International Journal of ChemTech Research
CODEN(USA): IJCRGG ISSN : 0974-4290
Vol.2, No.4, pp 2034-2042, Oct-Dec 2010

Development of validated stability indicating RP-HPLC method for the estimation of Niflumic acid

Nalini Kanta Sahoo[1,*], Subrata Sen[2], P. Srinivas Rao[1], S.K. Moitra[3], T.K. Laha[2], Siba Prasad pradhan[1], Madhusmita Sahu[4]

[1]Yalamarty Pharmacy College, Tarluwada, Visakhapatnam, A.P, 530052,India.
[2]College of Pharmaceutical Sciences, Mohuda, Berhampur, Orissa, 760002,India.
[3]School of Pharmaceutical Sciences, SOA University, Kalinganagar, BBSR, Orissa, 751003,India.
[4]Roland Institute of Pharmaceutical Sciences, Ambapua, Berhampur, Orissa, 760010,India.
*Corres.author: sahoo.nalini@gmail.com
Ph.No: 09550741536, 09396772373, 09090355293

Abstract: The present paper deals with the development of a stability indicating reverse phase HPLC with UV-Visible detector method for the determination of Niflumic acid using phenomenx RP-C18 (250x4.6mm, packed with Luna 5 µ) column. A mobile phase consisting of methanol: water (75:25%v/v) was employed in this study. The flow rate was kept at 1.0 ml/min and the injection volume was 20 µl. The separation was performed at ambient temperature. Eluents were monitored by UV detector set at 254 nm. The developed method was statistically validated for the linearity, precision, accuracy, robustness, specificity, LOD and LOQ. The specificity of the method was ascertained by force degradation studies by acid hydrolysis, alkali hydrolysis & degradation by oxidation. The degraded products were well resolved from the analyte peak with significant difference in their RT values.
Keywords: RP-HPLC, Niflumic acid, Forced degradation.

Introduction

Stability of pharmaceutical product may be defined as, the capacity of a particular formulation in a specific container or closure system, to remain within its physical, chemical, microbiological, therapeutic and toxicological specifications. Stability of a drug can also be defined as, the time from the date of manufacture and packing of the formulation until its chemical and biological activity is not less than a predetermined label of potency and its physical characteristics have not changed appreciably or deleteriously[1].

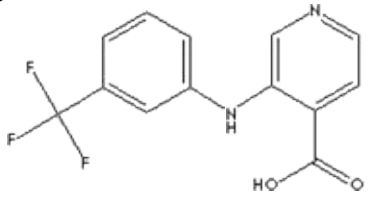

Fig 1: Niflumic acid

The ICH guideline requires that stress testing be carried out to elucidate the inherent stability characteristics of the active substances[2]. It is stated that testing should include the effect of temperature, humidity (where appropriate); oxidation, photolysis and susceptibility to hydrolysis across a wide range of pH values. An ideal stability-indicating method is one that quantifies the drug per se and also resolves its degradation products[3].

Niflumic acid [2-((3-(trifluoromethyl) phenyl) amino)-3-pyridinecarboxylic acid; 2-(3-(Trifluoromethyl) anilino) Nicotinic acid] (Fig 1) is a drug belongs to a class of NSAIDs (Nonsteroidal anti-inflammatory drugs), acts by inhibiting isoforms of cyclo-oxygenase 1 and 2. It is used to treat inflammatory rheumatoid diseases and in acute pain. It is effective against period pains, pain after surgery, and fever[4].

The present work was designed to develop a simple, precise and rapid analytical LC procedure, which would serve as stability indicting assay method for analysis of Niflumic acid API. Literature survey for Niflumic acid analysis revealed several methods based on different techniques like HPLC and UV for the quantification of Niflumic acid in human plasma and urine but no such stability indicating method was found [7-10].

Materials and methods

HPLC instrumentation & conditions

Quantitative HPLC was performed on a binary gradient HPLC with Shimadzu LC-10AT and LC-10AT VP series HPLC pumps, with a 20µl sample injection loop (manual) ,SCL-10A VP system controller,SPD-10A VP UV-Visible absorbance detector and ELSD(Evaporation Light scattering Detector) were used. The out put signal was monitored and integrated using Shimadzu CLASS-VP version 6.12 SP1 software. Phenomenex RP-C_{18} (250 x 4.6 mm, packed with Luna 5 micron) column was used for the separation.

Chemicals and reagents

Niflumic acid (API) was provided by Glenmark Pharmaceutical Ltd., Mumbai, India. Methanol, acetonitrile & water of HPLC grade were purchased from Rankem (Ranbaxy Fine Chemicals Ltd.), Delhi. Hydrogen peroxide I.P (3%) Solution and commercial formulation of Niflumic acid [NIFLURIL, Glenmark Pharmaceutical Ltd.] was purchased from local medicine store.

Standard & sample preparation

The standard & sample stock solutions were prepared separately by dissolving standard & sample in methanol & diluting with the same solvent.

Forced degradation studies

The drug was subjected to stress conditions in various ways to observe the rate and extent of degradation that is likely to occur in the course of storage and/or after administration to body. The various degradation pathways studied are acid hydrolysis, basic hydrolysis and oxidative degradation.

a. Acid Hydrolysis

An accurately weighed 10 mg of pure drug was transferred to a clean & dry round bottom flask. 30 ml of 0.1 N HCl was added to it & it was refluxed in a water bath at 60^0C for 4 hours. After reflux drug was solubilized using methanol and allowed to cool to room temperature. The sample was than neutralized using 2N NaOH solution & final volume of the sample was made up to 100ml with water to prepare 100ppm solution. Finally it was injected into the HPLC system against a blank of methanol: water (75: 25 %v/v).

b. Basic Hydrolysis

An accurately weighed 10 mg. of pure drug was transferred to a clean & dry round bottom flask. 30 ml of 0.1N NaOH was added to it & it was refluxed in a water bath at 60^0C for 4 hours and allowed to cool to room temperature. The sample was than neutralized using 2N HCl solution & final volume of the sample was made up to 100ml with water to prepare 100ppm solution. Finally it was injected into the HPLC system as above.

c. Oxidation with (3%) H_2O_2

Accurately weighed 10 mg. of pure drug was taken in a clean & dry 100 ml. volumetric flask. 30 ml. of 3% H_2O_2 and a little methanol was added to it to make it soluble & then kept as such in dark for 24 hours. Final volume was made up to 100 ml. using water to prepare 100 ppm solution. The above sample was injected into the HPLC system as above.

Method validation

i) Accuracy

Accuracy was best determined by the standard addition method. Previously analyzed samples of Niflumic acid API were added with standard drug solutions and are analyzed by the proposed method. Recovery (%), RSD (%) and bias (%) were calculated for each concentration.

Accuracy is reported as percentage bias, which is calculated from the expression

$$\%Bias = \frac{(\text{measured value} - \text{true value})}{\text{true value}} \times 100$$

ii) Precision

Precision was determined as both repeatability and intermediate precision, in accordance with ICH guidelines. Repeatability of

sample injection was determined as intra day variation and intermediate variation was determined by measurement of inter day variation. For these determinations, three concentrations of the solutions of Niflumic acid API were used (20, 300 and 500 µg/ml).

iii) Robustness

The concept of robustness of an analytical procedure has been defined by the ICH as "a measure of its capacity to remain unaffected by small but deliberate variations in method parameters". The robustness of a method is the ability to remain unaffected by small changes in parameters such as pH of the mobile phase, temperature, % organic solvent strength and buffer concentration etc. To determine the robustness of the method experimental conditions are purposely altered and chromatographic characters are evaluated. Influence of small changes in chromatographic conditions such as change in flow rate (± 0.1ml/min), temperature (±2^{0}C), wavelength of detection (±2nm) and water content in mobile phase (±2%) were studied to determine the robustness of the method

iv) Limit of detection (LOD)

The limit of detection (LOD) of an analytical method may be defined as the concentration, which gives rise to an instrument signal that is significantly different from the blank. For spectroscopic techniques or other methods that rely upon a calibration curve for quantitative measurements, the IUPAC approach employs the standard deviation of the intercept (Sa), which may be related to LOD and the slope of the calibration curve, b, by

LOD = 3 Sa / b

v) Limit of quantitation (LOQ)

The LOQ is the concentration that can be quantitated reliably with a specified level of accuracy and precision. The LOQ represent the concentration of analyte that would yield a signal-to-noise ratio of 10.

LOQ = 10 Sa / b

Where, Sa is the standard deviation of the peak area ratio of analyte to IS (5 injections) of the drugs and b is slope of the corresponding calibration curve.

vi) Specificity

The specificity of the method was determined by exposing the drug sample to acidic (0.1 N HCl), basic (0.1N NaOH) and oxidizing (3% H_2O_2) stress conditions. The resulting solutions were then analyzed and the analyte peak was evaluated both for peak purity and for resolution from the nearest eluting peak.

vii) Stability

Stability of Niflumic acid API was determined after storage of the drug solution for 24 hrs at room temperature (25± 2^{0}C).

Table 1: Summary of process optimization

Column Used	Mobile Phase	Flow Rate	Wave length	Observation	Result
Microbondapak C_{18}, 5µm, 50x4.6mm i.d.	Methanol only	0.5 ml/min	288 nm	Low resolution	Method rejected
Phenomenex RP-C_{18}, Luna5µm, 250 x 4.6mm i.d.	Methanol: water =9 : 1	0.8ml/min	289 nm	Resolution but not satisfied	Method rejected
Microbondapak C_{18}, 5µm, 50x4.6mm i.d.	Methanol : phosphate buffer(pH7.8)= 9:1	1ml/min	287 nm	Poor resolution	Method rejected
Phenomenex RP-C_{18}, Luna5µm, 250 x 4.6mm i.d.	Methanol:Acetronitrile= 9:1	1ml/min	288 nm	Poor resolution	Method rejected
Phenomenex RP-C_{18}, Luna5µm, 250 x 4.6mm i.d.	Methanol: water= 75:25	1ml/min	254nm	Good resolution	Method accepted

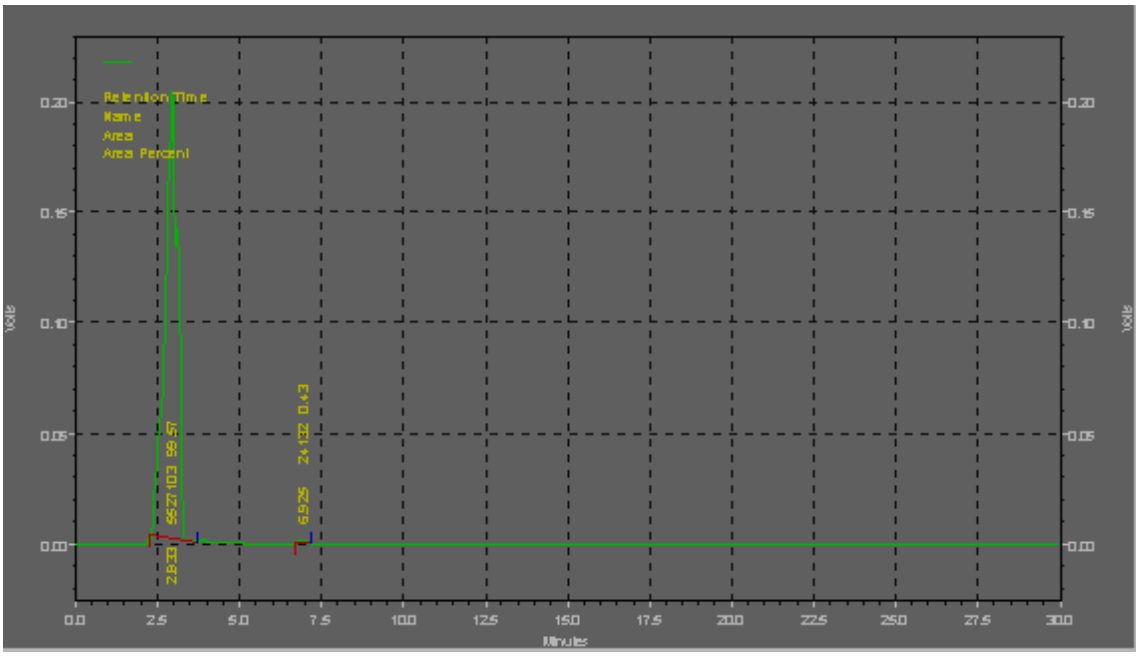

Fig 2: HPLC spectrum of Niflumic acid (100 ppm, R_t 2.833min)

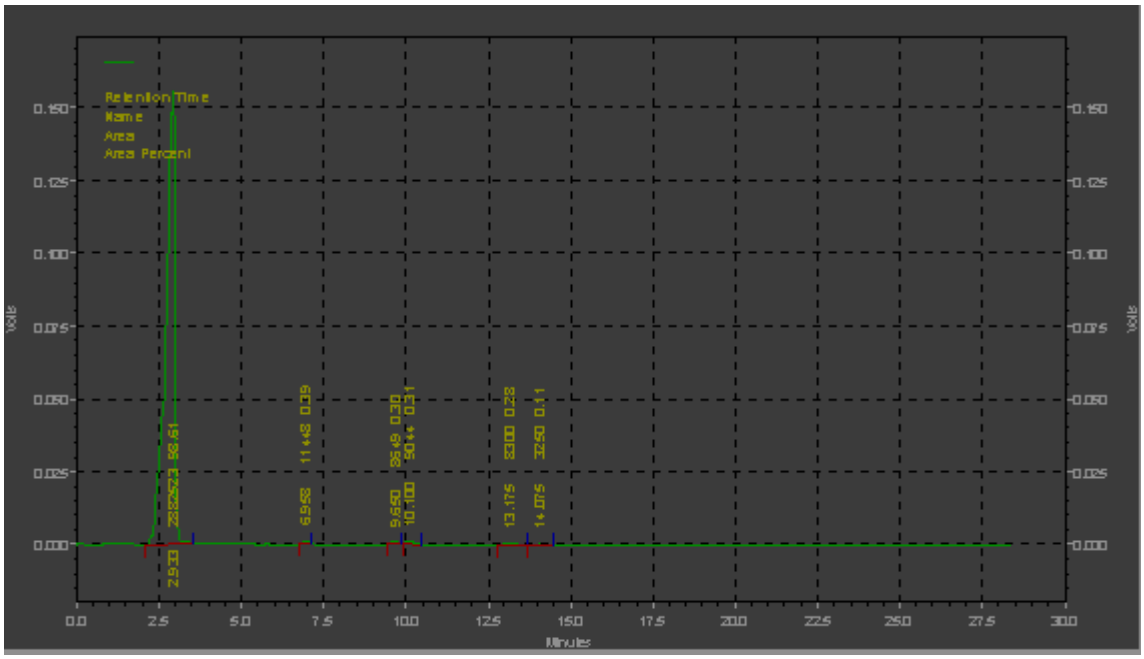

Fig 3: Chromatogram showing the acid degraded products

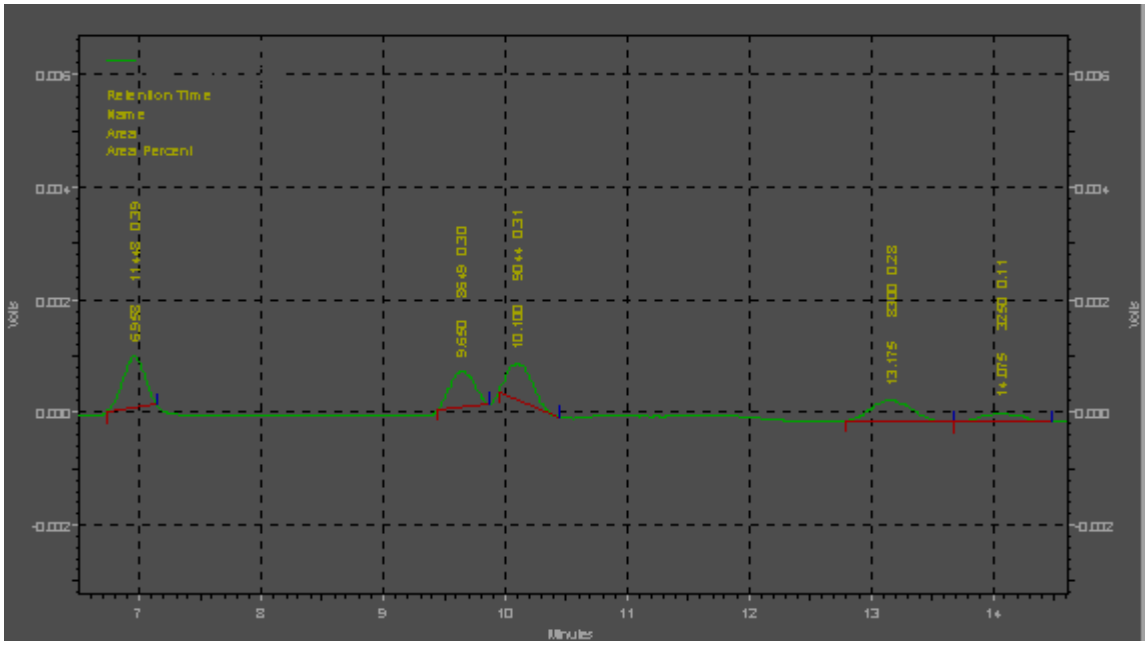

Fig 4: Chromatogram (enlarge view) showing the acid degraded products

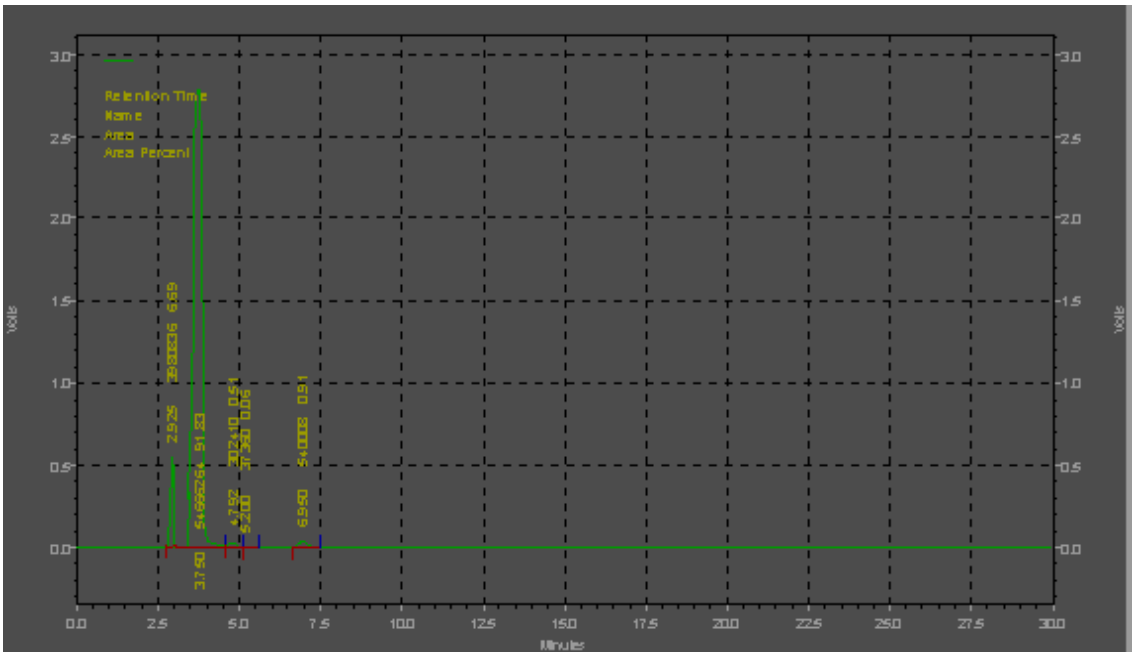

Fig 5: Chromatogram showing the base degraded products

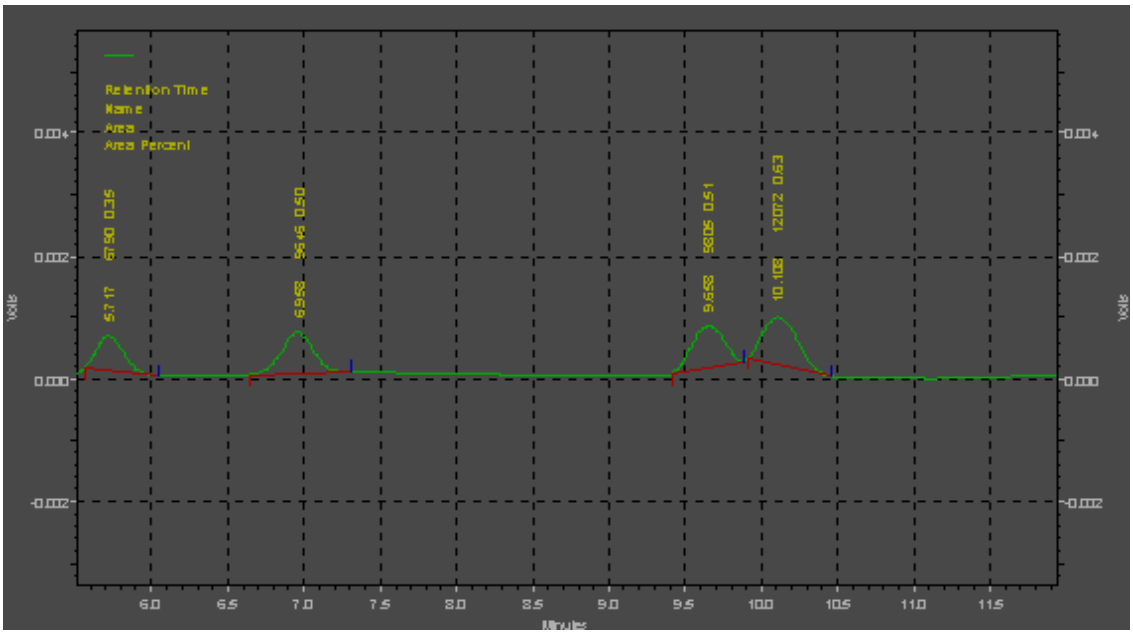

Fig 6: Chromatogram (enlarge view) showing the base degraded products

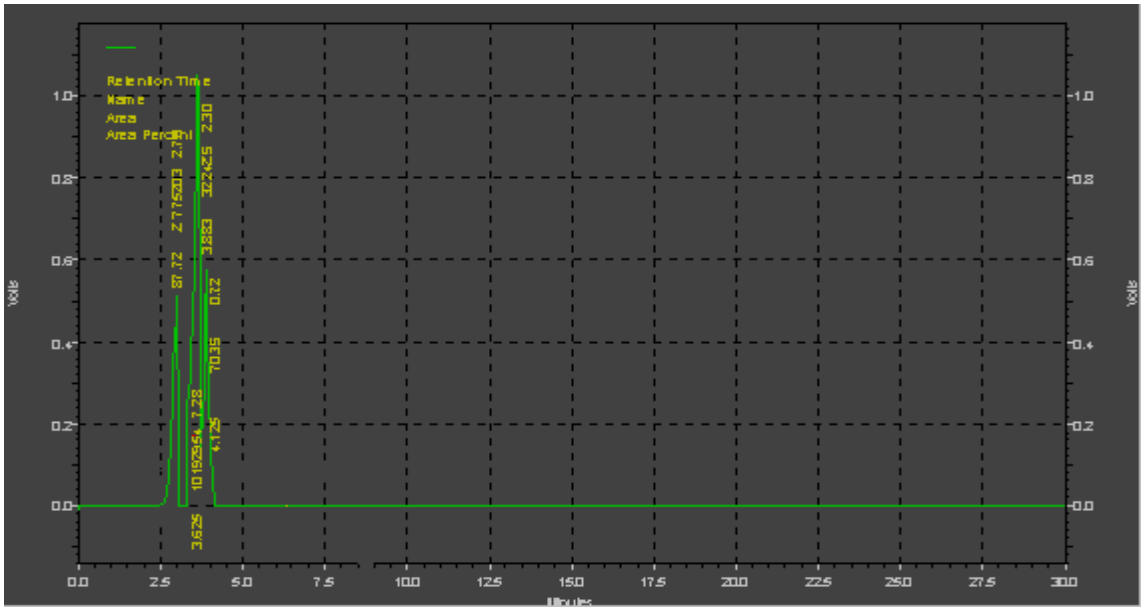

Fig 7: Chromatogram showing Niflumic acid, degraded product and H_2O_2

Table 2: Peak results for forced degradation studies

Stress conditions	Niflumic acid API		Degraded Product 1		Degraded Product 2		Degraded Product 3		Degraded Product 4		Degraded Product 5	
	Rt	Area	Rt	Area	Rt	Area	Rt	Area	Rt	Area	Rt	Area
0.1 N HCl	2.833	3138825	6.958	11448	9.650	8649	10.1	9044	13.175	8300	14.075	3250
0.1N NaOH	2.9	1531259	3.75	3117929	5.717	6790	6.958	9646	9.658	9805	10.108	12072
3% H_2O_2	2.9	3775203	3.625	10192954	3.883	32242587	4.125	7035219	____	____	____	____

Table 3: Results of force degradation studies of Niflumic acid API.

Stress condition	Time	Assay of active substance	Assay of degraded products	Mass Balance (%)
Acid Hydrolysis (0.1N HCl)	4 Hrs.	98.62	1.27	99.89
Basic Hydrolysis (0.1N NaOH)	4 Hrs.	97.97	1.19	99.16
Oxidation(3% H_2O_2)	24Hrs.	87.20	10.35	97.55

Table 4: Results of recovery study

Amount of drug added (µg) to analyte	Recovery from Formulation			
	Mean Amount (µg) found (n=6)	Mean % Recovery	% RSD	% Bias
160	159.35	99.37	0.31	-0.406
200	198.34	99.17	0.11	-0.83
240	239.03	99.59	0.21	-0.404

Table 5: Results of intra-assay & inter-assay

Conc. Of Niflumic acid (µg/ml)	Observed Conc. Of Niflumic acid (µg/ml) by the proposed method.			
	Intra-Day		Inter-Day	
	Mean (n=6)	% RSD	Mean (n=6)	% RSD
20	20.17	0.17	20.05	0.32
300	298.12	0.19	297.32	0.15
500	499.05	0.23	498.90	0.45

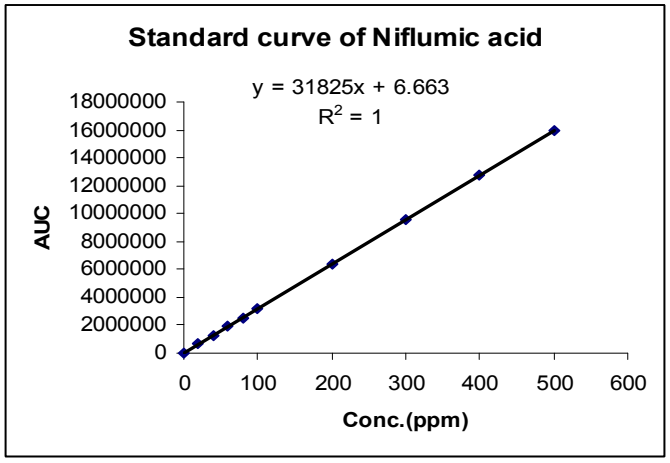

Fig 8: Calibration curve of Niflumic acid API.

Table 6: Result of robustness test

Change in parameter	% RSD(n=6)
Flow (0.9 ml/min)	0.14
Flow (1.1 ml/min)	0.02
Temperature (27^0C)	0.09
Temperature (23^0C)	0.13
Wavelength of Detection (256nm)	0.09
Wavelength of detection (252nm)	0.61

Table 7: Assay of Niflumic acid Ointment

Brand name of Ointment	Amount of Drug consider (mg)	Mean (±SD) amount (mg) found by the proposed method (n=6)	% Mean Assay
Nifluril (3%)	100	99.40 (±0.8)	99.40

Results and Discussion

Optimization of chromatographic conditions

The chromatographic conditions were optimized by different means i.e. using different column, different mobile phase, different flow rate, different detection wavelength and different diluents for sample preparation etc. and are summarized in Table 1.

Forced degradation studies

The results of the forced degradation studies were given in Table 2 and Fig 3-7. In all the three stress conditions, there is significant change in peak area but not in retention time of Niflumic acid API. Well separation of the degraded product from the parent peak shows the method is stability indicating. Fig 2 shows the chromatogram of pure Niflumic acid API.

Method validation:

Accuracy: Recovery study

The recovery of the method, determined by adding a previously analyzed test solution with additional drug standard solution, was 99.17- 99.59%. The values of recovery (%), RSD (%) and % Bias listed in Table 4 indicate the method is accurate.

Precision: Intra-assay & inter-assay

The intra & inter day variation of the method was carried out and the high values of mean assay and low values of standard deviation and % RSD (% RSD < 2%) within a day and day to day variations for Niflumic acid revealed that the proposed method is precise (Table 5).

Linearity & Range

The calibration curve showed good linearity in the range of 20-500 □g/ml, for Niflumic acid API with correlation coefficient (r^2) of 1.00 (Fig 8). A typical calibration curve has the regression equation of y = 31825x + 6.663 for Niflumic acid.

Robustness

Influence of small changes in chromatographic conditions such as change in flow rate (± 0.1ml/min), Temperature (±2^0C), Wavelength of detection (±2nm) & water content in mobile phase (±2%) studied to determine the robustness of the method are also in

favor of (Table 6, % RSD < 2%) the developed RP-HPLC method for the analysis of Niflumic acid API.

LOD & LOQ

The Minimum concentration level at which the analyte can be reliable detected (LOD) & quantified (LOQ) were found to be 1.13 & 3.46 □g/ml respectively.

Specificity & stability in analytical solution

The United Sates Pharmacopoeia (USP) and International Conference on Harmonization (ICH) guidelines define specificity as the ability of a method to assess unequivocally the analyte of interest in the presence of potential interferences[5-6]. The results of specificity indicated that the peak was pure in presence of degraded sample. It is important to mention here that the Niflumic acid API was stable in solution form up to 24 hrs at 25^0C.

The results of linearity, precision, inter & intra day assays, robustness, LOD, LOQ, specificity and stability in analytical solution established the validation of the developed RP-HPLC method for analysis of Niflumic acid.

Assay of Niflumic acid in dosage form

Assay was performed by using the regression equation ($y=31825x+6.663$, $R^2=1$) obtained from the standard curve of Niflumic acid API. Results obtained are given in table 7. The assay of Nifluril ointment containing Niflumic acid was found to be 99.40 % as per the method.

Conclusion

A sensitive & selective stability indicting RP-HPLC method has been developed & validated for the analysis of Niflumic acid. Based on peak purity results, obtained from the analysis of force degradation samples using described method, it can be concluded that the absence of co-eluting peak along with the main peak of Niflumic acid indicated that the developed method is specific for the estimation of Niflumic acid in presence of degradation products. Further the proposed RP-HPLC method has excellent sensitivity, precision and reproducibility.

Even though no attempt has been made to identify the degraded products, proposed method can be used as a stability indicating method for assay of Niflumic acid in commercial formulations.

Acknowledgements

The authors would like to acknowledge the contributions of Institute of Minerals & Materials Technology (IMMT), BBSR, Orissa & College of Pharmaceutical Sciences, Berhampur, Orissa for providing necessary facilities to carry out the research work.

References

1. Singh, S., and Bakshi, M., Development of validated stability-indicating assay method-Critical review, J. Pharmaceutical and Biomedical Analysis, 28, 2002; 1011-1040.
2. ICH, Q1A Stability Testing of New Drug Substances and Products, Int Conf on Harmonization. Geneva, October 1993.
3. Thomas, S., Kumar, R., Sharma, A., Issarani, R., and Nagori, B.P., Stability-indicating HPLC method for determination of vitamins B_1, B_2, B_3 and B_6 in pharmaceutical liquid dosage form, *Indian Journal of Chemical Technology,* 15, 2008; 598-603.
4. http://en.wikipedia.org
5. The United State Pharmacopeia 25/National Formulary 20, ch. 1225, pg. 2256-2259 (The United State Pharmacopeial Convention, Inc., Rockville, Maryland, 2002)
6. ICH, Q2B Validation of Analytical Procedure; Methodology (International Conferences on Harmonization of Technical requirements for the registration of Drugs for Human use, Geneva, Switzerland, May 1997)
7. Avgerinos, A., and Malamataris, S., High-performance liquid chromatographic determination of Niflumic acid in human plasma and urine, *J Chromatogr.B,* 1990, 533; 271-274.
8. Guechot, C., and Nicolle, P., Purity assay of Niflumic acid by reversed-phase high-performance liquid chromatography, *J Chromatogr. B,* 1984, 303; 440-443.
9. P., Gallo, Serena Fabbrocino, Floriana Vinci, Maurizio Fiori, Vincenzo Danese, Antonella Nasi, and Luigi Serpe, Multi-Residue Determination of Non-Steroidal Anti-Inflammatory Drug Residues in Animal Serum and Plasma by HPLC and Photo-Diode Array Detection, *Journal of Chromatographic Science,* 44, 2006; 585- 590.
10. H.W. Lee., K.J. Won., S.H. Cho., Y.H. Ha., W.S. Park., H.T. Yim., M. Baek., J.H. Rew., S.H. Yoon., S.V. Yim., J.H. Chung., and K.T. Lee., Quantitation of niflumic acid in human plasma by high-performance liquid chromatography with ultraviolet absorbance detection and its application to a bioequivalence study of talniflumate tablets, *Journal of Chromatography,* 821, 2005; 215-220.

*****