Sumatriptan			
	Standard Rt	Sample Rt	Standard area	Sample area	Standard Rt	Sample Rt	Standard area	Sample area
01	2.248	2.24	1523510	1521502	5.871	5.865	1566643	1561262
02	2.249	2.250	1531670	1529265	5.875	5.863	1573899	1564536
MEAN			1532594	1525384			1570271	1562899
STDEV			1527590	5488.8			1570271	2315.2
%RSD			0.377	0.36			0.327	0.15

Table (1) Linearity of Sumatriptan and Naproxen

SI.No	Conc.Taken in µg/ml	R.T in min Naproxen	R.T in min Sumatriptan	Peak area of Naproxen	Peak area of Sumatriptan
1	60ppm	2.247	5.842	1189032	1219297
2	70ppm	2.248	5.846	1497165	1531332
3	80ppm	2.245	5.866	1851004	1856868
4	90ppm	2.246	5.865	2186380	2252060
5	100ppm	2.247	5.869	2548658	2560402
Correlation Coefficient				0.999	0.999

Table (2): Assay of Sumatriptan and Naproxen by RP-HPLC

S.no	Naproxen		Sumatriptan	
01	Spl. Area	1525384	Spl. Area	1503.654
02	Std.Area	1532594	Std.Area	1457.193
03	Std. Wt	10mg	Std. Wt	10mg
04	Spl.Wt	117mg	Spl.Wt	19.8mg
05	LC	500mg	LC	85mg
06	Avg.Wt	997.5mg	Avg.Wt	997.5mg
07	Std.Purity	99.8	Std.Purity	99.6
08	Assay %	98.8	Assay %	98.6

Table (3): Assay of Sumatriptan and Naproxen by RP-HPLC

Linearity:-

Linearity data of sample:

nclusion

As the results are within the acceptance limits, the proposed method is found to be linear at concentration of 60-100 µg/ml for Sumatriptan and Naproxen.

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	984243	6.15	6.23	101.4%	101.0%
100%	1567396	10.0	9.98	99.8	
150%	2497228	15.5	15.79	101.9%	

Table (4) Accuracy of Sumatriptan

Assay:-

Conclusion:- As the results are within the acceptance limits 0f 95-105%

Accuracy

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	958196	6.15	6.25	101.7%	101.0%
100%	1532695	10.0	9.96	99.5%	
150%	2425792	15.6	15.8	101.7%	

Table (5) Accuracy of Naproxen

Conclusion:- As the results are within the acceptance limits 0f 98-102%

Precision:

SI.No	Injection number (80 mcg/ml)	Retention Time of Naproxen	Retention Time of Sumatriptan	Area of Naproxen	Area of Sumatriptan
1	Injection-1	2.412	6.374	1549491	1579612
2	Injection-2	2.259	5.900	1530248	1574521
3	Injection-3	2.259	5.884	1530713	1566564
4	Injection-4	2.261	5.863	1527834	1570849
5	Injection-5	2.257	5.857	1537667	1567180
	AVRG			1535191	1571745
	STDEV			8792.8	5433.0
	%RSD			0.57	0.35

Table (6) Precision of Sumatriptan and Naproxen

System Suitability

Proposed variations		USP Plate Count	USP Tailing
Variation in mobile phase composition	10% less	2396.9	1.8
	*Actual	2183.4	1.6
	10% more	2218.9	1.5
Variation in flow rate	**0.6ml/min**	2228.3	1.6
	0.8ml/min	2183.4	1.6
	1.0ml/min	2142.7	1.6

Table (7): System Suitability of Sumatriptan and Naproxen by RP-HPLC

Robustness
Sumatriptan

Table (8) Robustness of Sumatriptan

Proposed variations		USP Plate Count	USP Tailing
Variation in mobile phase composition	10% less	2963.1	1.2
	*Actual	2203.7	1.3
	10% more	2268.8	1.3
Variation in flow rate	**0.6ml/min**	2519.4	1.3
	0.8ml/min	2203.7	1.3
	1.0ml/min	2474.4	1.3

Naproxen

Drug name	USP tailing	USP theoretical plates
Naproxen	1.6	2187.1
Sumatriptan	1.3	2196.7
Acceptance criteria	In between 0.5 to 2.0	Above 2000

Table (9) Robustness of Naproxen

Ruggedness:

	Retention Time of Naproxen	**Retention Time of Sumatriptan**	**Area of Naproxen**	**Area of Sumatriptan**
Standard(80mcg)	2.293	5.951	1976857	1990948
Analyst(1)(80mcg)	2.291	5.955	1971778	1993690
Analyst(2)(80mcg)	2.290	5.944	1970279	1995386
Analyst(3)(80mcg)	2.290	5.935	1979007	1992472
Analyst(4)(80mcg)	2.286	5.923	1970631	1993363
AVRG			**1973711**	**1993172**
STDEV			**3966.9**	**1631.1**
%RSD			**0.20**	**0.08**

Table (10): Ruggedness of Sumatriptan and Naproxen

Conclusion:-
The results are within the acceptance limit, the proposed method is found to be rugged.

LIMIT OF DETECTION (LOD)
Sumatriptan:-
Calculation of S/N Ratio:
Average Baseline Noise obtained from Blank : 44 µV
Signal Obtained from LOD solution (0.9% of target assay concentration) : 148 µV
S/N = 148/44 = 3.36

Acceptance Criteria:
S/N Ratio value shall be 3 for LO
D solution.

Conclusion:-
The LOD for Sumatriptan was found to be 3.36

Naproxen:-
Calculation of S/N Ratio:
Average Baseline Noise obtained from Blank : 44 µV
Signal Obtained from LOD solution (0.16% of target assay concentration) : 141 µV
S/N = 141/44 = 3.20

Acceptance Criteria:
S/N Ratio value shall be 3 for LOD solution.

Conclusion:-
The LOD for Naproxen was found to be 3.20

LIMIT OF QUANTIFICATION (LOQ)
Sumatriptan:-
Calculation of S/N Ratio:
Average Baseline Noise obtained from Blank : 44 µV
Signal Obtained from LOQ solution (0.29% of target assay concentration) : 436µV
S/N = 436/44 = 9.90

Acceptance Criteria:
S/N Ratio value shall be 10 for LOQ solution.

Conclusion:-
The LOQ for Sumatriptan was found to be 9.90

Naproxen:-
Calculation of S/N Ratio:
Average Baseline Noise obtained from Blank : 44 µV
Signal Obtained from LOQ solution (0.49% of target assay concentration) : 434µV
S/N = 434/44= 9.86

Acceptance Criteria:
S/N Ratio value shall be 10 for LOQ solution.

Conclusion:-
The LOQ for Naproxen was found to be 9.86

DISCUSSION:

From the linearity Table 1, it was found that the drug obeys linearity within the concentration range of 60-100 µg/ml for Sumatriptan and Naproxen. From the results shown in accuracy Table 4, 5 it was found that the percentage recovery values of pure drug were in between 99.8 to 101.9, which indicates that the method was accurate and also reveals that the commonly used excipients and additives present in the pharmaceutical formulations were not interfering the proposed method. From the results shown in precision Tables 6, 7 it was found that % RSD is less than 2%; which indicates that the proposed method has good reproducibility. The system suitability parameters also reveal that the values were within the specified limits for the proposed method.

SUMMARY AND CONCLUSION:

In the present work, an attempt was made to provide a newer, sensitive, simple, accurate and low cost RP-HPLC method. It is successfully applied for the determination of Sumatriptan and Naproxen in pharmaceutical preparations without the interferences of other constituent in the formulations.

In HPLC method, HPLC conditions were optimized to obtain, an adequate separation of eluted compounds. Initially, various mobile phase compositions were tried, to get good optimum results. Mobile phase and flow rate selection was based on peak parameters (height, tailing, theoretical plates, capacity factor), run time etc. The system with Buffer : acetonitrile (50:50 v/v) with 0.7 ml/min flow rate is quite robust.

The optimum wavelength for detection was 285 nm at which better detector response for drug was obtained. The average retention time for Sumatriptan and Naproxen were found to be 5.87 and 2.24 min. System suitability tests are an integral part of chromatographic method. They are used to verify the reproducibility of the chromatographic system. To ascertain its effectiveness, system suitability tests were carried out on freshly prepared stock solutions. The calibration was linear in concentration range of 60 – 100 µg/ml**.** The low values of % R.S.D. indicate the method is precise and accurate. The mean recoveries were found 101.0 %.

Sample to sample precision and accuracy were evaluated using, three samples of five and three different concentrations respectively, which were prepared and analyzed on same day. Day to day variability was assessed using three concentrations analyzed on three different days, over a period of three days. These results show the accuracy and reproducibility of the assay.

Ruggedness of the proposed methods was determined by analysis of aliquots from homogeneous slot by different analysts, using similar operational and environmental conditions; the % R.S.D. reported was found to be less than 2 %.The proposed method was validated in accordance with ICH parameters and the results of all methods were very close to each other as well as to the label value of commercial pharmaceutical formulation. Therefore, there is no significant difference in the results achieved by the proposed method.

ACKNOWLEDGMENT:

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