RESEARCH ARTICLE

Optimization and Validation of an RP-HPLC Technique for the Concurrent Quantification of Sumatriptan and Naproxen in Raw Materials and Tablet Dosage Forms

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ABSTRACT
This study focuses on the development and validation of a Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) method for the simultaneous quantification of Sumatriptan (SUT) and Naproxen (NAP) in both raw materials and tablet formulations. The method validation was carried out in accordance with established guidelines, assessing critical parameters such as linearity, precision, accuracy, and recovery to ensure its suitability for pharmaceutical analysis.
The method demonstrated excellent linearity over a concentration range of 5-70 µg/mL for both SUT and NAP, with correlation coefficients (R^2) of 0.9992 for Sumatriptan and 0.9994 for Naproxen. Recovery studies showed that the method is highly accurate, with average recovery rates of 99.36% for Sumatriptan and 99.47% for Naproxen. In the analysis of commercial tablet formulations, the method consistently produced precise and reproducible results. The retention times for Sumatriptan and Naproxen were recorded at 4.918 minutes and 3.256 minutes, respectively. Furthermore, the method exhibited strong selectivity, with a resolution factor (Rs) of 2.05 between the two compounds, indicating effective separation.
Keywords – Sumatriptan, naproxen, validated simultaneous, stability indicating, tailing factor.
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INTRODUCTION

Accurate quantification of active pharmaceutical ingredients (APIs) in both raw materials and formulated dosage forms is critical in pharmaceutical analysis to ensure product quality, efficacy, and patient safety[1,2]. This underscores the need for robust analytical methods capable of reliably detecting and quantifying multiple components within complex matrices. Such methodologies are vital in pharmaceutical research, development, and quality control processes. Among the various analytical techniques available, RP-HPLC is particularly notable for its versatility and widespread use in pharmaceutical analysis[3,4]. RP-HPLC provides high selectivity, sensitivity, and reproducibility, making it especially effective for simultaneous quantifying multiple compounds in pharmaceutical formulations. This capability is crucial for drugs that incorporate multiple active ingredients to achieve synergistic therapeutic effects or to address various facets of a medical condition[5-8]. **Figure 1** shows the Chemical structure of Sumatriptan. **Figure 2** shows the chemical structure of Naproxen

Naproxen

MATERIALS AND METHODS

Chemicals and Reagents

Sumatriptan and Naproxen reference standards were sourced from IPCA Lab., Mumbai, India. All other reagents were of HPLC grade.

Instrumentation and Chromatographic Conditions

The analysis was conducted on a LC-10AT HPLC Shimadzu attached with a Rheodyne 7725i injector and a photodiode array (PDA) detector (SPD-10AVP UV-Visible). Data acquisition and system control were managed through CLASS-VP software installed on a PC workstation.

Standard Solutions Preparation

Stock Solutions

Precisely weigh 10 mg of Sumatriptan and transfer it into a 100 mL volumetric flask. Similarly, weigh 10 mg of Naproxen and place it in a separate 100 mL volumetric flask. Dissolve each compound in 25 mL of methanol to ensure complete solubility. Adjust the final volume in both flasks to 100 mL using methanol. The resulting concentration for both Sumatriptan and Naproxen is 100 mg/mL.

Working Solutions:

Prepare aliquots from the stock solutions and dilute them further with methanol. Create working concentrations of 5, 10, 15, 20, and 25 µg/mL.

Calibration Curve Construction:

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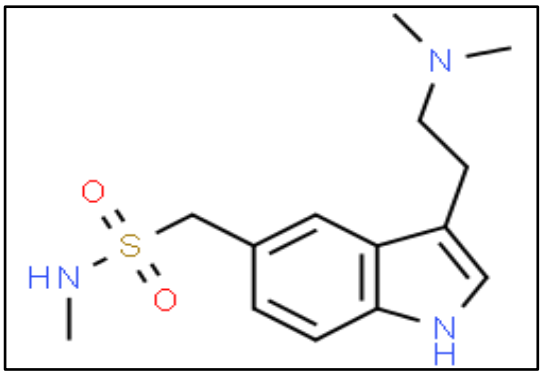

Figure 1: Chemical structure of Sumatriptan 1-[3(2Dimethylaminoethyl)-1H-indol-5yl]-N-methyl-methanesulfonamide

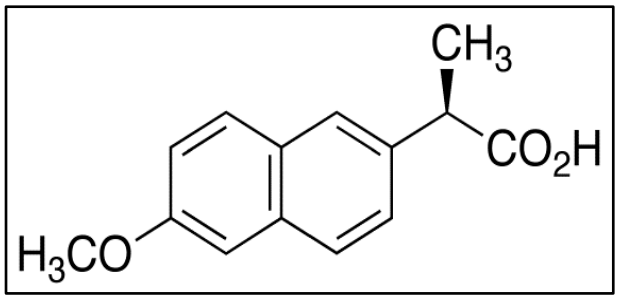

Figure 2: Chemical structure of Naproxen (+)-(S)-2-(6-Methoxynaphthalen-2yl) propanoic acid

Table 1: Linearity of SUT

Conc.	(Rep. 1)	(Rep. 2)	(Rep. 3)	(Rep. 4)	(Rep. 5)	(Rep. 6)	Mean + SD
5	1106683.6	1118804.5	1099024.3	1111765.7	1127348.2	1134449.8	1116491.2 + 12956.44
10	2270959.9	2261405.3	2264754.0	2254863.6	2286211.4	2272857.4	2270554.9 + 10854.59
15	3334061.5	3334312.3	3340109.4	3329345.6	3329114.8	3352837.4	3340962.0 + 8608.10
20	4412663.2	4405461.5	4413804.0	4442218.0	4410246.5	4448130.5	4422080.4 + 18107.37
25	5612388.2	5780508.9	5706683.4	5672169.8	5698279.3	5677730.3	5663056.4 + 31907.71

Table 2: Linearity of NAP

Conc.	REP 1	REP. 2	REP 3	REP 4	REP 5	REP 6	Mean ± SD
5	1406140.9	1376867.8	1387844.7	1381623.1	1414876.1	1404716.5	1395114.6 ± 4309.19
10	2673440	2702213.8	2665687.3	2729508	2647620.8	2690541.6	2685218.3 ±26083.22
15	3885468.4	3884231.9	3950481.7	3929659.8	3864341.1	3893369.8	3890832.2 ± 30396.46
20	5165761.7	5150542.2	5163018.3	5152871.9	4628995	5133610	5154798.5 ± 15713.52
25	6558313.2	6532586.5	6571005	6591643.3	6587419.3	6570136.9	6569281.2 ±23337.24

Prepare dilutions from the 100 mg/mL stock solutions. Inject a 20 µL sample from each dilution into the HPLC system.Plot the peak areas versus the concentrations (ranging from 5 µg/mL to 70 µg/mL) and generate calibration curves using linear regression analysis.

Tablet Sample Preparation:

Select twenty commercially available tablets containing both Sumatriptan and Naproxen. Determine the average tablet weight. Grind the tablets into a fine, uniform powder. Weigh an amount equivalent to 10 mg of Sumatriptan and its corresponding amount of Naproxen. Transfer this powder into a 50 mL volumetric flask. Add 30 mL of methanol and shake for 5-10 minutes to ensure complete dissolution. Filter the solution using Whatman #41 filter paper and collect the filtrate in a 100 mL volumetric flask. Rinse the remaining residue on the filter twice with methanol. Combine the filtrates and dilute to a final volume of 100 mL with methanol. From this solution, take 10 mL and dilute it further to 100 mL to obtain the final sample for HPLC analysis. Prepare six replicate samples of this final solution for consistent results.

Method Validation:

The developed RP-HPLC method was validated following ICH guidelines[9], with assessments based on the following parameters:

Linearity:

Inject standard solutions with concentrations of 5, 10, 15, 20, and 25 µg/mL into the HPLC system. Create calibration curves by plotting peak area versus concentration and performing linear regression analysis.

Precision:

Intra-Day Precision: Analyze three replicates of a 10 µg/mL solution at different times throughout the day.

Inter-Day Precision: Measure the same solution across three consecutive days. Report precision as % relative standard deviation (%RSD).

Accuracy (Recovery Study):

Pre-analyzed tablet solutions are spiked with standard Sumatriptan and Naproxen solutions at 80%, 100%, and 120% concentration levels. Calculate percentage recovery by comparing observed concentrations to expected values.

Limits of Detection (LOD) and Quantification (LOQ):

Calculate LOD and LOQ using the following formulas:

LOD = 3.3 × (S.D./slope)

LOQ = 10 × (S.D./slope)

System Suitability Testing:

Assess system suitability by running six replicates of the standard solution. Monitor retention time, resolution, theoretical plate count (N), and tailing factor (Tf). Confirm that the Rs value between Sumatriptan and Naproxen is acceptable, with Rs > 2 ensuring good separation.

Selectivity and Specificity:

Selectivity:

Analyze blank, placebo, and standard solutions to check for any interfering peaks at the retention times of Sumatriptan (4.918 minutes) and Naproxen (3.256 minutes).

Specificity:

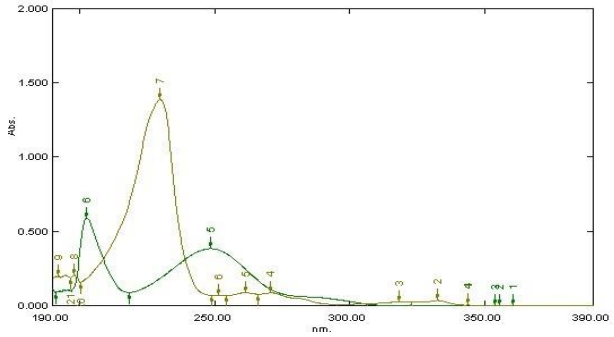

Figure 3: Overlain spectra of Naproxen and Sumatriptan

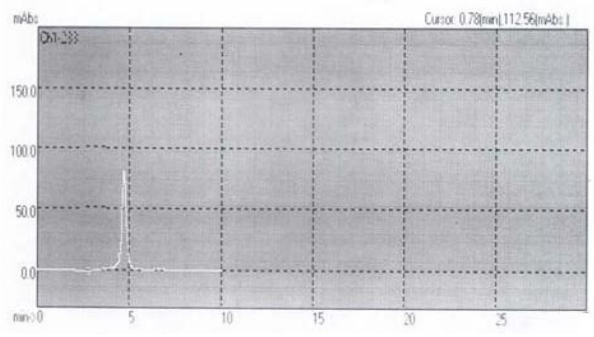

Figure 4: Chromatogram of SUT

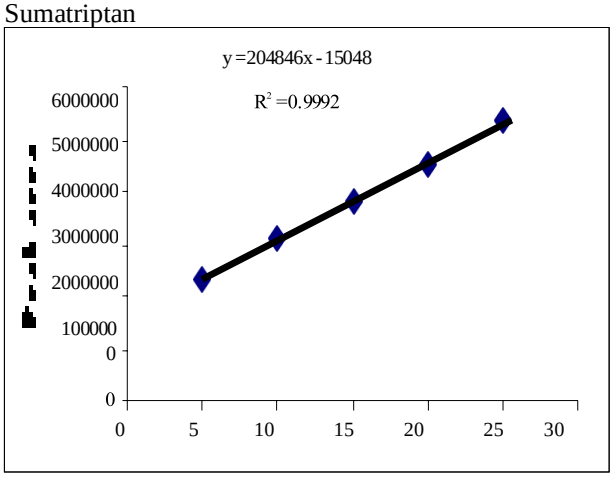

Figure 5: Calibration curve of SUT

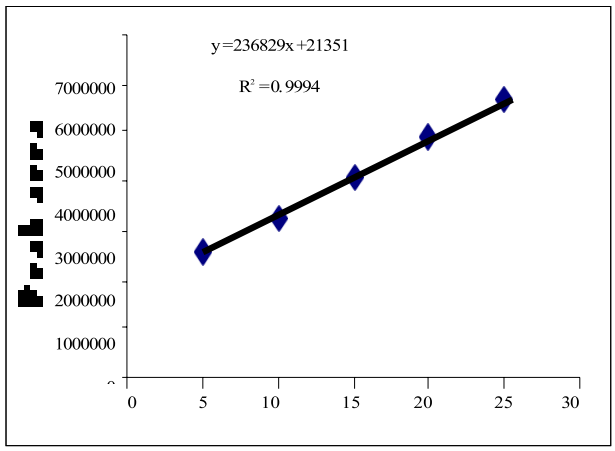

Figure 6: Chromatogram of NAP

Table 3: Analysis of mixed standard

S. No.	SUT	Amount Present (µg/ml)	Amount Found (%)	NAP	Amount Present (µg/ml)	Amount Found (%)
1	5	5.49	109.78	5	5.61	111.12
2	10	11.02	110.02	10	10.99	109.95
3	15	16.44	109.92	15	15.00	106.62
4	20	21.44	107.34	20	21.99	111.21
5	25	27.83	111.32	25	27.78	112.31
Mean	-	-	109.17	-	-	111.86
S.D.	-	-	1.50	-	-	1.99
%COV	-	-	1.37	-	-	1.98
S.E.	-	-	0.66	-	-	0.80

Compare chromatograms of tablet and standard solutions to confirm that the method accurately separates and quantifies both drugs without interference

Data Analysis and Results

The HPLC data were processed using CLASS-VP software to determine peak areas and retention times for both Sumatriptan and Naproxen. Calibration curves for both drugs demonstrated excellent linearity ($R^2 > 0.999$). Recovery rates were between 98% and 102%, indicating high accuracy. Precision studies exhibited %RSD values below 2%, demonstrating the method's repeatability and reliability. **Figure 3** shows the overlain spectra of Naproxen and Sumatriptan

Selection of sampling wavelengths

Assay of Sumatriptan (SUT) and Naproxen (NAP) in Combination

Preparation of Stock Solutions

Sumatriptan Stock Solution:

50 mg of Sumatriptan was accurately weighed. The drug was transferred into a 50 mL volumetric flask. Methanol was used as the solvent to dissolve the drug. The solution was diluted to the mark to achieve a final concentration of 1000 µg/mL.

Naproxen Stock Solution:

50 mg of Naproxen was accurately weighed. The drug was dissolved in 50 mL of methanol in a separate volumetric flask. This resulted in a stock solution of 1000 µg/mL.

Preparation of Working Solutions for Linearity Study

Appropriate volumes of the 1000 µg/mL stock solutions of Sumatriptan and Naproxen were diluted with methanol. Final concentrations achieved were 5, 10, 15, 20, and 25 µg/mL for both drugs. The working solutions were utilized to construct calibration curves for the assay.

Sample Preparation for Tablets

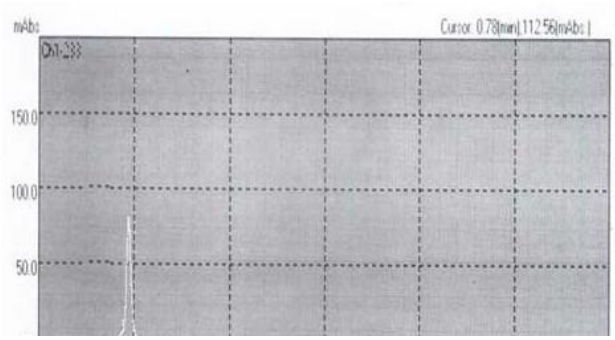

Figure 7: Calibration curve of NAP

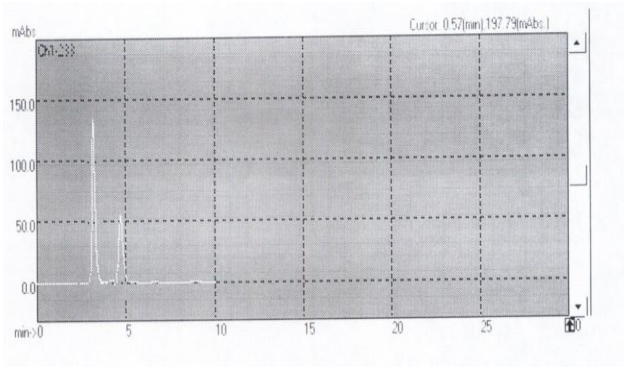

Figure 8: Chromatogram of SUT and NAP

Table 4: Results of Tablet Analysis

S. No.	SUT	Amount Present (µg/ml)	Amount Found (%)	NAP	Amount Present (µg/ml)	Amount Found (%)
1	10	11.12	111.21	10	10.97	109.70
2	10	11.03	110.33	10	11.06	110.60
3	10	10.98	109.78	10	10.96	109.63
4	10	10.98	109.78	10	11.01	110.10
5	10	11.03	110.33	10	10.98	109.86
6	10	10.92	109.30	10	10.98	109.84
Mean	-	-	110.12	-	-	109.90
+ S.D.	-	-	0.678	-	-	0.386
%CV	-	-	0.68	-	-	0.39
S.E.	-	-	0.277	-	-	0.157

Table 5: Result of recovery study

%	Drug	Mean % + S.D.	% CV	S.E.
80	SUT	99.21 + 0.384	0.38	0.34
	NAP	99.47 + 0.418	0.40	0.33
100	SUT	99.36 + 0.528	0.52	0.51
	NAP	99.47 + 0.541	0.40	0.40
120	SUT	98.90 + 0.618	0.60	0.31
	NAP	98.52 + 0.636	0.65	0.35

Twenty commercially available tablets containing both Sumatriptan and Naproxen were chosen for analysis. The average weight of the tablets was calculated. The tablets were finely ground into a homogeneous powder. A sample of the powder equivalent to 10 mg of Sumatriptan (and the corresponding amount of Naproxen) was accurately weighed. The weighed sample was transferred into a 50 mL volumetric flask. 30 mL of methanol was added, and the solution was shaken for 5-10 minutes to ensure complete dissolution of the drugs. The solution was filtered through Whatman #41 filter paper into a 100 mL volumetric flask. The residue was rinsed with methanol. The combined filtrates were diluted to a total volume of 100 mL with methanol. A 10 mL aliquot of this solution was further diluted to 100 mL with methanol to create the final sample solution. Six replicates of the sample solution were prepared for analysis.

HPLC Analysis

A 20 µL aliquot of each standard and sample solution was injected into the HPLC system under optimized chromatographic conditions. Retention times were recorded at: Sumatriptan: 4.918 minutes. Naproxen: 3.256 minutes

Results and Discussion

The assay results, along with statistical validation, are summarized below table 1:

Sumatriptan: The concentration of Sumatriptan in the tablet samples was found to be within the range of 109-111% of the labeled claim.

Naproxen: The concentration of Naproxen in the tablet samples was found to be within the range of 109-112% of the labeled claim.

Both drugs were quantified with high accuracy, as demonstrated by the mean recovery rates and low standard deviation values, confirming the method's reliability for accurate quantification. **Table 1** shows the Linearity of SUT and **Figure 4** shows the Chromatogram of SUT. **Figure 5** shows the calibration curve of SUT. **Table 2** shows the Linearity of NAP and **Figure 6** shows the Chromatogram of NAP. Figure 6 shows the calibration curve of NAP.

Analysis of Mixed Standards

From the standard stock solutions of 1000 µg/mL for each compound, various mixed standard solutions with specified concentrations were created. The corresponding chromatograms were captured. The concentrations of Sumatriptan and Naproxen were determined by relating the peak areas to their respective calibration curves. The analytical findings are compiled in the subsequent **table 3.**

Analysis of Tablet Formulation

Twenty tablets containing both Sumatriptan (SUT) and Naproxen (NAP) were selected for analysis. The average weight of these tablets was calculated to verify consistency. The selected tablets were finely ground to create a homogeneous powder. An exact amount of the powdered tablets, corresponding to 10 mg of Sumatriptan (along with the equivalent amount of Naproxen), was accurately weighed. This weighed powder was transferred into a 50

Table 6: Result of intraday precision study

Replicate	Conc. Found (µg/ml)			
	1st h.	SUT	NAP	
1	11.12	33.35	11.18	33.03
2	11.13	33.10	11.03	33.25
3	11.11	33.12	11.12	33.04
4	11.34	33.23	11.23	33.19
5	11.23	33.20	11.25	33.23
6	11.00	33.21	11.00	33.12
Mean	11.16	33.18	11.14	33.12
+ S.D.	0.095	0.901	0.084	0.089
% CV	0.85	0.27	0.75	0.28
S.E.	0.0388	0.0367	0.0310	0.0343

Table 7: Result of inter-day precision study

Replicate No.	Concentration Found (µg/ml)			
	1st day	SUT	NAP	
1	11.23	33.21	11.01	33.13
2	11.00	33.23	11.21	33.25
3	11.23	33.13	11.00	33.13
4	11.12	33.14	11.21	33.12
5	11.16	33.10	11.02	33.36
6	11.23	33.12	11.03	33.35
Mean	11.17	33.17	11.13	33.18
+ S.D.	0.061	0.052	0.100	0.066
% CV	0.55	0.18	0.99	0.22
S.E.	0.0242	0.021	0.041	0.027

Table 8: Result from system suitability study

Property (n=6)	NAP	SUT
Rt (Retention time)	4.918	3.256
Tf (Tailing facto)	1.37	1.22
K (Capacity factor)	1.26	1.43
N (Theoretical plate number)	8932	7856
Rs (Resolution)	-	2.05

mL volumetric flask. A 10 mL aliquot of the prepared solution was taken and further diluted to a total volume of 100 mL with methanol. This dilution ensured the appropriate concentration of both medications for subsequent analysis. Six replicate samples of the final solution were prepared to enhance accuracy and ensure repeatability in the analytical process.

Table 4 shows the results of tablet analysis and Figure 8 shows the chromatogram of SUT and NAP

Recovery Study:

The recovery study was conducted by spiking previously analyzed tablet solutions with standard solutions of Sumatriptan and Naproxen at three different concentration levels: 80%, 100%, and 120% of the actual drug content **(table 5)**. The samples were prepared in triplicate for each concentration level to ensure accuracy and reproducibility.

Precision Study

Intra-day Precision: Measurements were taken at three different time intervals (one-hour apart) within the same day. The concentrations of Sumatriptan and Naproxen were recorded for each replicate **(Table 6**.

Inter-day Precision: The same sample solutions were analyzed on three separate days (Day 1, Day 2, and Day 3) under identical chromatographic conditions. Six replicates were prepared and analyzed each day to determine the precision across different days **(Table 7)**.

Figure 9 shows the calibration curve of SUT and **Figure 10** shows calibration curve of NAP

Selectivity and Specificity

The selectivity of the developed technique was assessed by determining the resolution factor between the peaks of the active ingredients. Under the established chromatographic parameters, both Sumatriptan (SUT) and Naproxen (NAP) were successfully separated, achieving a resolution of 2.05 **(Table 8)**. This indicates a robust capability for the simultaneous quantification of these two compounds. The retention times for both substances remained consistent in the standard and tablet formulations, with no additional peaks identified, confirming the method's specificity[10, 11]. To further validate specificity, the chromatograms of the tablet formulation were compared with those of a placebo and the standard drug solutions. This analysis affirmed the method's suitability for accurately quantifying these medications in commercial preparations.

CONCLUSION

The developed RP-HPLC method successfully enables the simultaneous quantification of Sumatriptan and Naproxen in both raw materials and tablet formulations with high precision, accuracy, and reliability. The method demonstrated excellent linearity ($R^2 > 0.999$), accurate recovery rates (99.36% for Sumatriptan and 99.47% for Naproxen), and well-resolved retention times (4.918 min for Sumatriptan and 3.256 min for Naproxen). The resolution factor (Rs = 2.05) confirms effective separation of both compounds. The validated method meets regulatory requirements for pharmaceutical analysis, making it a

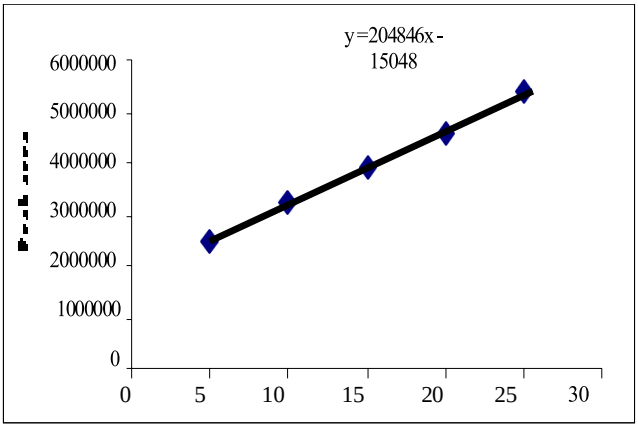

Figure 9: Calibration curve of SUT
Mean SD 20323.02
Slope 204656
LOD 3.3 x 20323.02/204656= 0.24µg/ml
LOQ 10 x 20323.02/204656= 0.84 µg/ml

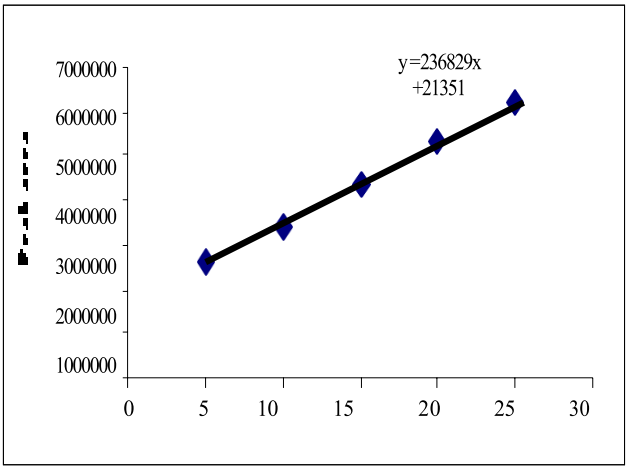

Figure 10: Calibration curve of NAP
Mean standard deviation 19426.6
Slope 233729
LOD 3.3 x 19426.6/233729= 0.25 µg/ml
LOQ 10 x 19426.6/233729= 0.81 µg/ml

robust and efficient tool for routine quality control of Sumatriptan and Naproxen in pharmaceutical formulations.

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