Int J Pharm Bio Sci 2012 Oct; 3(4): (P) 847 - 854

Research Article **Analytical Chemistry**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

A NOVEL RP-HPLC METHOD FOR THE QUANTIFICATION OF NEPAFENAC IN FORMULATION, PLASMA(IN VITRO)

V. PHANI KUMAR AND Y. SUNANDAMMA*

Department of chemistry, Acharya Nagarjuna University, Guntur.

ABSTRACT

A simple, precise and accurate RP-HPLC method was developed and validated for rapid assay of Nepafenac in tablet dosage form. Isocratic elution at a flow rate of 1ml/min was employed on a symmetry Chromosil C18 (250x4.6mm, 5µm in particle size) at ambient temperature. The mobile phase consisted of Methanol: ACN: THF 50:40:10 (V/V). The UV detection wavelength was 238 nm and 20µl sample was injected. The retention time for Nepafenac was 3.26 min. The percentage RSD for precision and accuracy of the method was found to be less than 2%. The method was validated as per the ICH guidelines. The method was successfully applied for routine analysis of Nepafenac in tablet dosage form, bulk drug and plasma sample.

KEY WORDS: **Nepafenac, RP-HPLC, UV detection, recovery, precise, 238 nm, Plasma sample**

Y. SUNANDAMMA
Department of chemistry, Acharya Nagarjuna University, Guntur.

INTRODUCTION

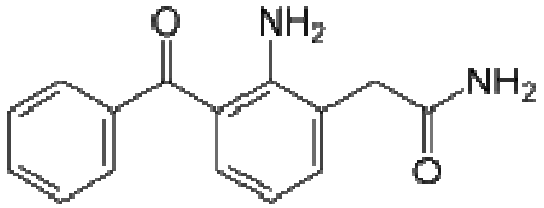


Figure.1

Nepafenac is a non-steroidal anti-inflammatory drug (NSAID). Nepafenac It is used to treat pain and inflammation associated with cataract surgery. Its side effects may include decreased visual acuity, a feeling that something is in the eye, increased eye pressure or a sticky sensation, as well as other effects. Dry eye; eye discomfort; eyelid crusting; headache; increased tear production; nausea; sensitivity to light; sinus inflammation; vomiting. Severe allergic reactions (rash; hives; difficulty breathing; tightness in the chest; swelling of the mouth, face, lips, or tongue); changes in vision; dizziness; eye itching, pain, swelling, or bleeding.

EXPERIMENTAL

Materials

Working standard of Nepafenac was obtained from well reputed research laboratories. HPLC grade water, methanol, Acetonitrile ,Tetrahydrofuran was purchased from E. Merck (Mumbai, India).

Apparatus

A Series HPLC system PEAK LC7000 isocratic HPLC with PEAK 7000 delivery system. Rheodyne manual sample injector with switch (77251),Analytical column Chromosil C18. 250×4.6mm, Electronic balance-DENVER (SI234), A manual Rheodyne injector with a 20 µl loop was used for the injection of sample., PEAK LC software were used. UV 2301 SPECOPHOTOMETER was used to determine the wavelength of maximum absorbance

Determination of wavelength of maximum absorbance

The standard solutions of Nepafenac were scanned in the range of 200 -400 nm against mobile phase as a blank. Nepafenac showed maximum absorbance at 238 nm. So the wavelength selected for the determination of Nepafenac was 238 nm.

Chromatographic equipment and conditions

The development and validation of the assay was performed on A Series 200 HPLC system PEAK LC7000 isocratic HPLC with PEAK 7000 delivery system. Rheodyne manual sample injector with switch (77251), Analytical column Chromosil 100-5 C18. 250×4.6mm, , manual injector rheodyne valve) with 20µL fixed loop, PEAK LC software were used.

The mobile phase consist of Methanol, Acetonitrile Tetrahydrofuran 50:40:10 (v/v). Injections were carried out using a 20 µl loop at room temperature (20 + 2 °C) and the flow rate was 1 ml/min. Detection was performed at 238 nm with 6 min runtime.

Standard and sample solutions

A 10 mg amount of Nepafenac reference substance was accurately weighed dissolved in10ml mobile phase in a 10 ml volumetric flask to obtain 1000 ppm concentrated solution. From standard solution by the serial dilution we prepared required concentrations of 100ppm.3ml of

above sample was taken and diluted to 10ml using mobile phase.
A composite of 20 tablets was prepared by grinding them to a fine, uniform size powder. 10 mg of Nepafenac was accurately weighed

and quantitatively transferred into a 100 ml volumetric flask. Approximately 26 ml mobile phase were added and the solution was sonicated for 15 min. The flask was filled to volume with mobile phase, and mixed. After filtration, an amount of the solution was diluted with mobile phase to a concentration of 100 µg/ml.

Method validation
Method validation was performed following ICH specifications for specificity, range of linearity, accuracy, precision and robustness

RESULTS AND DISCUSSION

System Suitability
Having optimized the efficiency of a chromatographic separation the quality of the chromatography was monitored by applying the following system suitability tests: capacity factor, tailing factor and theoretical plates. The system suitability method acceptance criteria set in each validation run were: capacity factor >2.0, tailing factor ≤2.0 and theoretical plates >2000 13. In all cases, the relative standard deviation (R.S.D) for the analytic peak area for two consecutive injections was < 2.0%. A chromatogram obtained from reference substance solution is presented. System suitability parameters were shown in Table.1. Standard chromatogram was given in Figure.2

Table.1
System suitability parameters

Mobile phase	MethanolAcetonitrile:Tetrahydrofuran 50:40:10 (v/v)
Pump mode	Isocratic
pH	5.8
Diluents	Mobile phase
Column	Zodiac C18 column (250 X 4.6 mm, 5µ)
Column Temp	Ambient
Wavelength	238nm
Injection Volume	20 µl
Flow rate	1 ml/min
Run time	6 minutes
Retention Time	3.26 minutes
Area	227411
T.Plates	9675
Tailing Factor	1.21
Pump Pressure	11.5MPa

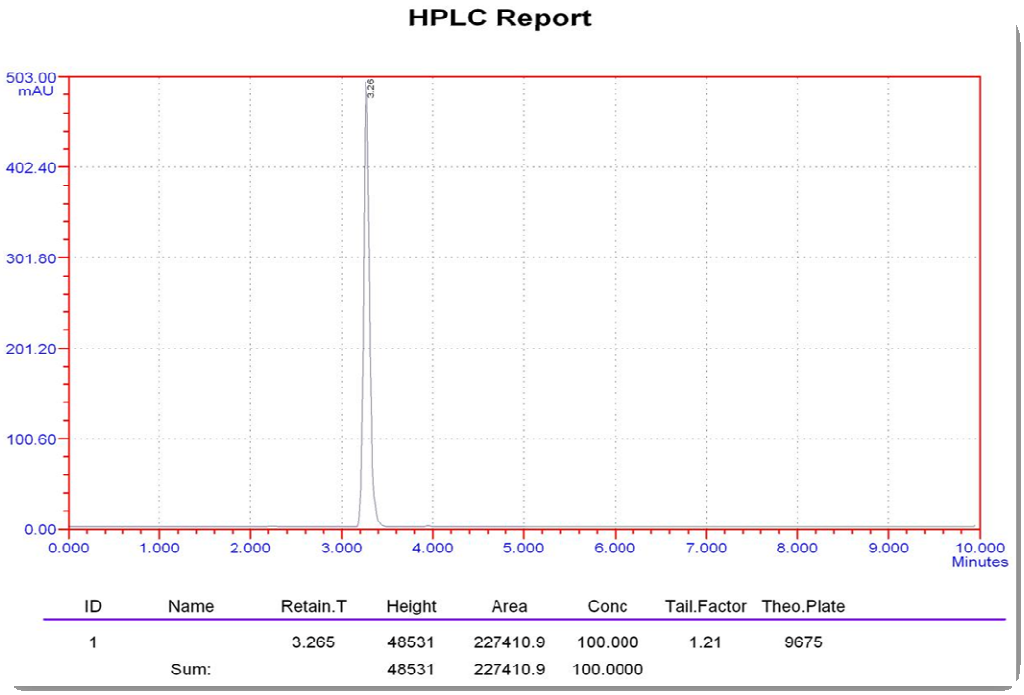


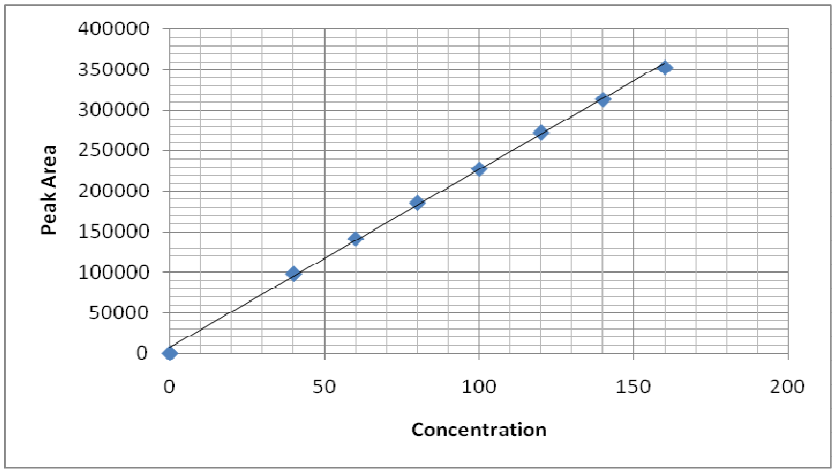
Figure.2
Standard Chromatogram

Range of linearity

Standard curves were constructed using seven standard concentrations in a range of 40, 60 , 80, 100, 120, 140, 160µg/ml for Nepafenac. The linearity of peak area responses versus concentrations was demonstrated by linear least square regression analysis. The linear regression equation was y = 6868 + 2194.114x (r= 0.9994). Linearity values can shown in Table: 2

Table.2
Linearity results.

Level	Concentration of Nepafenac In ppm	peak area
Level - 1	40	98038
Level - 2	60	141362
Level - 3	80	185649
Level - 4	100	227411
Level - 5	120	272510
Level – 6	140	313221
Level - 7	160	352633
Range:40-120ppm	Slope Intercept Correlation coefficient	2194.114 6868 0.9994



Graph.1
Calibration curve

Precision

To study precision, six replicate standard solutions of Nepafenac (100 ppm) were prepared and analyzed using the proposed method. The percent relative standard deviation (% RSD) for peak responses was calculated and it was found to be 0.63 for intraday and 0.34 for interday precision, which is well within the acceptance criteria of not more than 2.0%. Results of system precision studies are shown in Table.3

Precision Results for Nepafenac

Table.3
Precision results

Sample	Conc. (in ppm)	Injection No.	Peak Areas	INTRADAY RSD (Acceptance criteria ≤ 2.0%)
Nepafenac	100	1	227411	**0.63**
		2	228179	
		3	230175	
		4	229956	
		5	230048	
		6	231355	

Sample	Conc. (in ppm)	Injection No.	Peak Areas	INTERDAY RSD (Acceptance criteria ≤ 2.0%)
Nepafenac	100	1	238772	0.34
		2	238410	
		3	236822	
		4	238521	
		5	239137	
		6	238533	

Limit of Detection and Limit of Quantification

To determine the Limit of Detection (LOD) sample was dissolved by using Mobile phase and injected until peak was disappeared. After 0.03 ppm dilution Peak was not clearly observed, based on which 0.03 ppm is considered as Limit of Detection and Limit of Quantification is 0.1 ppm.

Table.4

Parameter	Measured Value
Limit of Quantification	0.1ppm
Limit of Detection	0.03 ppm

Robustness

Typical variations in liquid chromatography conditions were used to evaluate the robustness of the assay method. In this study, the chromatographic parameters monitored were retention time, area, capacity factor, tailing factor and theoretical plates. The robustness acceptance criteria set in the validation were the same established on system suitability test describe above.

Table.5
Robustness results

S.NO	PARAMETER	CONDITION	AREA	% OF CHANGE
1	Standard	Standard conditions	227411	……
2	Mobile phase	Methanol 40%,ACN 50%,THF 10%.	228670	0.55
		Methanol60%,ACN 30%,THF 10%.	223653	1.65
3	Mobile phase pH	5.6	223494	1.72
		6.0	223176	1.86
4	Wavelength	234 nm	223885	1.55
		242	224290	1.37

Recovery

Recover test was performed at 3 different concentrations i.e. 30ppm,60ppm,120ppm. Results are given in table.6

Table.6
Recovery results

	Nepafenac				
% of Recovery	Target Conc., (ppm)	Spiked conc, (ppm)	Final Conc, (ppm)	Conc., Obtained	% of Assay
50%	40	20	60	59.31	98.85
	40	20	60	60.25	100.42
	40	20	60	15.03	59.83
99.72	40	40	80	79.59	99.48
	40	40	80	79.48	99.35
	40	40	80	79.64	99.55
150%	40	60	100	98.89	98.89
	40	60	100	100.57	100.57
	40	60	100	101.51	101.51

FORMULATION ANALYSIS

Table.7
Formulation results.

S.NO	Formulation	Dosage	Sample conc	Drug estimated	% of Drug Estimated in Tablet
1	NEVANAC	1 mg	100 ppm	98.77	98.77

Quantification of Fulvestrant in Plasma

Blood sample was procured from Local blood bank and prepared plasma by using the method PROIMMUNE protocols[6]. 100 ppm sample solution was prepared with plasma, and injected in to HPLC at our developed conditions. Chromatogram was recorded.

Table.8
Plasma sample analysis result

S.NO	Sample	Sample concentration	Sample estimated	% of Drug Estimated in Tablet
1	Plasma	100 ppm	94.52 ppm	94.52 %

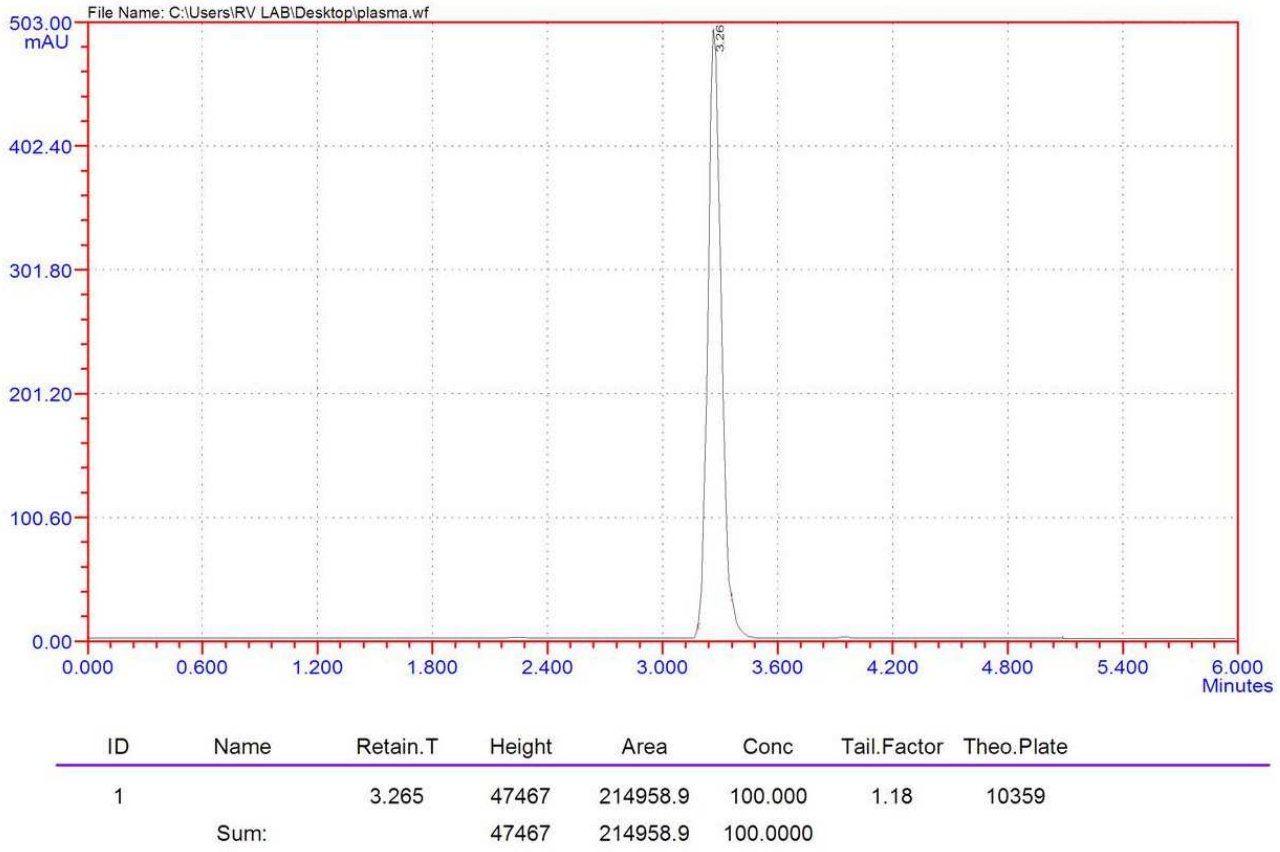


Figure.3
Plasma sample chromatogram

CONCLUSION

The proposed method for the assay of Nepafenac in tablets or capsules is very simple and rapid. The method was validated for specificity, linearity, precision, accuracy and robustness. Although the method could effectively separate the drug from its products, further studies should be performed in order to use it to evaluate the stability of pharmaceutical formulations.

REFERENCES

1. Nepafenac, a Unique Nonsteroidal Prodrug with Potential Utility in the Treatment of Trauma-Induced Ocular Inflammation: I. Assessment of Anti-Inflammatory Efficacy, Daniel A. Gamache, Gustav Graff, Milton T. Brady, Joan M. Spellman and John M. Yanni, INFLAMMATION, Volume 24, Number 4 (2000), 357-370.
2. Inflammation-Mediated Retinal Edema in the Rabbit Is Inhibited by Topical Nepafenac, M. A. Kapin, J. M. Yanni, M. T. Brady, T. J. McDonough, J. G. Flanagan, M. H. Rawji, D. C. Dahlin, M. E. Sanders and D. A. Gamache, INFLAMMATION, Volume 27, Number 5 (2003), 281-291.
3. Comparative Effects of the Nonsteroidal Anti-inflammatory Drug Nepafenac on Corneal Sensory Nerve Fibers Responding to Chemical IrritationM. Carmen Acosta , Carolina Luna , Gustav Graff ,Victor M. Meseguer , Felix Viana , Juana Gallar and Carlos Belmonte, Invest. Ophthalmol. Vis. Sci.January 2007 vol. 48 no. 1182-188
4. Topical Administration of Nepafenac Inhibits Diabetes-Induced Retinal Microvascular Disease and Underlying Abnormalities of Retinal Metabolism and Physiology, Timothy S. Kern1, Casey M. Miller1Yunpeng DuLing ZhengSusanne MohrSherry L. Ball,M. Kim, Jeffrey A. Jamison andDavid P. Bingaman, Diabetes February 2007 vol. 56 no. 2 373-379
5. Prostaglandin E_2 Inhibition and Aqueous Concentration of Ketorolac 0.4% (Acular LS) and Nepafenac 0.1% (Nevanac) in Patients Undergoing Phacoemulsification, Frank A. Bucci et al, American Journal of Ophthalmology,Volume 144, Issue 1 , Pages 146-147, July 2007 http://www.proimmune.com/ecommerce/pdf_files/PR31.pdf
6. Protocols for the preparation of blood plasma and serum, PROIMMUNE, September, 2009.