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Bioanalytical Validated LC-MS Method for Determination of Naproxen in Human Plasma

Anil Kumar Veeragoni[1] | Vasudeva Murthy Sindgi[2] | Shoba Rani Satla[3]

[1]Department of Pharmaceutical Analysis, Pathfinder Institute of Pharmacy Education and Research, Warangal, Telangana, India.
[2]Jayamukhi College of Pharmacy, Narsampet, Warangal, Telangana, India.
[3]Center for Pharmaceutical Sciences, Institute of Science and Technology, JNTUH, Hydreabad, Telangana, India.

ABSTRACT

Naproxen is an anti-inflammatory drug belongs to the category of analgesics and antipyretics. Naproxen has the ability to bind and inhibit the synthesis of prostaglandins and produces anti-inflammatory effect. Naproxen also inhibits COX-II which is involved in the inflammation. Bioanalytical method for naproxen has been developed using human plasma. Standard stock solution of naproxen was prepared using methanol. Zidovudine was used as internal standard. The linearity of the proposed method was developed in the range between 100 and 10000 ng/ml. The slope of the curve was found to be 111.46 and intercept was 6395.2 and correlation coefficient was 0.999. The proposed method was evaluated with validation parameters like accuracy, precision and stability. The developed method is suitable for pharmacokinetic studies.

KEYWORDS: *LC MS Method, naproxen, bioanalytical, zidovudine, validation*

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I. Introduction

Naproxen, (+)-(*S*)-2-(6-methoxynaphthalen-2-yl) propanoic acid (Figure 1) is one of the non-steroidal anti-inflammatory drug and belongs to the category of acetic acid group of analgesics and antipyretics. Naproxen has the ability to bind and inhibit the synthesis of prostaglandins and produces anti-inflammatory effect. Naproxen also inhibits COX-II which is involved in the inflammation. [1] Naproxen shows high affinity for plasma proteins and binds up to 99%. The major side effects of naproxen may include diarrhea, nausea, rashes, mild insomnia, vomiting, blurred vision, weight loss, mood changes, problems in urination, yellowing of skin and eyes. Naproxen is recommended for rheumatoid arthritis, bursitis, osteoarthritis [2] and acute gout. Naproxen is also recommended for dysmenorrhea. Naproxen has little drug interaction with cyclosporine where it does cause nephrotoxicity; shows diminished antihypertensive effect of timolol in combination with naproxen. The severe adverse events include myocardial infarction, heartburn, bleeding in stomach and interfere with antidepressants [3].

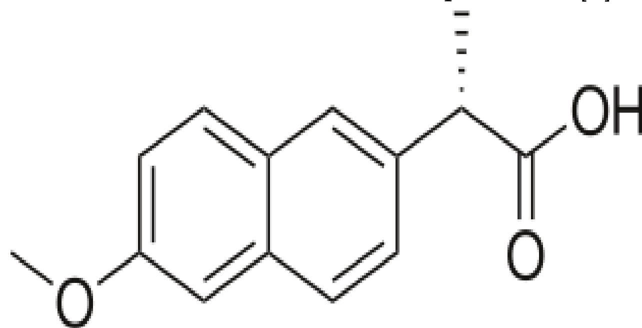

Figure 1. Structure of naproxen

The literature survey revealed that various HPLC methods were reported such as simultaneous determination of naproxen [4], naproxen is determined with graphite column [5], in tablet formulations [6], stability assays method [7], chemometric HPLC [8], RP-HPLC method [9], but methods with lowest limit of detection were not reported. The present study is aimed to develop LC-MS method with minimum limit of detection.

II. Materials and Methods

Ammonium acetate, Sodium Hydroxide, Potassium di hydrogen phosphate, acetonitrile HPLC grade was purchased from Sigma-Aldrich, India. Naproxen was determined using API 3000 in negative ion mode. Naproxen has showed m/z value at 228.9 showed in Figure 2. Similarly the internal standard zidovudine has showed m/z value at 265.8 showed in Figure 3. Ammonium acetate was prepared by adding 77.08 mg in 500 ml of Millipore water and further sonicated about 20 min. Mobile phase is prepared by adding 100 ml of 20 mM ammonium acetate to 900 ml of HPLC grade acetonitrile. Agilent Zorbax 4.6 x 75 mm with 3.5 micron particle diameter was selected for the study. The shape of chromatogram and peak symmetry was achieved and suitable for the determination of drug in human plasma. Zidovudine has showed good peak symmetry along with naproxen and been selected as internal standard.

Naproxen was determined using liquid –liquid extraction method in which 100 µL of plasma is added to 200 µg/ml of zidovudine (25 µL) and vortexed. Furthermore, 500 µL of TBME was added and shaken for 30 min. The organic layer is separated after centrifugation at 5000 rpm and evaporated to dryness at 40 °C and residue is reconstituted with suitable volume of mobile phase and injected to LC-MS system. The retention time for naproxen was found to be 2.2 ± 0.25 min and for zidovudine was 1.8 ± 0.35 min.

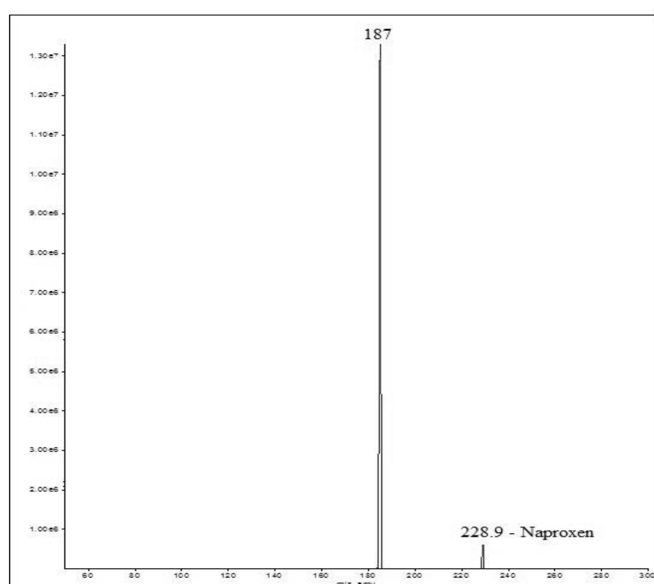

Figure 2. Mass spectrum of naproxen

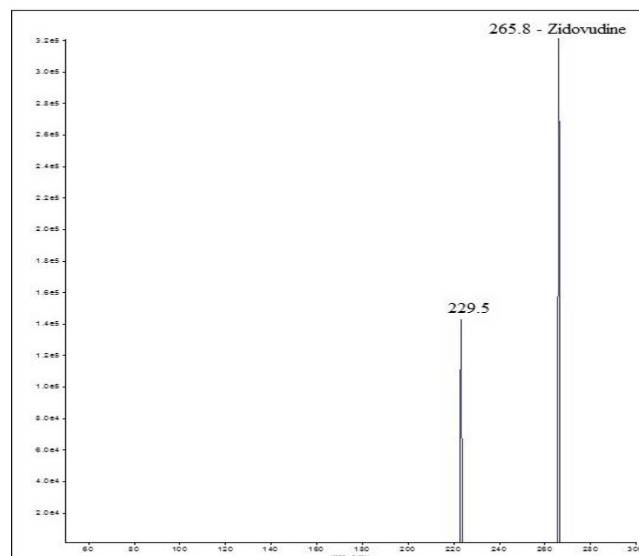

Figure 3. Mass spectrum of zidovudine

2.1 Stock solution – Naproxen

Accurately weighed 4 mg of naproxen was transferred to 1.5 ml capacity centrifuge tube and diluted to 1 ml with HPLC graded methanol. The stock solution is kept at below 6 °C. This prepared stock solution was diluted further to suitable concentrations and analyzed.

2.2 Stock solution – *Zidovudine* (Internal Standard)

Accurately weighed 1 mg of zidovudine was transferred to 1.5 ml capacity centrifuge tube and diluted to 1 ml with HPLC graded methanol. The stock solution is kept at below 6 °C. This prepared stock solution was diluted further to suitable concentration and analyzed at constant concentration.

2.3 Analysis of human plasma sample

Dipotassium EDTA was selected to isolate plasma and selected in such a way that no interference was observed against naproxen and zidovudine. 200 µl of plasma sample is spiked to 200 µl of calibration concentration of naproxen and further diluted to suitable concentrations and analyzed. This spiked solution was kept at subzero temperature.

2.4 Calibration concentrations of naproxen

Naproxen stock solution was further diluted and spiked with plasma to obtain concentration ranges between 100 and 10000 ng/ml. The prepared calibration concentrations were consisting of 100 ng/ml as LLOQ, 500 ng/ml as LQC, 3000 ng/ml as MQC and 8000 ng/ml as HQC. Ten samples of each were prepared and stored at sub-zero

temperature and analysed after thawing whenever required.

Spiking Dilution Concentration	Stock Aliquot	Plasma Volume	Final Volume of Plasma	Final Concentration in Spiked Plasma
(ng/mL)	(mL)	(mL)	(mL)	(ng/mL)
100 – LLOQ	0.5	9.5	10	100.0
500 – LQC	0.5	9.5	10	500
1000	0.5	9.5	10	1000
3000 – MQC	0.5	9.5	10	3000
5000	0.5	9.5	10	5000
8000 – HQC	0.5	9.5	10	8000
10000	0.5	9.5	10	10000

Table 1. Plasma concentrations of naproxen

III. Results and Discussion

3.1 Selectivity

LLOQ sample of naproxen was selected and chromatogram area was determined. The method was found to be more selective for naproxen as showed in the table 2.

LLOQ	Naproxen Area	IS Area
Mean	12537.5	142504.4
Standard Deviation	1036.54	9544.27
% Coefficient of Variance	8.26	6.7

Table 2. Selectivity - Naproxen

3.2 Linearity

The linearity of the method was determined by taking the respective concentrations of naproxen and injected into LC-MS system. The area of each concentration was correlated to its concentration and a scatter graph was plotted. The value of correlation coefficient greater than 0.999 represents the curve is linear. The values were showed in the table 3.

Concentration ng/ml	Mean area	Standard Deviation	% Coefficient of Variance
100	12537.5	2556.24	20.38
500	65425.1	8452.48	12.91
1000	114315.7	11112.19	9.72
3000	355870	35625.75	10.01
5000	556760	45875.86	8.23
8000	887649	89858.48	10.12
10000	1128538	102415.3	9.07

Table 3. Linearity of spiked naproxen calibration concentrations

The linear regression parameters were given in the following table.

Slope	Intercept	r^2
111.46	6395.2	0.999

Table 4. Linear regression equation parameters

3.3 Precision and Accuracy for Naproxen

Statistic	LLOQ	LQC	MQC	HQC
Mean	17012.71	64248.45	355812.75	847514.42
S.D.	2812.86	8424.75	34419.526	89547.56
C.V.%	16.53	13.11	9.67	10.56
% Nominal	90.12	103.80	104.44	94.32
N	18	18	18	18

Table 5. Precision and accuracy of spiked naproxen calibration concentrations

The precision and accuracy of the proposed method was determined using LLOQ, LQC, MQC and HQC sample. Each sample was prepared (N=18), extracted and analysed at inter day and intraday time points. Naproxen has showed 90.12% for LLOQ and has the precision of 17%.

3.4 Solution stability

3.4.1 Stock solution stability

The stock solution stability (N=10) of naproxen 3000 ng/ml was determined at ambient temperature (25 ± 2°C)and performed for a period of 9 hours for naproxen and zidovudine and analysed (Table 6.)

Drug	Hours	%Stability
3000 ng/ml of Naproxen	0	99.78
	9	99.14
3000 ng/ml of zidovudine	0	99.82
	9	99.65

Table 6. Stock solution stability of naproxen and zidovudine

3.4.2 Refrigeration stability

The similar concentrations were prepared as mentioned in stock solution stability and maintained the temperature between 2°C and 8°C respectively for a period of seven days and observed that there was no significant change in the concentration of drug samples in plasma. The details were showed in the table 7.

Drug	Day	%Stability
3000 ng/ml of Naproxen	0	100.14
	7	99.57
3000 ng/ml of zidovudine	0	99.43
	7	99.07

Table 7. Refrigeration solution stability of naproxen and zidovudine

3.4.3Freeze thaw stability

The freeze thaw stability of naproxen and zidovudine were determined. Four freeze thaw cycles were performed and analyzed for drug content. Firstly, the sample was frozen to -20°C and immediately thawed to liquid state. This procedure was repeated continuously for four times and analyzed subsequently. The details of the analysis were showed in the table 8.

Drug	Freeze Thaw Cycle	%Stability
3000 ng/ml of Naproxen	0	100.03
	4	99.48
3000 ng/ml of zidovudine	0	99.81
	4	99.59

Table 8. Freeze thaw stability of naproxen and zidovudine

IV. Conclusion

The results of selectivity, linearity, precision and accuracy, stabilities were found to be within the acceptance range for bioanalytical acceptance criteria as per USFDA 'Guidance for Industry - Bioanalytical Method Validation (May 2001)'.The LC-MS bioanalytical method for the estimation of naproxen in human plasma is valid for future pharmacokinetic studies.

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