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# Stability Indicating RP-HPLC Method Development and Validation for Estimation of Safinamide in Bulk Drug and Dosage Form

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## Abstract
After Alzheimer's disease, Parkinson's disease (PD) is the second most common chronic progressive neurological condition in the elderly. Safinamide (SAF) is an orally available derivative of the alpha-aminoamide chemical class with multiple mechanisms of action including monoamine oxidase B and dopamine reuptake inhibition used in the treatment of epilepsy and Parkinson's disease. safinamide potently modulates dopamine (DA), a substrate of MAO-B, suppresses DA uptake, and reversibly binds to MAO-B, thereby blocking MAO-B function, leading to relief of PD symptoms. In addition to MAO-B inhibition, safinamide exhibits novel anticonvulsant activities, including sodium channel blockade, calcium channel blockade, and inhibition of glutamate release. This research article emphasizes on this research, a novel, sensitive, convenient, clear, accurate, and robust reverse-phase high-performance liquid chromatography (RP-HPLC) method was developed and validated for the determination of safinamide in drug and tablet formulation. Separation was performed by HPLC with UV detector and Open lab EZchrome workstation program, as well as Kromasil C18 (250 mm X 4.6 mm i.d.) 5 µm. Methanol: 0.025% TFAA (45:55) was pumped at a flow rate of 1.0 mL/min and detected at 226 nm.

**Keywords**
RP-HPLC, safinamide, Parkinson's disease, Development, Validation.

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## 1. INTRODUCTION:
After Alzheimer's disease, Parkinson's disease (PD) is the second most common chronic progressive neurological condition in the elderly. Safinamide (SAF) is an orally available derivative of the alpha - aminoamide chemical class with multiple mechanisms of action including monoamine oxidase B and dopamine reuptake inhibition used in the treatment of epilepsy and Parkinson's disease. safinamide potently modulates dopamine (DA), a substrate of MAO-B, suppresses DA uptake, and reversibly binds to MAO-B, thereby blocking MAO-B function, leading to relief of PD symptoms. In addition to MAO-B inhibition, safinamide exhibits novel anticonvulsant activities, including sodium channel blockade, calcium channel blockade, and

inhibition of glutamate release. It was approved in Europe in February 2015, in the United States in March 2017, and in Canada in January 2019.

In this research, a novel, sensitive, convenient, clear, accurate, and robust reverse-phase high-performance liquid chromatography (RP-HPLC) method was developed and validated for the determination of safinamide in drug and tablet formulation. Separation was performed by HPLC with UV detector and Open lab EZchrome workstation program, as well as Kromasil C18 (250 mm X 4.6 mm i.d.) 5 µm. Methanol: 0.025% TFAA (45:55) was pumped at a flow rate of 1.0 mL/min and detected at 226 nm. The developed RP-HPLC method gave a suitable retention time for safinamide of 3.96 min, which was optimized by trial and error. The linearity of the established method was verified with a correlation coefficient (r2) of 0.9999 in the concentration range of 10.00-150.00 µg/mL. The percent RSD for method precision was found to be less than 2.0 percent. The percentage recoveries were found to be within the limit. 0.744 µg/ml and 2.255 µg/ml were found to be the LOD and LOQ. [1][2][3][4][5]

**Chemistry:**

Chemically, safinamide is (s)-(+)-2-[4-(3-fluoro benzyloxybenzylamino)propanamide] methane sulfonate (1:1 salt) (Figure No.01)

Safinamide is a unique molecule with many mechanisms of action and a very high therapeutic index. It combines strong, selective and reversible inhibition of MAO-B with blockade of voltage-dependent Na+ and Ca2+ channels and inhibition of glutamate release. Safinamide has neuroprotective and neurorescue effects in MPTP-treated mice, in kainic acid rats, and in a gerbil model of ischemia. [6][7]

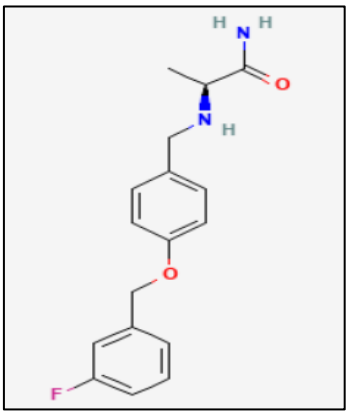



**Fig.01. Molecular Structure of Safinamide**

## 2. METHODS:

2.1. Instrumentation and software:

An Agilent 1260 Infinity II HPLC system with a DEAX02386 pump and an autosampler with a UV-visible detector served as the chromatographic system (DEACX16446). For data collection and processing, chromatograms were registered using Open lab EZ Chrome Workstation on a Windows computer system. Safinamide concentrations were determined using a Kromasil C18 column (250 mm x 4.6 mm ID 5 µm).

2.2. Ultraviolet (UV) spectroscopy:

Water was chosen as the solvent for dissolving safinamide mesylate.

Water as blank and safinamide standard solution (20 PPM) were scanned from 400 nm to 200 nm. Absorption maxima were determined for the drug. Safinamide showed maximum absorbance at 226 nm shown in the results.

## 3. EXPERIMENTAL WORK:

3.1. Method development using RP – HPLC:

Preparation of standard stock solution for chromatographic development:

A standard safinamide stock solution was prepared by dissolving 13.18 mg of safinamide mesylate (equivalent to 10 mg of safinamide) in a 10 mL clean and dried volumetric flask, adding about 7 mL of water, sonicating to dissolve completely, and making up to the mark with water (1000 ppm).

Next, 1 ml of stock solution is diluted to 10 ml with mobile phase (100 PPM). It was prepared in the mobile phase of each study and injected in the development studies.

Choice of Analytical Wavelength for HPLC Method Development: Analytical wavelength for examination was selected from the wavelength of maximum absorption from spectrophotometric analysis and was 226 nm.

**3.2. Optimization of the HPLC method**

Six trials were used to estimate safinamide from that trial #06 which gives the optimized result. Thus, all parameters are used for the development of the RP-HPLC method. (Table.01.)

Principle: Reverse phase liquid chromatography with isocratic elution and UV detection.

**Table.01. Optimized chromatographic conditions**

| Parameter | Description |
|---|---|
| Mode | Isocratic |
| Column Name | Inertsil ODS-3V (C18), 150 mm X 4.6mm ID, 5 µm |
| Detector | UV Detector |
| Injection Volume | 20 µl |
| Wavelength | 226 nm |
| Column Oven temp | 40ºC |
| Mobile Phase | Methanol: 0.025% TFAA (45:55) |
| Flow Rate | 1.0 ml/min |

**3.3. METHOD VALIDATION:**

The optimized method for the estimation of safinamide was validated for the following parameters using the ICH Q2(R1) guidelines. [12-20]

**3.3.1. CONTROL STRATEGY:**

**SYSTEM SUITABILITY TEST** (Safinamide Standard Solution):

Weighed about 26.36 mg of safinamide mesylate (equivalent to 20 mg of safinamide) and transferred to a 50 mL volumetric flask, added 30 mL of water, sonicated to dissolve, made up to the mark with water. Pipette 5 ml of the standard stock solution into a 20 ml volumetric flask and make up to the mark with the mobile phase. (100 µ/mL = working concentration)

**FILTRATION STUDY:**

This study was performed with a test sample of safinamide. (Xafinact tablet sample solution). The filtration study was performed with unfiltered and filtered test solution. During the filtration activity, 0.45 µm PVDF and 0.45 µm nylon syringe filters were used by removing a 5 mL aliquot.[8]

**STABILITY OF THE ANALYTICAL SOLUTION:**

A stability study was performed for the standard and test solutions. The stability study was performed under normal laboratory conditions. The solution was stored under normal light laboratory conditions and analyzed after 12 hours and 24 hours. The stability study of the standard and test solution was performed by calculating the difference between the results of the test solution at each stability time point and the initial one.

**3.3.2. Analysis of the test sample sold:**

A marketed test sample named Xafinact 50 mg tablets is selected for analysis and to perform validation.

Average weight of the test sample (Xafinact 50 mg):

20 tablets were weighed at a time and the average tablet weight was calculated according to the following formula:

Average weight (mg) = weight of 20 tablets (mg) / 20

**Table.02. Sample Prepared in duplicate. A summary of the sample preparation follows.**

| Sample | Sample (mg) | Diluted to (mL) | Volume taken | Diluted to (mL) |
|---|---|---|---|---|
| Sample 1 | 252.9 | 100 | 2 | 20 |
| Sample 2 | 252.6 | 100 | 2 | 20 |

**Formula for % Assay calculation:**

$$\%\ Assay = \frac{\text{Safinamide } Spl\ area}{\text{Safinamide } Std\ avg\ area}\ X\ \frac{\text{Safinamide } STD\ wt\ (mg)}{50}\ X\ \frac{5}{20}\ X\ \frac{100}{Tablet\ sample\ weight\ (mg)}\ X\ \frac{20}{2}\ X\ \frac{Avg\ wt\ of\ tablet\ (mg)}{Label\ claim\ of\ \text{Safinamide (mg)}}\ X\ Factor\ X\ 100$$

**3.3.3. METHOD VALIDATION PARAMETERS:**

**3.3.3.1. Specificity:**

To demonstrate the specificity of the method, the following solution is prepared and injected. (Checked maximum purity for standard and test sample solution)

I. Blank (mobile phase as diluent)
II. Placebo
III. Safinamide standard solution
IV. Tablet test sample solution

The analyzed test sample on the market contains auxiliary substances (additives) that are completely

unknown. Thus, the placebo was prepared at the laboratory level using the following formula:

**Table.03. Placebo preparation**

| Sr. No. | Ingredients | Role | Qty (mg) |
|---|---|---|---|
| 1 | Lactose | Filler | 80 |
| 2 | Starch | Binder | 5 |
| 3 | Magnesium stearate | Lubricant | 5 |
| 4 | Talc | Glidant | 5 |
| 5 | crospovidone | Disintegrants | 5 |
| **Total** | | | **100 mg** |

**Preparation of placebo sample solution:**
120.65 mg of placebo material (equivalent to 100 mg of safinamide) was weighed and transferred to a clean and dried 100 ml volumetric flask. Add 70 ml of water, sonicate for 15 minutes with intermittent shaking. After 15 minutes, the solution is allowed to cool to room temperature and the volume is made up to the mark with water. The solution was filtered through a suitable 0.45µ syringe filter, removing 3-5 ml of the initial filtrate. Next, 2 ml of the filtered stock solution is diluted to 20 ml with the mobile phase.

**3.3.3.2. LINEARITY AND RANGE:**
5 levels of linearity from 10% to 150% working concentration were performed
Linearity of safinamide stock solution:
52.70 mg of safinamide mesylate (equivalent to 40 mg of safinamide) was weighed and dissolved in 20 mL of water. Next, 5 ml is diluted to 25 ml with the mobile phase. (400ppm)

**3.3.3.3. DETECTION LIMIT:**
According to ICH Q2R1 guidelines, the LOD and LOQ were determined using a calibration curve approach in which the residual standard deviation of the regression line was calculated and the LOD and LOQ were determined using the following formula:
LOD = 3.3 σ / S
LOQ = 10σ/S
Where,
σ = residual standard deviation of the regression line
S = Slope of the regression line

**3.3.3.4. ACCURACY:**
Accuracy will be carried out in the range from 50% to 150% of the working concentration. The solution of each precision level was prepared in triplicate. Calculated %Recovery for each sample, Mean %Recovery for each level and total recoveries as well as calculated %RSD for each level and %RSD for total recovery.

**3.3.3.5. ACCURACY:**
a) Repeatability: Preparation of sample solution (6 prepared samples)
b) Intermediate precision: Analyzed on a different day to check the reproducibility of the results. Samples prepared in the same way as for the Repeatability parameter (6 samples prepared).

**3.3.3.6. ROBUSTNESS:**
The robustness of an analytical procedure is a measure of its ability to remain unaffected by small but intentional variations in method parameters and provides an indication of its reliability in routine use. Determination: Standard solutions were injected under different chromatographic conditions as shown below.
i. Flow rate changes of ±10%. (± 0.1ml/min)
ii. Temperature change in the column oven. (± 2ºC)
iii. Wavelength change (± 3 nm) [9]

**3.3.3.7. FORCE DEGRADATION OF SAFINAMIDE:**
Sample selection for FD:
The sold Xanifact 50 mg tablet contains excipients unknown to us. If we have done FD on the tablet, there may be a chance of excipient degradation and we may get a peak of excipient degradation. We could not distinguish between the breakdown products of safinamide and the breakdown products of excipients. Therefore, we performed FD on Safinamide API.

**1) Performed Force degradation by two methods.**
I. Physical degradation
   - Photolytic
   - Thermal

II. Chemical degradation
   - Acid
   - Base
   - Peroxide

**2) To achieve degradation in the range of 5 % to 20% of assay value.**

**3) Preparation of Degradants:**
5 N Hydrochloric acid: 42.5 mL of HCl diluted to 100 mL with water.
5 N NaOH solution: 20 gm of NaOH dissolved in 100 mL of water.
0.1 N NaOH solution: 0.4 gm of NaOH dissolved in 100 mL of water.

0.1 N Hydrochloric acid: 0.85 mL of HCl diluted to 100 mL with water.
30% Hydrogen peroxide solution: Commercially ready made available.
30% Sodium sulfite solution: 30 gm of Sodium sulfite dissolved in 100 mL of water.

**Formula for % Assay of API in FD samples:**

$$\%\ Assay = \frac{\text{Safinamide degradation } Spl\ area}{\text{Safinamide } Std\ avg\ area}\ X\ \frac{\text{Safinamide } STD\ wt\ (mg)}{50}\ X\ \frac{5}{20}\ X\ \frac{100}{API\ weight\ taken\ for\ FD\ (mg)}\ X\ \frac{10}{1}\ X\ 100$$

**4. RESULT:**

**i) Selection of Analytical Wavelength:**

The standard solution was scanned from 400 nm to 200 nm. The wavelength of maximum absorption was determined for the drug. Safinamide showed maximum absorbance at 226 nm. It is shown in Fig.04. Therefore, 226 nm is considered the analytical wavelength for further determination.

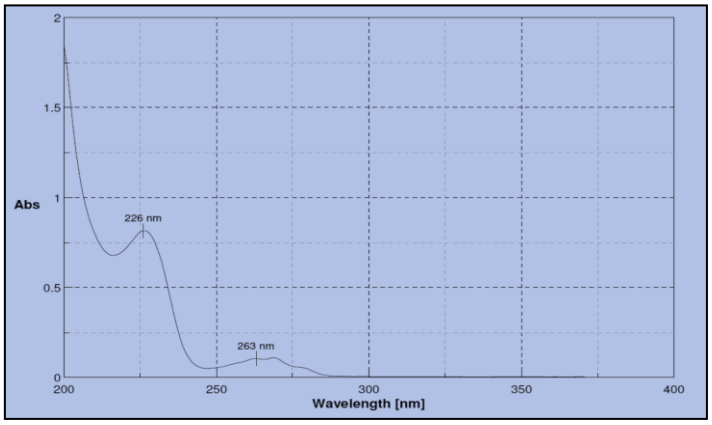



**Fig. 04. UV spectrum of Safinamide 20 PPM.**

**ii) Method Development by RP – HPLC:**

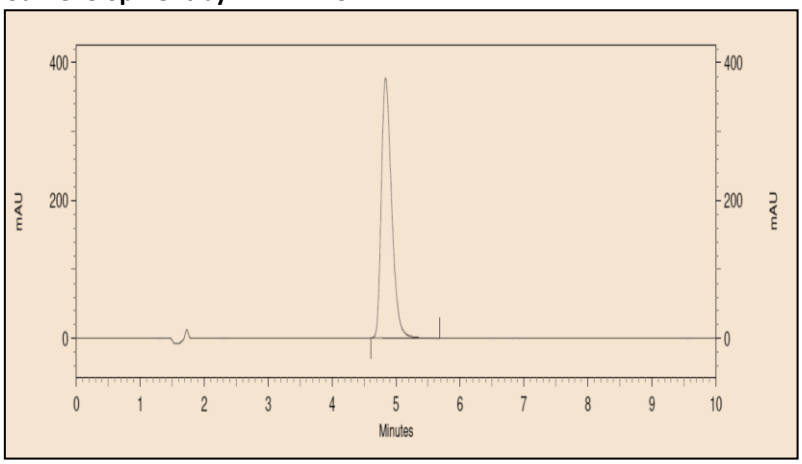



**Fig. 05. Typical chromatogram of Optimized method.**

**Observation:** Safinamide is eluted by good chromatography.

**Conclusion:** From the observation of experiments one to six, it was concluded that the chromatographic conditions in experiment six provide a better peak, good retention time, tailing factor, therefore the chromatographic conditions in experiment six were subjected to method validation.

**iii) CONTROL STRATEGY:**

**a) System suitability test:**

**Table.04. Results of the suitability test of the safinamide system.**

| Parameter | Acceptance Criteria | Result |
|---|---|---|
| %RSD | NMT2.0%. | **0.04** |
| Theoretical plates | More than 2000 | **4782** |
| Tailing factor | NMT2.0 | **1.21** |

It was observed from the data tabulated above; the method complies with system suitability parameters. Hence, it can be concluded that the chromatographic method is adequate for intended analysis. Typical chromatogram with Analytical data of SST for safinamide is shown in fig. 06.

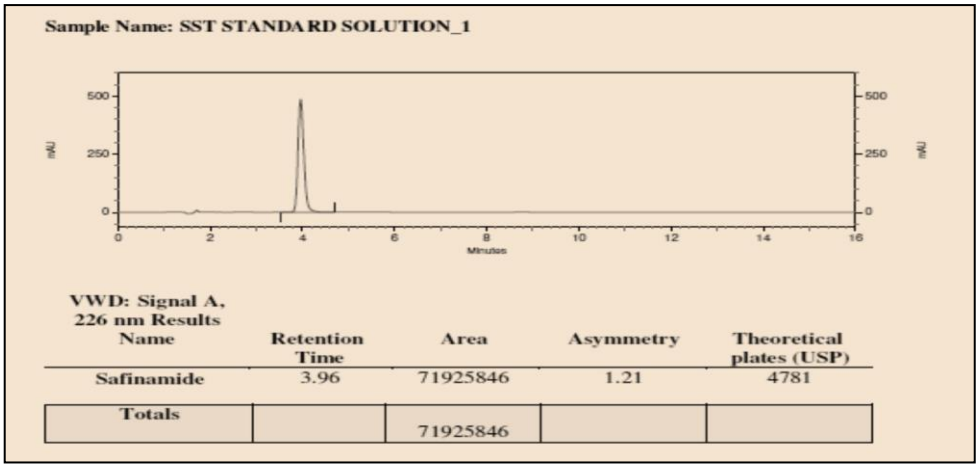



**Fig. 06: Typical chromatogram Standard solution 1 of system suitability solution.**

**a) FILTRATION STUDY:**

**Table.no.05. Analytical data of Filter Test**

| Sample | Area | %Absolute difference | Acceptance Criteria | Conclusion |
|---|---|---|---|---|
| **Unfiltered** | 70564931 | NA | | |
| **0.45µ PVDF filter** | 70426184 | 0.20 | % AbsolutedifferenceNMT2.0 | Both PVDF and Nylon filters passes the criteria for filter |
| **0.45µ Nylon filter** | 69551344 | 1.44 | | study |

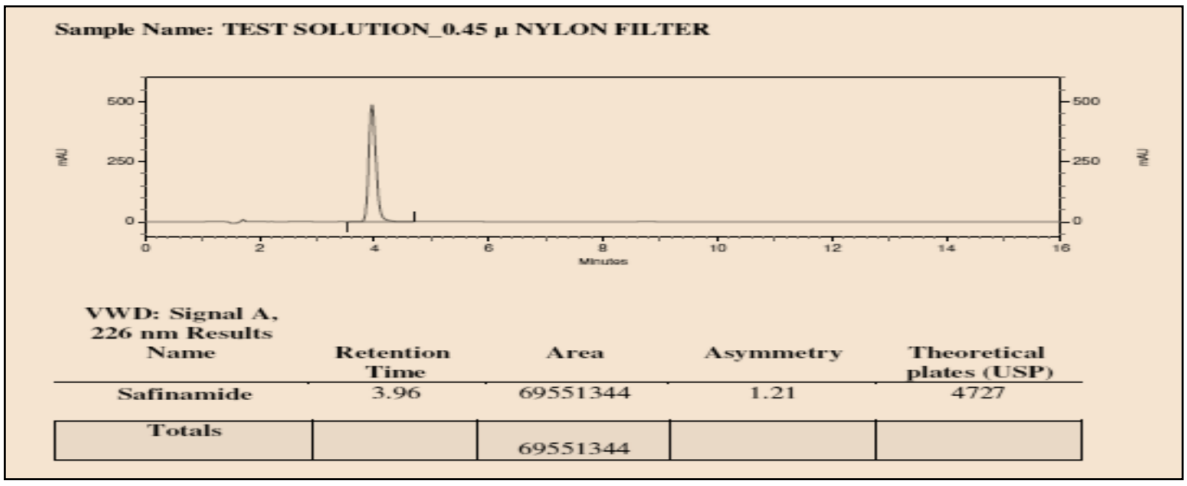



**Fig. 06: Typical chromatogram Standard solution 1 of system suitability solution.**

**a) FILTRATION STUDY:**

**Table.no.05. Analytical data of Filter Test**

| Sample | Area | %Absolute difference | Acceptance Criteria | Conclusion |
|---|---|---|---|---|
| **Unfiltered** | 70564931 | NA | | |
| **0.45µ PVDF filter** | 704261840.20 | % AbsolutedifferenceNMT2.0 | | Both PVDF and Nylon filters passes the criteria for filter study |
| **0.45µ Nylon filter** | 695513441.44 | | | |

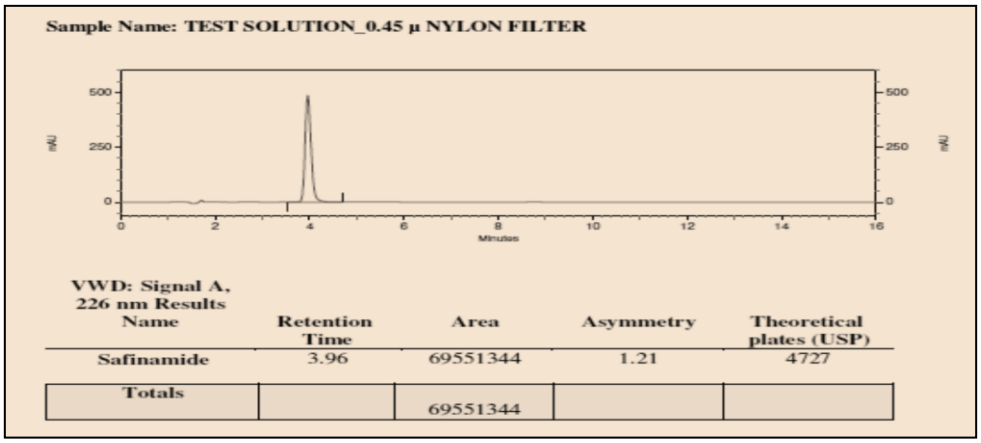



**Fig. 07. Typical chromatogram of sample filtered through 0.45µ Nylon filter.**

**b) SOLUTION STABILITY:**

**Table.06. Analytical data of safinamide for solution Stability**

| Test solution | | | Standard solution | | | Acceptance Criteria | Conclusion |
|---|---|---|---|---|---|---|---|
| Time point | Area | % Absolute difference | Time point | Area | % Absolute difference | %Absolute difference | |
| **Initial** | 70493204 | NA | **Initial** | 71903158 | NA | NMT2.0 | Both standard and sample solution were found stable for24hours |
| **12 Hrs** | 70035149 | 0.65 | **12 Hrs** | 71563079 | 0.47 | | |
| **24 Hrs** | 69563041 | 1.32 | **24 Hrs** | 71065237 | 1.17 | | |

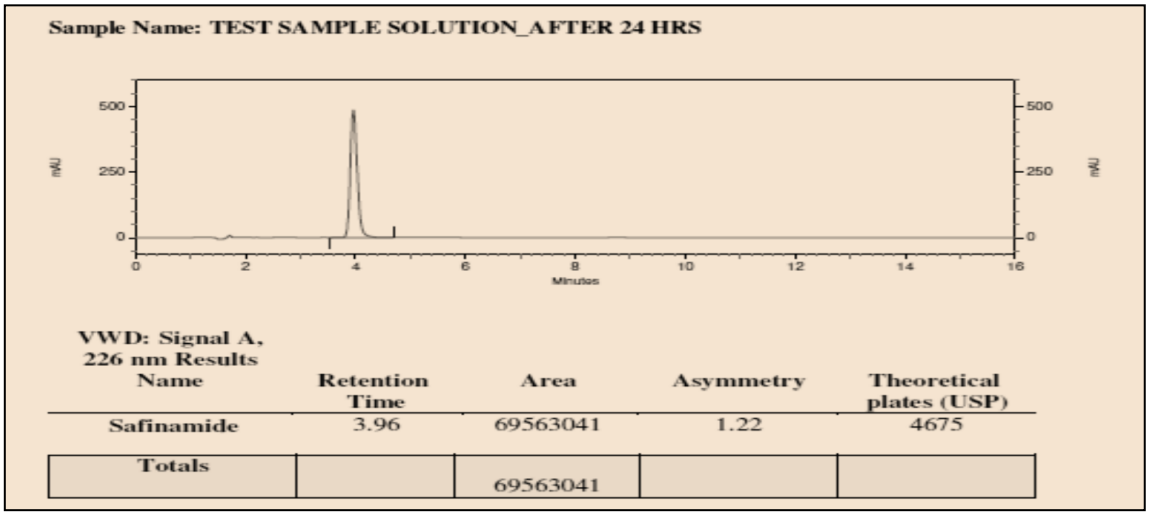



**Fig. 09. Typical chromatogram of Test solution After 24 Hrs.**

**i. ASSAY OF MARKETED TEST SAMPLE (Xafinact 50):**

**a) Average weight of tablets (Xafinact 50):**

Weight of 20 tablets: 2.5240 gm

Average weight of tablet = 2.5240 / 20 = 0.1262 gm = 126.2 mg

Average weight of tablet = 126.2 mg

**Table.07. Assay of Xafinact 50 tablet:**

| Sample | Area | % Assay | Mean % Assay | Acceptance Criteria | Conclusion |
|---|---|---|---|---|---|
| Sample 1 | 70429651 | 97.78 | 97.44 | % Assay found should be in the range of 90-110%. | Assay is passed. |
| Sample 2 | 69863694 | 97.11 | | | |

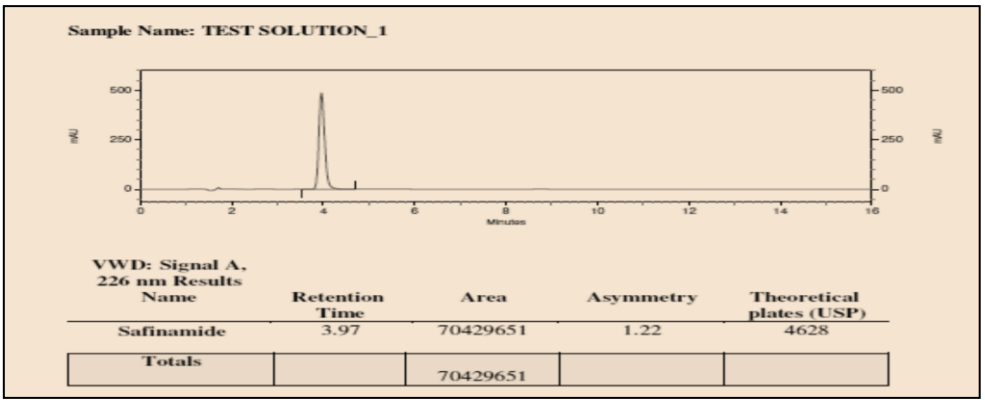



**Fig. 10. Typical chromatogram of Test solution of Xafinact 50 tablet.**

**ii. VALIDATION PARAMETERS:**

**i. SPECIFICITY:**

**Table. 08. Results of Specificity for Safinamide**

| Description | Observation | Acceptance criteria | Conclusion |
|---|---|---|---|
| Blank | No interference at R.T. of Safinamide due to blank | no Interference at R.T. | Developed chromatographic method passed the criteria for specificity. |
| Placebo | No interference at R.T. of Safinamide due to placebo | no Interference at R.T. | |
| Standard solution | Peak purity was 0.991 | Peak purity: NLT 0.95 | |
| Test Solution | Peak purity was 0.986 | Peak purity: NLT 0.95 | |

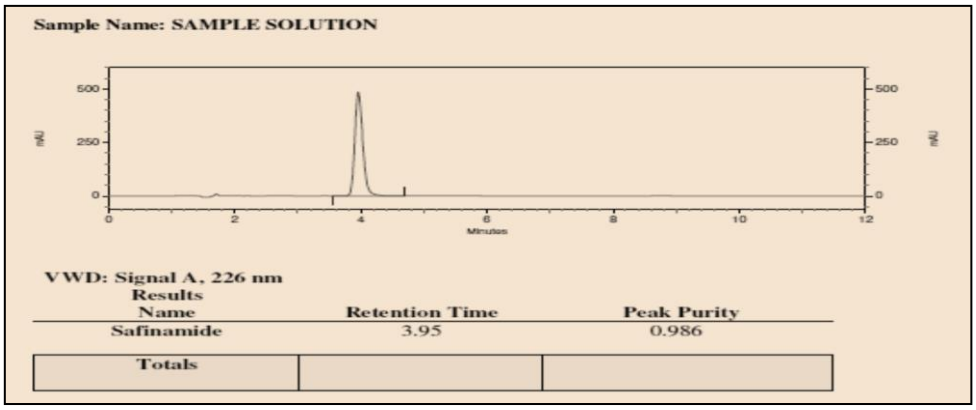



**Fig.11. Typical chromatogram of Peak purity of Test solution.**

**ii. Linearity and range**

The linearity of an analytical method is its ability to produce test results that are proportional to the concentration of the analyte in the samples within a given range. From the calibration curve, we had to conclude that safinamide shows a linear response in the range of 10 to 150 µg/ml. The regression value was found to be well within the limits. The result and statistical data of safinamide linearity are shown in Table 09. The linearity graph of safinamide is shown in Fig. 12. [10][11][12][13]

**Table. 09. Linearity Data for safinamide**

| Level | Conc (µg/mL) | Area | Mean | % RSD |
|---|---|---|---|---|
| 10% | 10.00 | 7182480<br>7152604<br>7126934 | 7154006 | 0.389 |
| 50% | 50.00 | 36056550<br>36119873<br>36195081 | 36123835 | 0.192 |
| 100% | 100.00 | 71958336<br>71860639<br>71768260 | 71862412 | 0.132 |
| 125% | 125.00 | 90027492<br>90136501<br>90163504 | 90109166 | 0.080 |
| 150% | 150.00 | 107778087<br>107593813<br>107698244 | 107690048 | 0.086 |

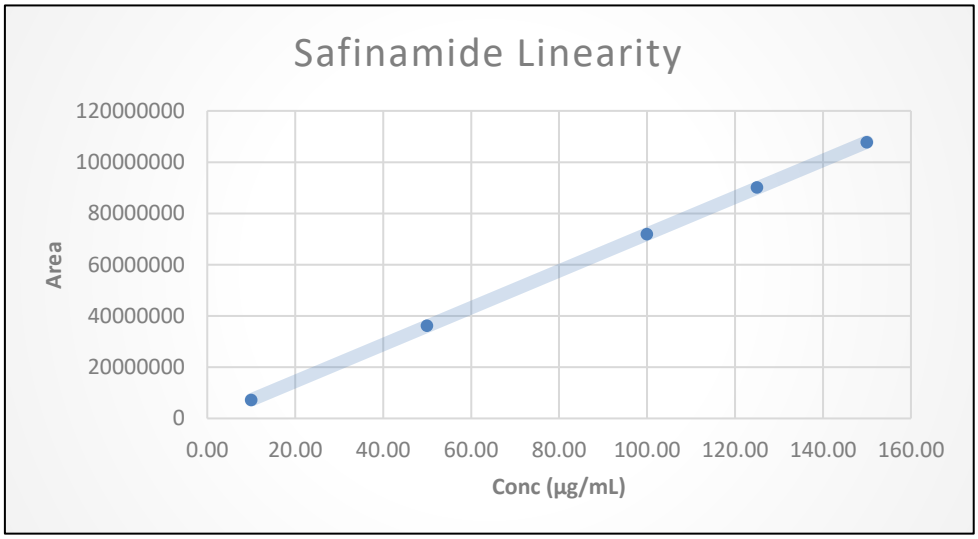



**Fig. 12. Calibration curve of Safinamide.**

**Table.10. Data of linearity of Safinamide**

| Sr no. | Parameter | Result value | Acceptance criteria |
|---|---|---|---|
| 1 | Beer's linearity range | 10.00-150.00 µg/mL | NA |
| 2 | Correlation coefficient ($R^2$) | 0.99999 | NLT 0.98 |
| 3 | Intercept | 70879.984 | To be report |
| 4 | Slope | 718586.361 | To be report |
| 5 | % RSD for area at each level | NA | NMT 2.0 |

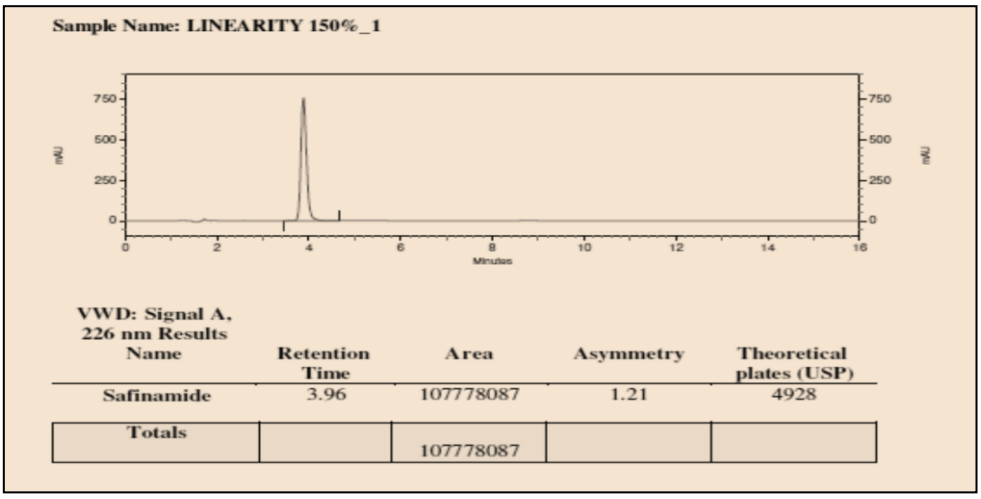



**Fig. 13. Typical chromatogram of Linearity 150%.**

**i. Limit of Detection (LOD) and Limit of Quantitation (LOQ):**

σ = 162060.11 (Residual standard deviation of a regression line)

s = 718586.361 (Slope)

**Detection limit (LOD):**

LOD = 3.3 σ / S

LOD = 3.3 x 162060.11 / 718586.361

**LOD = 0.744 µg/mL**

**Quantitation limit (LOQ):**

LOQ = 10 σ / S

LOQ = 10 x 162060.11 / 718586.361

**LOQ = 2.255 µg/mL**

**iv. ACCURACY (RECOVERY):**

The recovery of the analytical procedure was found to be well within the acceptance criteria at all 3 levels. % recovery is not limited by changing analyte concentration.

**Table.11. Result and statistical data of Accuracy of Safinamide**

| Level (%) | Area | % Recovery | Mean Recovery | % RSD | Acceptance Criteria | Conclusion |
|---|---|---|---|---|---|---|
| | 36193417 | 100.64 | | | | |
| **50** | 36263914 | 100.80 | 100.43 | 0.512 | | |
| | 35862941 | 99.84 | | | | |
| | 71865099 | 99.83 | | | % Recovery: 98.00 %to 102.0 % | % Recovery was found well within acceptance range at all three levels. |
| **100** | 71036091 | 98.89 | 99.70 | 0.756 | | |
| | 72163504 | 100.38 | | | | |
| | 107869341 | 99.97 | | | | |
| **150** | 106925975 | 99.17 | 100.04 | 0.901 | | |
| | 108969417 | 100.97 | | | | |

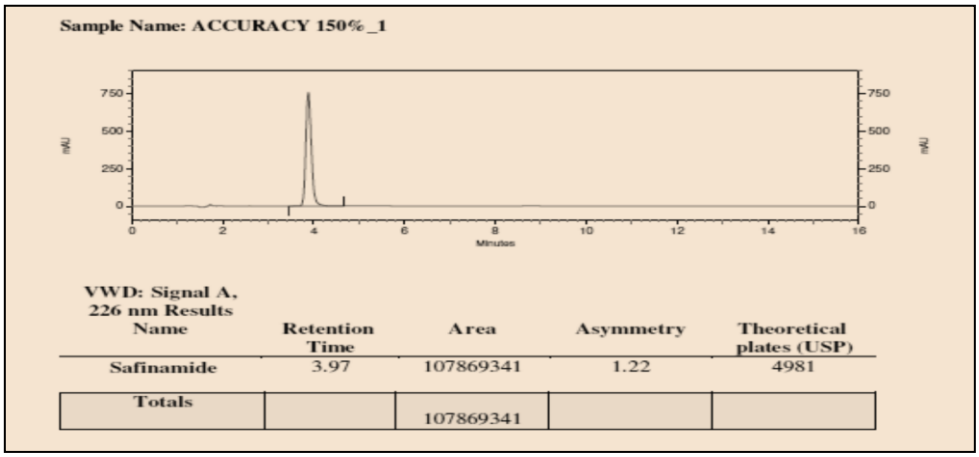



**Fig. 14. Typical chromatogram of Accuracy 150%.**

**i. PRECISION:**

**Table.12. Result of Intra- day and Inter- Day Precision for Safinamide test sample (Xafinact 50 tablet) assay**

| Parameters | Intraday Precision | Interday Precision | Acceptance criteria | Conclusion |
|---|---|---|---|---|
| **Mean** | **97.22** | **97.38** | | |
| **SD** | **0.9089** | **1.1534** | % RSD for the six samples NMT2.0 | HPLC method for the determination of Safinamide is precise |
| **%RSD** | **0.935** | **1.184** | | |

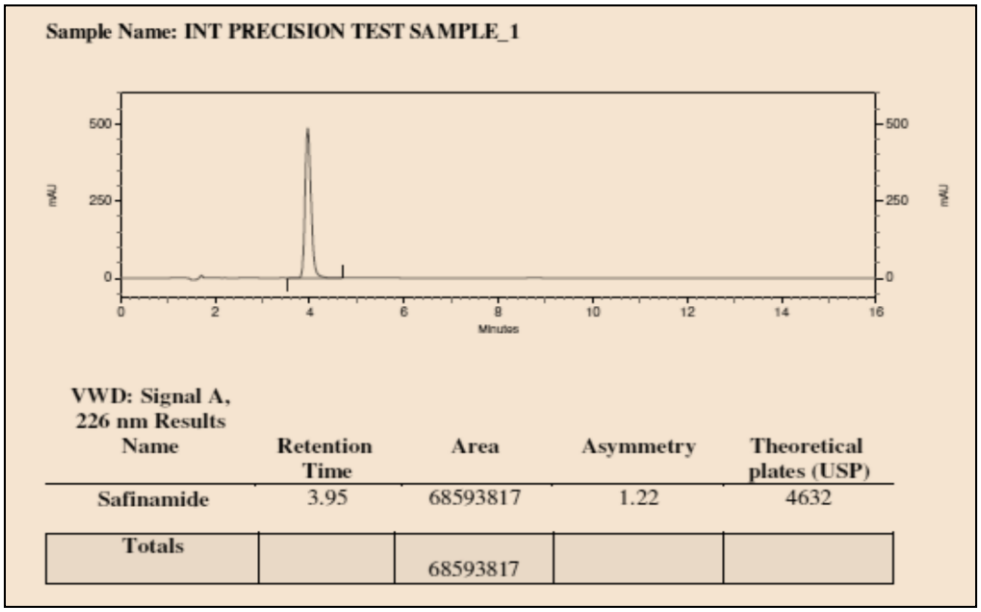



**Fig.15. Typical chromatogram of Inter-day precision (Sample 1).**

**vii. ROBUSTNESS:**

The robustness of an analytical method is a measure of its ability to remain unaffected by small but intentional variations in method parameters and provides an indication of its reliability in routine use.

The following changes made to the Robustness section:

- Change in wavelength
- Change in flow rate
- Temperature change in the column furnace. [14][15][16][17]

**Table.13. Result of robustness for safinamide**

| Sr. no. | Parameter | Observations | | | | | | Limit |
|---|---|---|---|---|---|---|---|---|
| | | Changes in flow Rate (mL/min) | | Change in Wavelength (nm) | | Change in Column Oven temperature | | |
| | | **1.10** | **0.90** | **229 NM** | **223 NM** | **42 ºC** | **38 ºC** | |
| 1 | Theoretical Plate | 4543 | 5301 | 4822 | 4797 | 4934 | 4572 | |
| 2 | Peak area response | 652855657 | 979983171 | 863287659 | 370727196 | 391771216 | 8712000 | NMT |
| 3 | Tailing factor | 1.20 | 1.17 | 1.18 | 1.19 | 1.20 | 1.22 | NMT 2.0 |
| 4 | R.T.(Min) | 3.51 | 4.28 | 3.97 | 3.96 | 3.95 | 3.98 | |

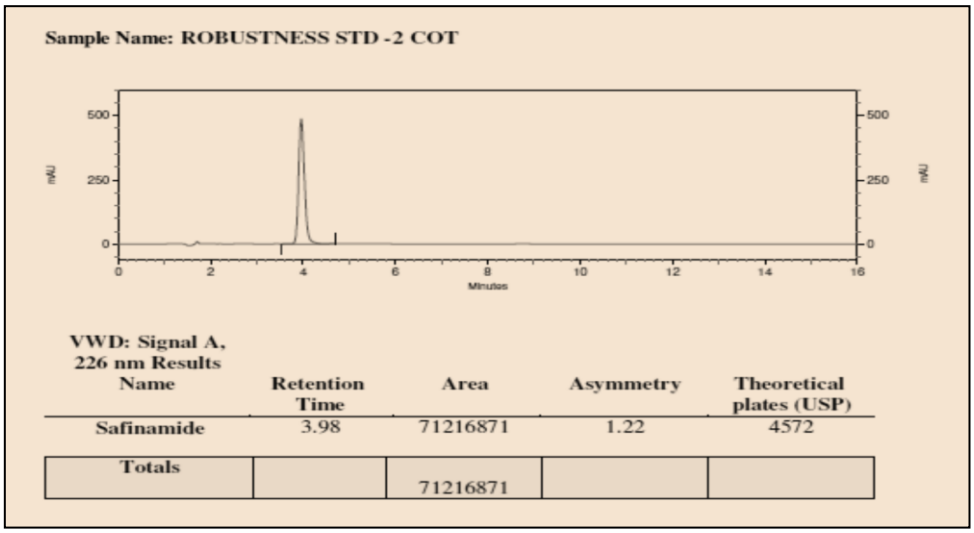



**Fig. 16. Typical chromatogram of Standard -2ºC C.O.T.**

**i. FORCE DEGRADATION OF API:**

**Table.14. Result summary of Force degradation of API**

| Sample Name | Treatment | Exposure condition | % Assay | % Degradation |
|---|---|---|---|---|
| API | Sample as such | NA | 99.94 | Nil |
| | Thermal | 105°C for 48 Hours | 99.37 | Nil |
| | Photolytic | Direct sunlight for 72 hours | 99.16 | Nil |
| | Acid | 2.5 mL of 5 N HCl for 12 Hour at R.T. | 99.60 | Nil |
| | | 2.5 mL of 5 N HCl for 24 Hour at R.T. | 99.04 | Nil |
| | Base | 2.5 mL of 5 N NaOH for 12 Hour at R.T. | 0.00 | 100.00 |
| | | 2.5 mL of 5 N NaOH for 30 Minutes at R.T. | 81.24 | 18.71 |
| | | 2.5 mL of 0.1 N NaOH for 5 Minutes at R.T. | 91.76 | 8.19 |
| | Peroxide | 2.5 mL of 30% $H_2O_2$ for 12 Hour at R.T. | 94.15 | 5.80 |

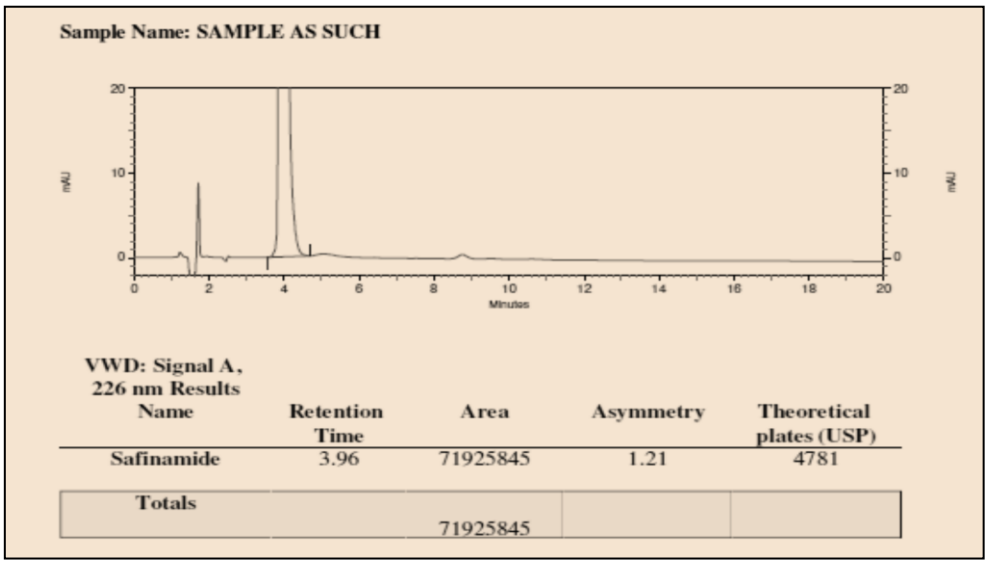
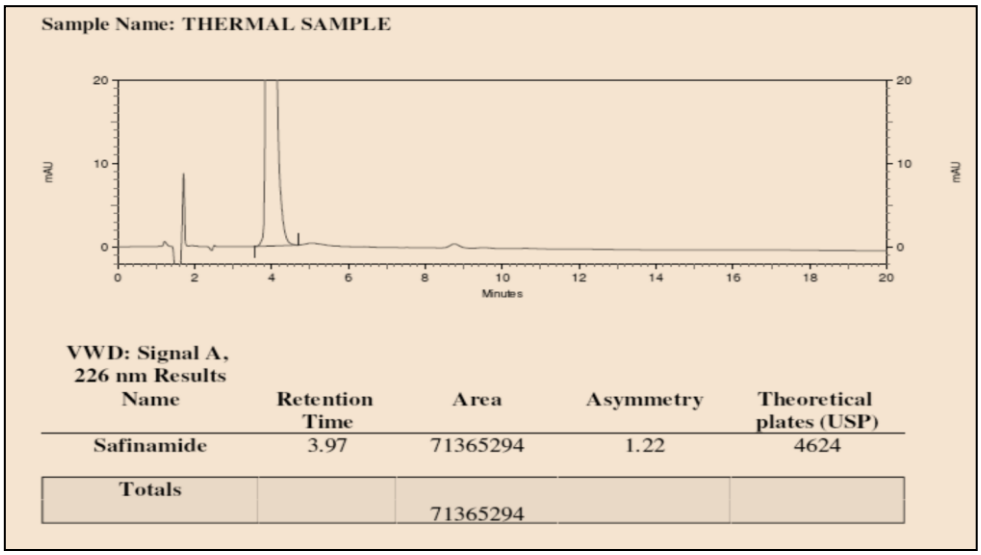


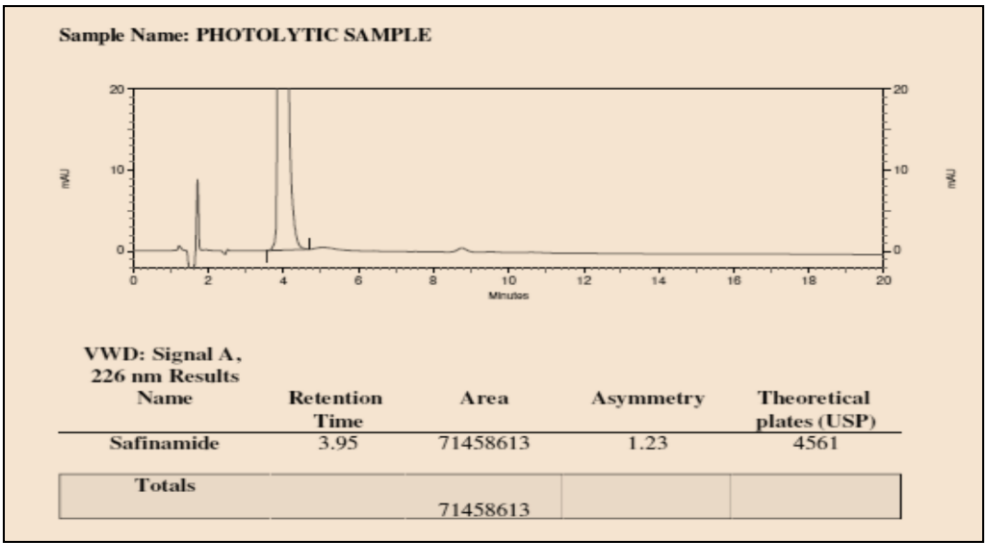

**Fig. 17. Typical chromatogram of API Sample as such.**




VWD: Signal A, 226 nm Results

| Name | Retention Time | Area | Asymmetry | Theoretical plates (USP) |
|---|---|---|---|---|
| Safinamide | 3.97 | 71365294 | 1.22 | 4624 |
| Totals | | 71365294 | | |

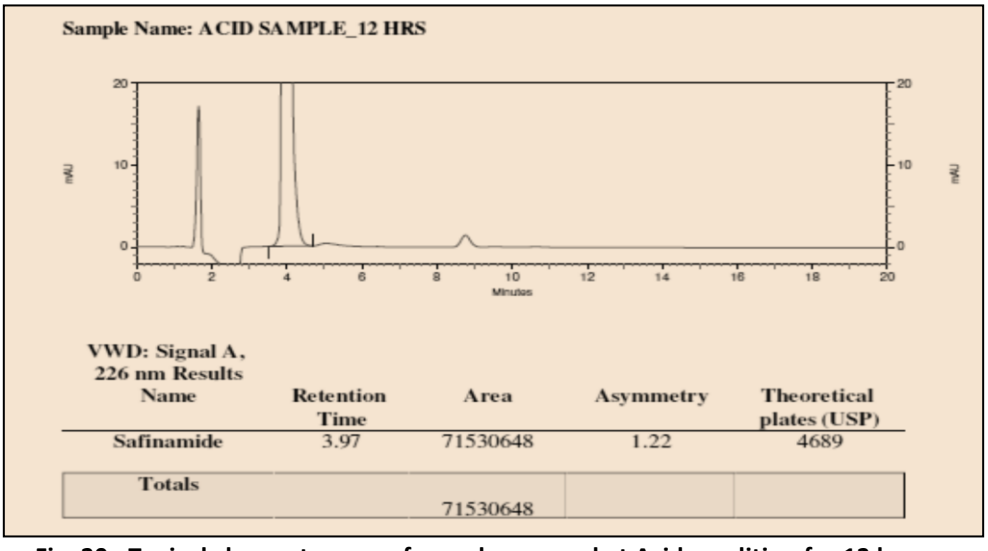
**Fig. 18. Typical chromatogram of Thermal sample.**




VWD: Signal A, 226 nm Results

| Name | Retention Time | Area | Asymmetry | Theoretical plates (USP) |
|---|---|---|---|---|
| Safinamide | 3.95 | 71458613 | 1.23 | 4561 |
| Totals | | 71458613 | | |

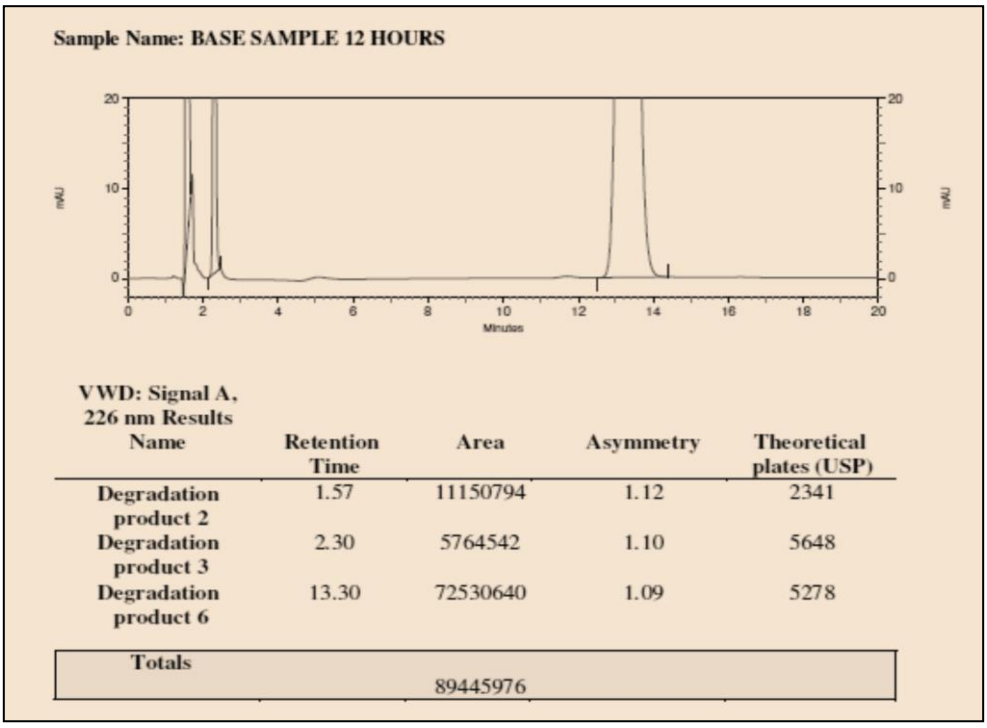
**Fig. 19. Typical chromatogram of Photolytic sample.**




VWD: Signal A, 226 nm Results

| Name | Retention Time | Area | Asymmetry | Theoretical plates (USP) |
|---|---|---|---|---|
| Safinamide | 3.97 | 71530648 | 1.22 | 4689 |
| Totals | | 71530648 | | |

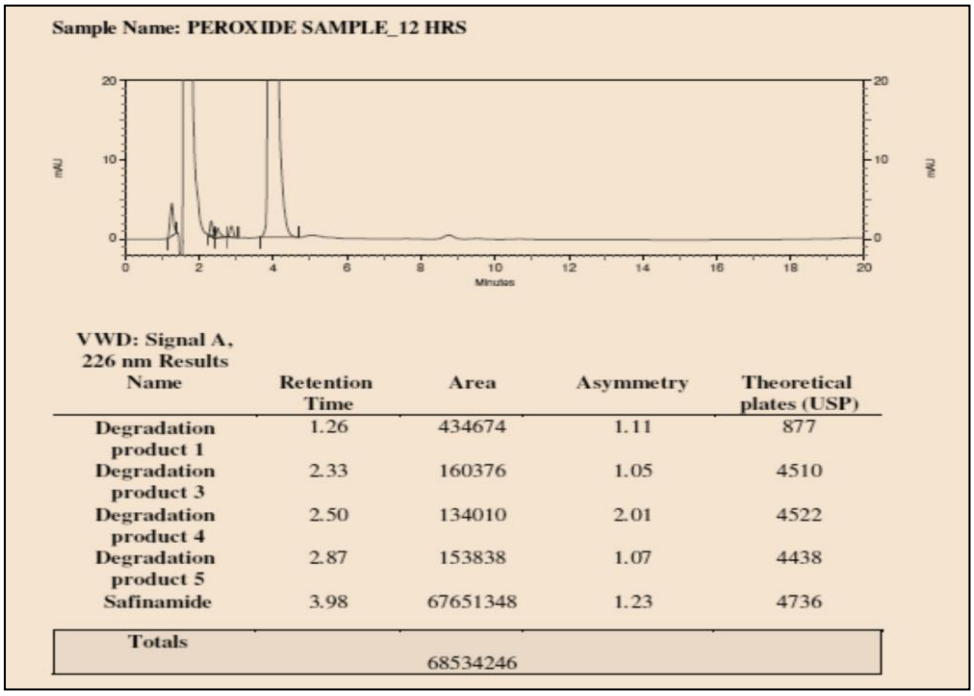
**Fig. 20. Typical chromatogram of sample exposed at Acid condition for 12 hours.**




VWD: Signal A, 226 nm Results

| Name | Retention Time | Area | Asymmetry | Theoretical plates (USP) |
|---|---|---|---|---|
| Degradation product 2 | 1.57 | 11150794 | 1.12 | 2341 |
| Degradation product 3 | 2.30 | 5764542 | 1.10 | 5648 |
| Degradation product 6 | 13.30 | 72530640 | 1.09 | 5278 |
| Totals | | 89445976 | | |

**Fig. 21. Typical chromatogram of sample exposed at Basic condition for 12 hours.**




VWD: Signal A, 226 nm Results

| Name | Retention Time | Area | Asymmetry | Theoretical plates (USP) |
|---|---|---|---|---|
| Degradation product 1 | 1.26 | 434674 | 1.11 | 877 |
| Degradation product 3 | 2.33 | 160376 | 1.05 | 4510 |
| Degradation product 4 | 2.50 | 134010 | 2.01 | 4522 |
| Degradation product 5 | 2.87 | 153838 | 1.07 | 4438 |
| Safinamide | 3.98 | 67651348 | 1.23 | 4736 |
| Totals | | 68534246 | | |

**Fig.22. Typical chromatogram of sample exposed at Peroxide condition for 12 hours.**

**5. DISCUSSION:**

The aim of this project was to create a simple, reliable, accurate and convenient RP-HPLC system. The analysis results of the established method were validated in terms of linearity, precision, accuracy and robustness, as well as limits of detection and quantification. The developed method has many advantages, including reproducibility of findings, rapid interpretation, ease of sample preparation, and improved selectivity and sensitivity. The developed method can be used for routine research in the pharmaceutical industry for the bulk drug Safinamide as well as for the pharmaceutical dosage type as it is stable and reproducible and takes less time. [18][19][20]

## 6. CONCLUSION:

According to the above experimental results, this newly developed method for the estimation of safinamide was found to be simple, precise and accurate with a shorter retention time, which makes it more acceptable and cost-effective, and can be effectively used for routine analysis in quality control research institutions. departments in industry and approved testing laboratories.

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