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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF NAPROXEN USING RP-HPLC

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ABSTARCT

A sensitive and specific isocratic RP-HPLC was developed for quantitative estimation of naproxen in Tablet formulation. Naproxen is chemically (+)-(S)-2-(6-methoxynaphthalen-2-yl) propanoic acid. Naproxen is a non steroidal anti-inflammatory drug (NSAID) 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. The developed method consist of mobile phase Phosphate Buffer and Methanol in (40:60) with isocratic programming, Symmetry C_{18}, 250×4.6mm, 5µm column as stationary phase with a flow rate of 1.3 ml/minute. Proposed method was found to be linear in the concentration range of 2 to 20 ppm levels, the correlation coefficient was found to be 0.999. System suitability parameters were studied by injecting the standard solution five times and results were well under the acceptance criteria, the proposed method is found to be sensitive, rapid, reproducible, and accurate. Keywords—Naproxen, RP-HPLC, stationary phase, chromatograpy, purity, validation, devlepment,

INTRODUCTION

The compound (+)-(*S*)-2-(6-methoxynaphthalen-2-yl) propanoic acid (Naproxen) is a member of Cyclooxygenase Inhibitors. Naproxen (marketed as Artagen®, Arthopan®, and Napexar®) manufactured by Ranbaxy. Naproxen is a non steroidal anti-inflammatory drug (NSAID) with analgesic and antipyretic properties. The mechanism of action of naproxen, like that of other NSAIDs, is believed to be associated with the inhibition of Cyclooxygenase activity. 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 to relieve pain from various conditions such as headaches, muscle aches, tendonitis, dental pain, and menstrual cramps. It also reduces pain, swelling, and joint stiffness caused by arthritis, bursitis, and gout attacks.

LITERATURE REVIEW

Lotfi Monser at al., developed a simple, selective and sensitive high performance liquid chromatographic (HPLC) method for the simultaneous determination of naproxen and its main degradation products such as 1-(6-methoxy-2-naphthyl) ethanol (MNE), 2-methoxy-6-ethyl naphthalene (MEN) and 2- acetyl-6-methoxy naphthalene (AMN). The separation of these compounds was achieved on porous graphitic carbon (PGC) column using tetrahydrofuran–methanol as the mobile phase, and the effluent from the column was monitored at 272 nm. At a flow rate of 1 ml min^{-1}, the retention time of the last eluting compound was less than 10 min. Correlation coefficient for calibration curves in the ranges 2–25 μg ml^{-1} for all compounds studied were greater than 0.999. The sensitivity of detection is 0.05 μg l^{-1} for naproxen, MNE and MEN and 0.20 μg ml^{-1} for AMN. The reproducibility of the peak area of these compounds using isocratic elution were quite high, and the standard deviations (S.D.) were below 2% (*n*=5). The reproducibility of retention times of these compounds was within 1% (*n*=5). The proposed liquid chromatographic method was successfully applied to the analysis of commercially available naproxen sodium (NS) dosage forms with recoveries of 98.8–102%. A comparative study shows that the selectivity of these compounds on PGC column was different to that obtained with octadecyl silica (ODS) columns.

Patricia Damiani at al., reported a rapid, selective, sensitive and simple fluorescence method for the direct determination of naproxen in tablets. The tablets were triturated, dissolved in either NH_3 or NaOH solution, sonicated, filtered and then direct fluorescence emission was read at 353 nm (exciting at 271 nm). In order to validate the method the results were compared with those obtained by the USP XXIV NF 19 Pharmacopeia reference method (high performance liquid chromatography). The slope, intercept and variances which are associated with the regression coefficient calculated with bivariate least square (BLS) regression indicate that both methods are statistically comparable. The recoveries were excellent, except in tablets containing the antibiotic tetracycline. In this latter case a correction procedure is necessary.

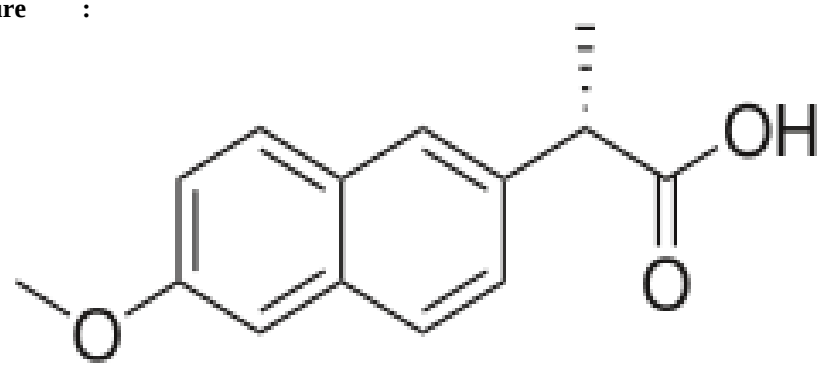
Islam Ullah Khan et al., reported that Naproxen reacts with 1-naphthylamine and sodium nitrite to give an orangish red color having maximum absorbance at 460-480 nm (working wavelength 480 nm). The reaction is selective for naproxen with 0.001 mg/ml as visual limit of quantitation and provides a basis for a new spectrophotometric determination. The reaction obeys Beer's law from 0.01mg to 6.5 mg/10ml of naproxen and the relative standard deviation is 1.5%. The quantitative assessment of tolerable amount of other drugs is also studied.

Rajesh Nuni, A reverse phase HPLC method is developed for the determination of Sumatriptan and naproxen in pharmaceutical dosage forms. Chromatography was carried out on a C8 column [4.6 x 150mm, 3.5mm, Make: XTerra] using a mixture of potassium di hydrogen ortho phosphate buffer and acetonitrile (50:50v/v) as the mobile phase at a flow rate of 0.7ml/min. Detection was carried out at 285 nm. The retention time of the drug Naproxen and sumatriptan was 2.24 minand 5.871 min. The method produced linear responses in the concentration range of 60 to 100μg/ml of Sumatriptan and naproxen. The LOD values for HPLC method for naproxen and sumatriptan were found to be 3.20 and 3.36 ng/ml. The LOQ for Naproxn and Sumatriptan were foud to be 9.86 and 9.90 ng/ml respectively. The method was found to be applicable for determination of the drug in tablets.

DRUG PROFILE OF NAPROXEN

Naproxen is a non steroidal anti-inflammatory drug (NSAID) 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.

Structure :



Systematic IUPAC name : (+)-(*S*)-2-(6-methoxynaphthalen-2-yl) propanoic acid
Formula : $C_{14}H_{14}O_3$
Category : Cyclooxygenase Inhibitors Gout Suppressants
Physical state : Solid (Powdered solid and crystalline powder)
Solubility : Insoluble in cold water
Melting point : 152°C (305.6°F)
Molecular Weight : 230.26 g/mole

MECHANISM OF ACTION

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.

Pharmacokinetic data

Bioavailability: Naproxen itself is rapidly and completely absorbed from the GI tract with an in vivo bioavailability of 95%. Although naproxen itself is well absorbed, the sodium salt form is more rapidly absorbed resulting in higher peak plasma levels for a given dose. Food causes a slight decrease in the rate absorption.

Protein binding: At therapeutic levels naproxen is greater than 99% albumin-bound.

Metabolism: Naproxen is extensively metabolized to 6-0-desmethyl naproxen and both Parent and metabolites do not induce metabolizing enzymes.

Half life: The observed terminal elimination half-life is approximately 15 hours.

Excretion: The clearance of naproxen is 0.13 mL/kg. Approximately 95% of the naproxen from any dose is excreted in the urine, primarily as naproxen(Less than 4%), 6-0-desmethyl naproxen (less than 1%) or their conjugates (66 % -92%).

Clinical use: Naproxen is used to relieve pain from various conditions such as headaches, muscle aches, tendonitis, dental pain, and menstrual cramps. It also reduces pain, swelling, and joint stiffness caused by arthritis, bursitis, and gout attacks.

Adverse Effects: Naproxen has side effects similar to other NSAIDs. A 2011 meta-analysis published in the British Medical Journal states that, of all NSAIDs evaluated, naproxen was associated with the smallest overall cardiovascular risks. As with other NSAIDs, naproxen can cause gastrointestinal problems such as heartburn, constipation, diarrhea, ulcers, and stomach bleeding. It may interfere and reduce the efficiency of SSRI antidepressants

OBJECTIVE

The Literature survey indicates that there are very few methods for the determination of Naproxen. Therefore an attempt was made to develop and validate a simple and economical RP-HPLC method as per ICH guidelines for the estimation of Naproxen in pharmaceutical dosage forms.

EQUIPMENTS AND CHEMICALS

A simple reverse phase HPLC method was developed for the determination of Naproxen pharmaceutical dosage form of 400mg. column used kromosil (150*4.6µm packed with 5µm) in an isocratic mode with mobile phase Buffer: Methanol (40:60) was used. The flow rate was 1.3ml/ min and effluent was monitored at 266 nm and column temperature of 25^0C.

Equipment and Apparatus used:

1. HPLC with PDA detector (Waters)
2. Sonicator (Ultrasonic sonicator)
3. P^H meter (Thermo scientific)
4. Micro balance (Sartorius)
5. Vacuum filter pump

Reagents used:

1. Methanol HPLC Grade (RANKEM)
2. Acetonitrile HPLC Grade (RANKEM)
3. HPLC grade Water (RANKEM)
4. Glacial Acetic acid

Optimized conditions

Colum : Symmetry C_{18}, 250×4.6mm, 5µm or Equivalent
Mobile phase: Buffer: Methanol (40:60)
Buffer : 0.02M Sodium acetate P^H 5.5 adjusted with Glacial acetic acid
Flow rate : 1.3ml/min
Detector : UV at 230nm
Run time : 8 minutes
Diluent : Methanol: Water (10:90)
Temperature : 25^0C
Injection Volume :10µL

Preparation of Mobile phase:

Mobile Phase: Phosphate Buffer: Methanol (40:60)

Buffer Preparation:

2.999gms of ammonium acetate and 2 ml of triethyl amine in 1000 ml of water and adjust the Ph to 6.5 using orthophosphoric acid.

Stock and Standard Solution Preparation: 100 microgram/ml

Weigh accurately about 10mg Naproxen working standard and transfer into a 100 mL volumetric flask, add 70 mL of diluent and sonicate to dissolve for about 5 min solution was filtered through 0.45µ filter, further volume was made up with diluent (Stock Solution). From this solution 2 m was taken in 10 volumetric flask and volume made up with diluent (Standard Solution 20ppm)

Preparation of Linearity Solutions

By appropriate aliquots of the standard naproxen solution with mobile phase, five working solutions ranging between 2-20 ppm were prepared. Each experiment was performed in triplicate according to optimized chromatographic conditions. The peak areas of the chromatograms were plotted against the concentration of naproxen to obtain the calibration curve.

Sample Preparation:

20 tablets were weighed and crushed into powder. Weighed powder equivalent to 5 mg of the naproxen transferred into a 100 mL. Volumetric flask, 70 mL of diluent added and sonicated for 15 min, further volume was made with diluent. Solution was filtered through 0.45µ Nylon filter. 4 ml of this solution was transferred to 10 ml volumetric flask and volume was made up with diluent.

SYSTEM SUITABILITY

A Standard solution of Naproxen working standard was prepared as per procedure and was injected five 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 five replicate injections.

LINEARITY

To demonstrate the linearity of assay method, inject 5 standard solutions with concentrations of about 2 ppm to 20 ppm of Naproxen. Plot a graph to concentration versus peak area. The results were summarised in table 1&2.

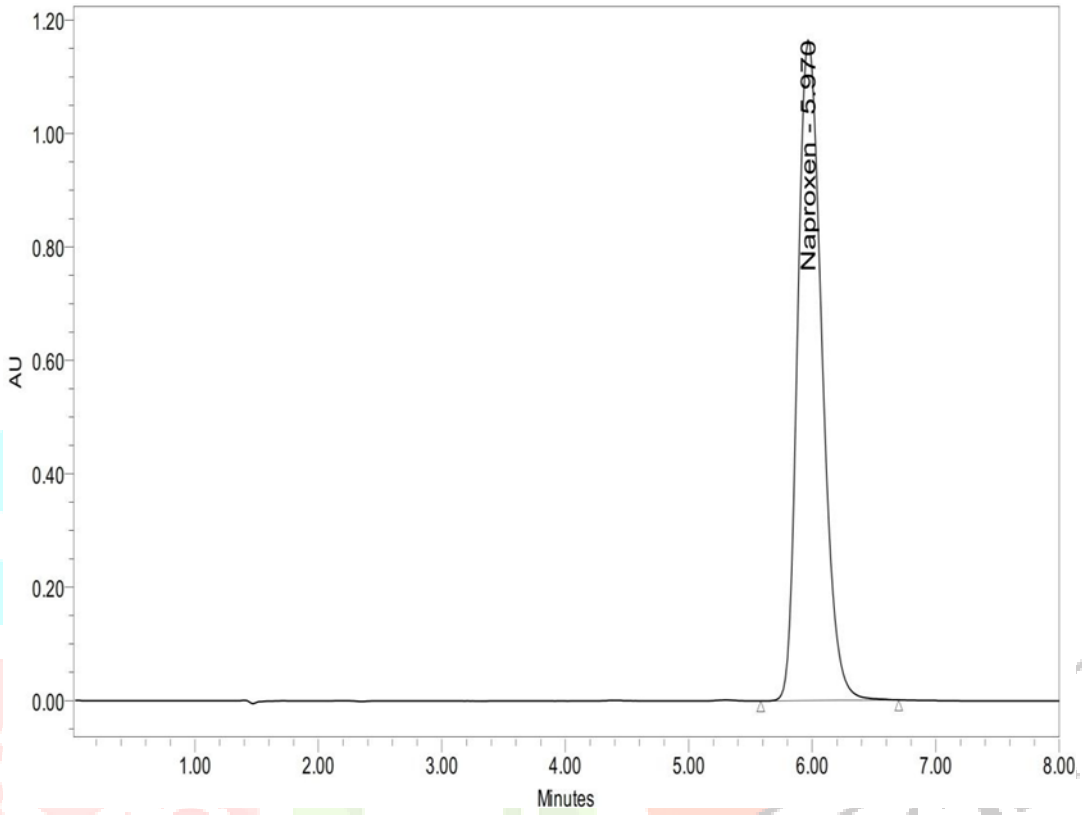


fig no: 1 chromatogram of standard naproxen

ASSAY MARKETED FORMULATION

Standard solution and sample solution were injected separately into the system and chromatograms were recorded and drug present in sample was calculated using afore mentioned formula

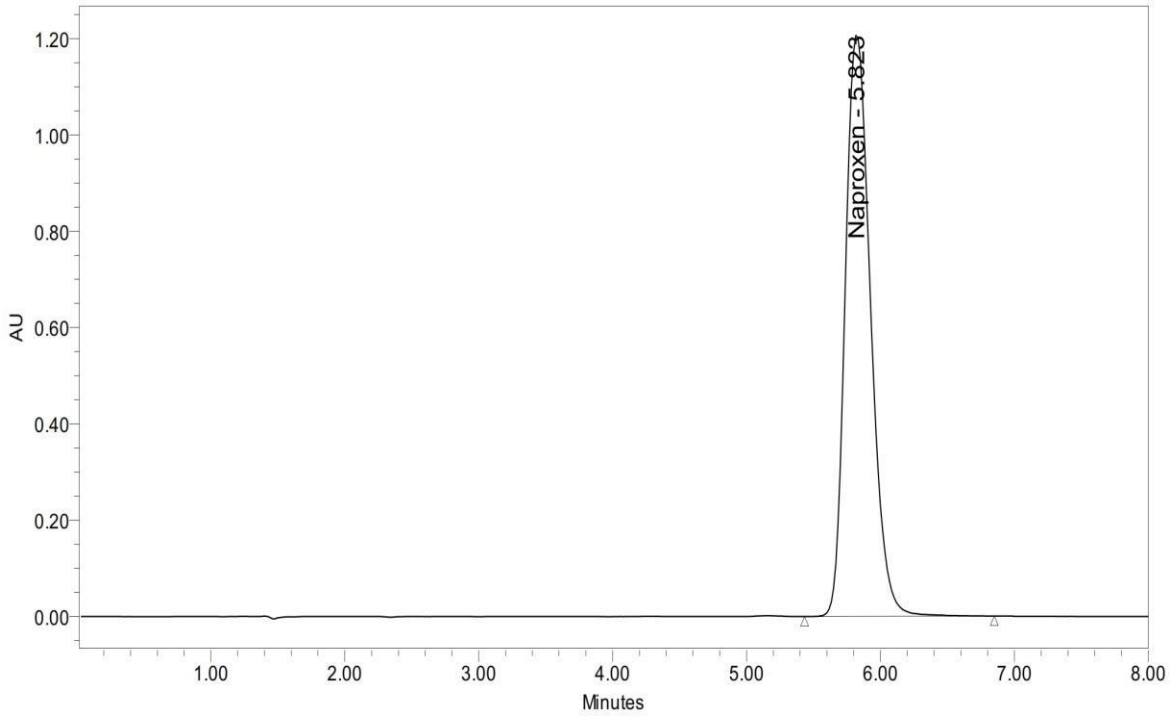


fig no: 2 chromatogram of formulation

RESULTS AND DISCUSSIONS

Naproxen is the drug mainly used to an anti-inflammatory agent with analgesic and antipyretic. A simple reverse phase HPLC method was developed for the determination of Naproxen. Column (250 x 4.6 mm, packed with 5µm) in an isocratic mode with mobile phase Phosphate Buffer: Methanol (40:60) was used. The flow rate was 1.3ml/ min and effluent was monitored at 230 nm. The column temperature was 25°C. The Retention time was found to be 5.82.

System Suitability Parameters

	Retention Times	Peak Area	Tailing Factor	Theoretical Plates
1	5.745	1625233	1.2	5770
2	5.759	1606708	1.2	5707
3	5.759	1588213	1.2	5288
Mean	5.754	1606718	-	-
SD	0.00808	18510	-	-
%RSD	0.14	1.152	-	-

table no: 1

Linearity Level (%)	Concentration (ppm)	Area
20	2	226723
50	5	686907
100	10	1591140
120	12	1890941
200	20	3239201

table no: 2

Optimized characteristics for linearity of Naproxen by RP-HPLC

Parameters	Observed values
Linearity concentration	2 - 20 ppm
Slope	16832
Intercept	-12257
Correlation coefficient	0.999

table no: 3

CONCLUSION

Naproxen is the drug used in the treatment of AIDS. It is a potential Xanthine oxidase inhibitor. From literature review and solubility analysis initial chromatographic conditions were set and different trials were run to Naproxen get eluted with good peak symmetric properties. Mobile phase Phosphate buffer: Methanol (40:60), Column, Symmetry C_{18}, 250×4.6mm, 5µm and flow rate 1.3 ml/min, detection wave length 230nm, column temperature 25^{o}c and diluent Methanol: Water (50:50) conditions were finalized as optimized method. System suitability parameters were studied by injecting the standard five times and results were well under the acceptance criteria. Linearity study was carried out between 2 to 20 ppm levels, R^2 value found 0.999. By using above method assay of marketed formulation was carried out, 99.7% was present. Full length method was not performed; if it is done this method can be used for routine analysis of Naproxen.

BIBLIOGRAPHY

[1].Ewing G. W., Instrumental Methods of Chemical Analysis, McGraw Hill Publishing Company Ins., 2nd Ed., 1960: 3.
[2].Lurie S. Ira and Wittwer, D. John Jr., HPLC in Forensic Chemistry, vii.
[3].Skoog D. A., West D. M. and Holler F. J., Fundamentals of Analytical Chemistry, Saunders College Publishing, New York, 6th Ed., 713
[4].Jeffery G. H., Bassett J., Medham J.and Denney R. C., Vogel's Textbook of Quantitative Chemical Analysis, English Language Book Society/ Longman, 5th Ed., 1989 :668.
[5].International Conference on Harmonization, Validation of Analytical Procedures: Methodology,Federal Register, Nov. 1996:1-8. [6]. International Conference on Harmonization, Draft Guideline on Validation of Analytical Procedures, Definitions and Terminology, Federal Register (26), 1995: 11260.
[7].United State Pharmacopoeia, Vol. I & II, Asian edition, United Pharmacopoeial Convention, Inc., Rockville, 2000: 2149.
[8].United State Pharmacopoeia, Vol. I & II, Asian edition, United Pharmacopoeial Convention, Inc., Rockville, 2000:1923.
[9]. Chatwal, G.R and anand, S.K., In; instrumental method of chemical analysis,5th Edn., 2005,2.107.
[10].Sharma, B.K., In; instrumentalmethod of chemical analysis,15th Edn., 1996,453.
[11]. Beckett, A.H., and stenlake, J.B., Inpractical pharmaceutical chemistry, 4th Edn, 2002,2,85.
[12]. Willard merit, H.H, Dean, Jr., and J.A., In; instrumental method of analysis, 6th Edn., 1986, 504.
[13]. Dr. Ravi Shankar. S., In; text book of pharmaceutical analysis, 3rd Edn., 1999,13-1.
[14].Remington the science and practice of pharmacy, 20th edition, 2000, 1, 587.
[15]. Jen martens – lobenhoffer, j., and bode-boger, S.M., J chromatogr B analyt technol biomed life sci, 2005, may 5, 819(1),197.
[16].Rao B.M., Ravi R., Shyamsundar reddy B., Sivakumar S., Gopichand, Praveen kumar, K., Acharyulu, P.V., Reddy, G.Om, and Srinivasu, M.K., J pharm Biomed Anal, 2004, Aug 18,745(2), 325.
[17]. Tiegong Guo, Lisa Oswald, M., Damodar Rao Mendu, and StevenSolidin, J., Clin Chim Acta.2007, Jan 375(1-2) 115.
[18]. Chidambaram Saravanan et al, Method Development and Validation for Determination of Naproxen

by UV Spectrophotometer, International Research Journal of Pharmacy, 1 (1), 2010, 314-323.
[19]. D. Ramakanth Reddy et al., Validated Spectrophotometric Method for Simultaneous estimation of Naproxen and Lamivudine in Combined Pharmaceutical dosage form International Journal of PharmTech Research, Vol.4, No.1, pp 311-314, Jan-Mar 2012.
[20]. B. Agaiah Goud et al., Quantitative Estimation of Naproxen by UV Spectrophotometry, International Journal Of Pharmacy & Technology Dec-2010 | Vol. 2 | Issue No.4 | 1328-1333.
[21]. C. H. Sharada et al., Development of a Spectrophotometric Method for the Quantitative Estimation of Naproxen Concentration in Bulk and Pharmaceutical Dosage Forms, KMITL Sci. Tech. J. Vol. 10 No. 1 Jan. - Jun. 2010.
[22]. J. Nijamdeen et al., Method development and validation of RP-HPLC method for simultaneous determination of Lamivudine and Naproxen J. Chem. Pharm. Res., 2010, 2(3):92-96.
[23]. D. Anantha Kumar et al., Simultaneous Determination of Lamivudine, Naproxen and Nevirapine in Tablet Dosage Forms by RP- HPLC Method, RASAYAN J. ChemVol.3, No.1 (2010), 94-99.
[24]. P. Venkatesh et al., Simultaneous estimation of Naproxen and Lamivudine tablets by RP-HPLC method, International Journal of Chem Tech Research, Vol. 3, No.1, pp 376-380, Jan-Mar 2011.
[25]. Maria Inês R. M. Santoro et al., Stability-Indicating Methods for Quantitative Determination of Naproxen and Stavudine in Capsule Quim. Nova, Vol. 29, No. 2, 240-244, 2006
[26]. K. Anand Babu et al., Analytical Method Development and Validation for Simultaneous Estimation of Naproxen, Lamivudine and Navirapine Tablets by RP-HPLC, International Journal of Pharmaceutical Research and Development, Vol 3 (7) Sep 2011, 9-14.
[27]. Nandini Pai, et al., Simultaneous reverse phase HPLC estimation of some antiretroviral drugs from tablets Indian Journal of Pharmaceutical Sciences. 2007, 69 (1). 118-120