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RP-HPLC Method Development and validation for estimation of sumatriptan and naproxen in spiked human plasma

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ABSTRACT

A rapid, simple, selective, and specific reverse phase high performance liquid chromatography (RP-HPLC) method was developed and validated for estimation of Naproxen and Sumatriptan from tablet using spiked human plasma. The chromatographic separation was performed on Phenomenex Luna C18 column (5µm, 25cmx4.6mm id) with a mobile phase comprised of Acetonitrile: Methanol: phosphate buffer pH 6 (50:10:40 v/v), at a flow rate of 1.0ml/min. The calibration curve was linear in the range of 1-3 µg/ml. The developed method was found to accurate and sensitive. Results of recovery studies prove the extraction efficiency. Stability data indicated that Sumatriptan and Naproxen was stable in plasma after three freeze thaw cycles and upon storage at −20°C for 30 days.

Keywords: Sumatriptan, Naproxen, Human Plasma, RP-HPLC

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INTRODUCTION

Naproxen sodium (NAP) is chemically, (S)-6-methoxy-α- methyl-2-naphthaleneacetic acid, sodium salt. NAP is a non-steroidal anti-inflammatory drug (NSAID) commonly used for the reduction of moderate to severe pain, fever, inflammation and stiffness. It works by inhibiting both the COX-1 and COX-2 enzymes. Like other NSAIDs, naproxen is capable of producing disturbances in the gastrointestinal tract. Several chromatographic methods have been reported for determination of NS in raw material[1], tablets[2-4], plasma[5-7], urine[8], intestinal perfusion samples [9] and pharmaceutical preparations [10-11]. Sumatriptan succinate (SUMA) is chemically, 3-[2-(dimethylamino)ethyl]-N-methyl-indole- 5- methanesulfonamide succinate (1:1). SUMA is a selective 5-hydroxytryptne1 receptor subtype agonist.

Sumatriptan succinate is official in European Pharmacopoeia [12] and United States Pharmacopoeia [13], where chromatographic methods for sumatriptan succinate in bulk and tablet formulation were reported. Several analytical techniques like HPLC [14-15] and LS-MS [16-19] have been reported for sumatriptan succinate in combination with other drugs.

Aim of the present study was to develop and validate HPLC method for the determination of Sumatriptan and Naproxen in plasma. This report describes optimization and validation of a simple HPLC assay method with ultraviolet detection which permits the bioanalytical determination of Sumatriptan and Naproxen. Sample handling and chromatographic run times were minimized to provide fast quantitative results while maintaining the specificity, accuracy and precision required for evaluation of Sumatriptan and Naproxen.

MATERIALS AND METHOD

Reagents

Sumatriptan succinate and Naproxen sodium were obtained as gift samples from Sun Pharmaceutical Ltds., Vadodara (Gujarat). HPLC Grade solvents (Acetonitrile, Methanol, water) were obtained from S. D. Fine Chemicals Ltd., India.

A Shimadzu HPLC, CP224S analytical balance (Sartorius) and ultra sonic cleaner (Frontline FS 4) were used, Injector(Rheodyne,20μl), Sonicator, pH meter, Vaccum filter pump, Millipore filtration kit, mobile phase reservoir, Water bath, Sample filtration assembly and glassware were used throughout the experiment.

Chromatographic Conditions

Analysis was carried at 229nm using a C 18 column (Phenomenex-Gemini 250mm x 4.6mm, 5 μm) at ambient temperature. The mobile phase consisted of Acetonitrile: Methanol: phosphate buffer pH 6 (50:10:40 v/v) was set at a flow rate of 1.0 ml/min.

Sample preparation

Blood samples were collected in heparinized tubes and immediately placed on ice and taken to the lab where they were centrifuged at 5000 rpm for 5 min at room temperature. The resulting plasma samples were stored at -30°C until analysis.

Protein Precipitation

Blood sample was withdrawn from human volunteer in Na2-EDTA tube. The samples were then centrifuged at 5000 rpm for 20 minutes for separation of plasma; subsequently the supernatant plasma was separated and stored at -80°C till the time of analysis.

In a 5 mL borosilicate test tube, 500 µL of plasma was taken. To this, 500 µl of known concentration of drug was added. Further, 2 mL of organic solvent (Acetonitrile) was added to precipitate proteins. It was subjected to centrifugation at 5000 rpm for 10 min. After that, the supernatant, which contains the analyte of interest, was collected into a clean test tube. Appropriate volume of the solvent was taken into vial and allowed to evaporate under the stream of N_2 gas. The residue was reconstituted with 2 mL of diluents and 20 µL was injected into HPLC.

Equipment

The chromatographic system used in the study was a Shimadzu LC-20 HPLC system. The separation of compounds was achieved using a Phenomenex Luna C18 column (5µm, 25cmx4.6mm id).

The mobile phase was prepared by mixig 500 ml of Acetonitrile, 100 ml of methanol and 400 ml of phosphate buffer pH 6. Then the mobile phase was degassed by sonicating for 20min.

Quantification of drugs in plasma:

A Standard curve was prepared by injecting various concentrations of Sumatriptan succinate and Naproxen sodium in plasma. The concentrations of the plasma and quality control samples were calculated by using the regressed equation of the straight line y = ax + b. The individual chromatograms of blank plasma and the plasma containing Sumatriptan succinate and Naproxen sodium are presented in Fig. 1 and 2.

METHOD VALIDATION

The developed method was validated for specificity, linearity, accuracy, method and system precision, limit of detection (LOD), limit of quantification (LOQ), ruggedness, and robustness in accordance with ICH guideline Q2(R1).

Linearity

Linearity Standard stock solution was further diluted to obtain 1 µg/mL, 2 µg/mL, 3 µg/mL, 4 µg/mL, 5 µg/mL, 6 µg/mL of Sumatriptan Succinate and Naproxen sodium in combination. Twenty microlitre of the each standard solution was injected and chromatograms were recorded.

Precision

Precision of the method was verified by repeatability (system precision) and intermediate precision (method precision) studies. Repeatability studies were performed by six replicate injections of 2 µg/ml of Sumatriptan Succinate and 2 µg/ml solution of Naproxen sodium on the same day. The studies were replicated on different days to determine intermediate precision.

Accuracy

Accuracy of the method was carried out by applying the method to drug sample to which known amount of SUMA and NAP standard powder corresponding to 50%, 100% and 150% of label claim had been added (standard addition method), the solutions are analyzed by optimized method. The absolute recovery of Sumatriptan and naproxen was determined by comparison of the peak areas from non extracted and extracted samples of QC-3 in triplicate. The intra-day accuracy and precision were determined at three different concentrations from six replicate QC.

LOD and LOQ

Limit of detection (LOD) and Limit of quantification (LOQ) The limit of detection (LOD) is the lowest amount of analyte in a sample that can be detected, but not necessarily quantified, under the stated experimental conditions. LOD & LOQ was calculated by using standard deviation and slope values obtained from calibration curve.

$\text{LOD} = 3.3\ \sigma/\ S$

$\text{LOQ} = 10\ \sigma/\ S$

Where, σ is standard deviation (intercept of calibration line); S is slope

Robustness

Robustness of the method To evaluate robustness of the HPLC method, Slight variations were made in pH of Mobile phase was changed 6 to 5.8 and 6.2. Slight variations were made in amount of organic phase and mobile phase.

Stability

The stability of sumatriptan succinate and naproxen sodium in plasma was determined by spiking human plasma with known amounts of sumatriptan succinate and naproxen sodium (1 and 3 µg/mL). The prepared samples were aliquot (2 mL) into polypropylene plastic tubes, capped, and stored at -70°C, -20°C, 4°C, and room temperature. Five replicates of each pool were assayed from each storage condition at set time points following initial storage.

Short term stock stability

A stock solution of sumatriptan succinate and naproxen sodium in plasma was kept at room temperature for 8 hours and checked for its stability.

Long term stock stability

A stock solution of sumatriptan succinate and naproxen sodium in plasma was kept at room temperature for 45 days and checked for its stability.

Bench top stability

The three replicate concentration of low and high quality concentration samples were determined by comparing the mean area ratio of freshly thawed samples which have been kept at room temperature for about 6 hours.

Coolant stability

The three replicate concentration of low and high quality concentration samples were determined by comparing the mean area ratio of freshly thawed samples which have been kept at room temperature for about 24 hours.

Freeze thaw stability

The stability of low and high quality concentration samples was determined after three freeze thaw cycles. The percent degradation was determined by comparing the mean ratio of area of sumatriptan succinate and naproxen sodium in plasma.

Long term plasma stability

Three aliquots of each of low and high concentrations at same conditions were analyzed on three separate occasions. Storage time not exceeds the time between the date of first sample analysis and the date of last sample analysis.

RESULTS AND DISCUSSION

The method was validated in terms of limit of quantification, Recovery, Selectivity, Precision, accuracy and stability.

Results of study indicated that there were no interfering peaks of diluents or biological matrix at the retention time of analyte (Figure 1 & 2). Thus, developed method of estimation was well fitted to the acceptance criteria limit.

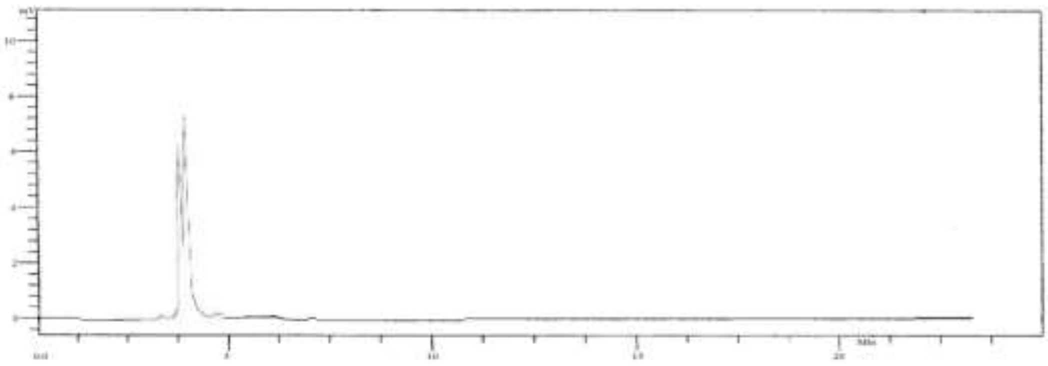

Figure 1: Chromatogram for Blank Human plasma

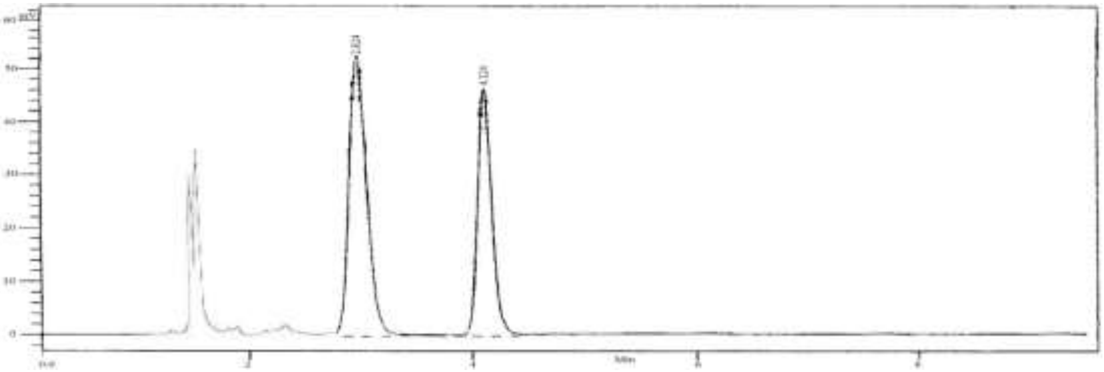

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Figure 2: Chromatogram of Blank human plasma spiked with Sumatriptan succinate and naproxen sodium

Linearity

The method was validated over the linearity range of 1-3 µg/ml for Sumatriptan and Naproxen(Figure 3 & 4).

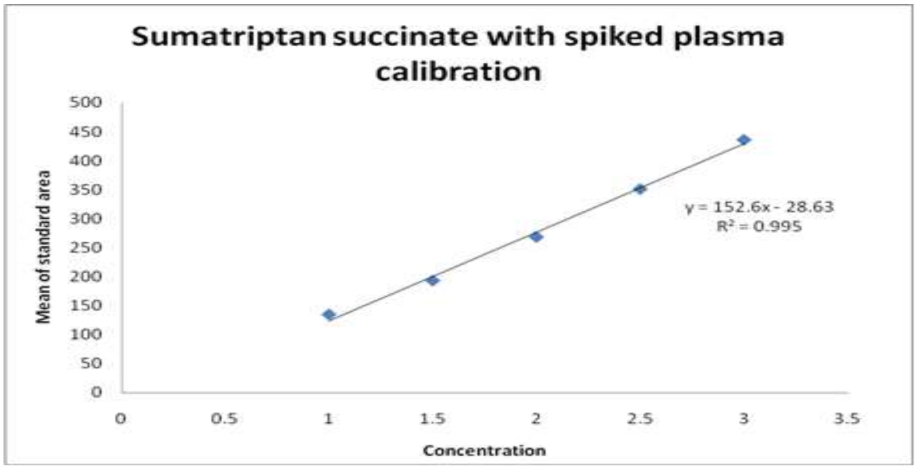

Figure 3: Sumatriptan succinate with spiked plasma calibration

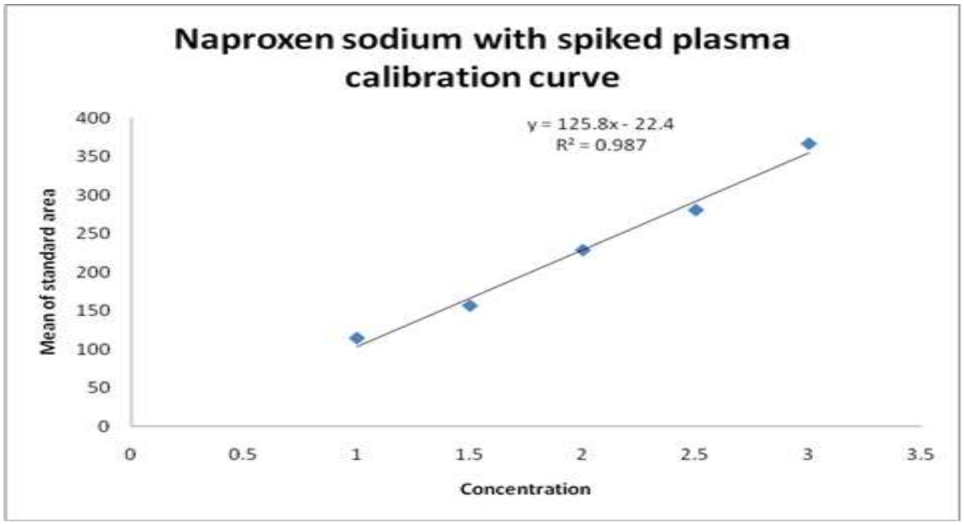

Figure 4: Naproxen sodium with spiked plasma calibration curve

Sensitivity

From the linearity graph of Sumatriptan succinate and Naproxen sodium, LLOQ for Sumatriptan succinate and Naproxen sodium were found to be 0.65 µg/ml and 0.98 µg/ml respectively (Table 1).

Table 1: L.O.Q. data for Sumatriptan succinate and Naproxen sodium

Parameters	**Sumatriptan succinate**	**Naproxen sodium**
Standard deviation of the 5 calibration curves	6.36	9.0258
Mean slope of the 5 calibration curves	97.40	91.8
L.O.Q. = 10 × (SD/Slope) (µg/mL)	0.65	0.98

recovery

The recovery was determined by comparing the aqueous solution and the spiked drug. The percentage recovery of the drug and the internal standard was calculated and it was found to be 97.22% and 99.57% respectively (Table 2 & 3).

Table 2: Recovery data of LQC, MQC and HQC for sumatriptan succinate

Sr. No.	**Concentration (µg/mL)**	**Area Mean ± S.D. (n=5)**	**Spiked Area ± SD S.D. (n=5)**	**% Nominal Value**	**% Mean Nominal Value**
1	1	155.15± 2.76	134.2 ±5.62	86.50	86.50
2	2	311.16± 8.96	268.4±5.34	86.26	
3	3	503.33± 8.25	436.6±7.85	86.74	

Table 3: Recovery data of LQC, MQC and HQC for Naproxen sodium

Sr. No.	Concentration (µg/mL)	Area Mean ± S.D. (n=5)	Spiked Area ± SD S.D. (n=5)	% Nominal Value	% Mean Nominal Value
1	1	130.25 ± 2.86	114.2±5.33	87.68	87.06
2	2	266.88 ± 6.19	228.4±6.75	85.58	
3	3	456.92 ± 6.36	366.6±4.62	87.93	

Precision and Accuracy

The accuracy, precision and intraday precision were carried out by preparing 6 individual samples of HQC, MQC and LQC and the percentage CV and percentage nominal was calculated (Table 4 & 5) .

Table 4: Precision data for bioanalytical method of sumatriptan succinate

Sr. No.	Concentration (µg/mL)	average of standard area	Standard deviation	% RSD	% Mean RSD
1	1	135.52	4.86	3.59	3.44
2	2	264.78	3.16	1.19	
3	3	433.18	23.99	5.54	

Table 5: Precision data for bioanalytical method of Naproxen sodium

Sr. No.	Concentration (µg/mL)	Average of standard area	Standard deviation	% RSD	% Mean RSD
1	1	120.70	6.77	5.61	4.33
2	2	234.11	7.88	3.37	
3	3	360.27	14.49	4.02	

Stability

Stability of the method was carried out by performing short term and long term stock stability. The percentage mean ratio of the drug and internal standard were calculated.

Stability of the plasma samples was carried out by performing coolant, bench top and freeze thaw stability studies. The percentage mean ratio of the HQC and LQC were calculated (Table 6 & 7).

% Deviation for different type of stability studies i.e. stock solution stability, short term stability, freeze and thaw stability and bench top stability was found to be within the acceptance range.

Table 6: Stability Data for Bioanalytical Method of sumatriptan Succinate

Stability	Theoretical Concentration (ng/mL)	Storage Time	Storage Temp.	Mean assay result	% assay	% Deviation
Stock solution	1	6	R.T.	0.96	96.41	3.59
	3			2.97	99.07	0.93
Short term	1	48	- 4 ^{0}C	0.98	98.12	1.88
	3			0.29	99.77	0.23

Freeze and Thaw	1	72	- 20 ^{0}C	0.93	93.24	6.76
	3			2.99	92.21	7.79
Bench Top	1	24	R.T.	0.93	93.13	6.87
	3			2.89	0.96	3.38

Table 7: Stability Data for Bioanalytical Method of sumatriptan Succinate

Stability	**Theoretical Concentration (ng/mL)**	**Storage Time**	**Storage Temp.**	**Mean assay result**	**% assay**	**% Deviation**
Stock solution	1	6	R.T.	0.93	93.00	1.95
	3			2.92	97.33	2.64
Short term	1	48	- 4 °C	0.93	93.00	2.45
	3			2.74	91.33	3.75
Freeze and Thaw	1	72	- 20 °C	0.94	94.00	2.53
	3			2.84	94.67	4.63
Bench Top	1	24	R.T.	0.96	96.00	1.34
	3			2.94	98.00	3.95

CONCLUSION

Substantiation of developed bioanalytical method for estimation of Sumatriptan succinate and naproxen sodium in human plasma was good at both its specificity and recovery; the results of bioanalytical method validation fulfills the FDA guideline, thus method can be employed in further clinical study to estimate the amount of Sumatriptan succinate and naproxen sodium in human plasma. This developed method provide selective quantification of drug without any interference from blank and placebo and are highly sensitive, reproducible, reliable, rapid and specific. Analysis by these methods were found to be simple, accurate, reproducible, precise, and in good agreement with labeled claim of the drug.

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