International Research Journal of Pharmaceutical and Applied Sciences
Available online at **www.irjpas.com**
Int. Res J Pharm. App Sci., 2012; 2(2):92-98

IRJPAS **ISSN-2277-4149**

Research Article

DEVELOPMENT AND VALIDATION OF NABUMETONE BY ISOCRATIC RP- HPLC METHOD

Lakshmi Prasanna. B[1*], Mahesh.M[1], Deepthi Jasti[2],
[*1]Department of Pharmaceutical Analysis, JNTUOTRI Campus, JNTU, Anantapur, Andhra Pradesh, India
[2]Veerayatan Institute of Pharmacy, Jakhaniya, Bhuj-Mandvi Road Kutch, Gujarat, India
(Received: 7 March, 2012; Accepted: 22 March, 2012; Published: 29 April, 2012)
**Corresponding author's email:* meghavath9@gmail.com

ABSTRACT
Nabumetone is a Non –Steroidal Anti-inflammatory drug which has good analgesic and anti-rheumatic properties. A new , simple and precise RP-HPLC method was developed and validated for the estimation of Nabumetone in bulk and tablet dosage forms using a hypersil C_{18},4.6mm×150mm,5µmcolumn from waters with a mobile phase consisting of methanol: phosphate buffer(65:35)at a flow rate of 1.0ml/min.The retention time was 5.01min.Linearity for Nabumetone was in the range of 20-120 µg/ml and the calibration curve was linear(r2=0.999),the recovery was in the range of 99.942-100.15%.The proposed method found to be simple ,precise ,accurate and economical.
Keywords: Nabumetone, RP-HPLC, methanol, validation

INTRODUCTION

Nabumetone a naphthyl alkanone (1)designated chemically as 4-(6-methoxy-2-naphthyl)-butan 2-one(fig-1).It is a non-steroidal anti-inflammatory drug .It shows pharmacological activities like anti-inflammatory(2) ,analgesic, and antipyretic properties. It plays a vital role in the treatment of rheumatoid arthritis (3) and osteoarthritis. It is a drug of arylalkanoic acid (4) family used to treat pain or inflammation caused by arthritis. This is official in Indian pharmacopoeia (5), United States of Pharmacopoeia (6), British pharmacopoeia (7).

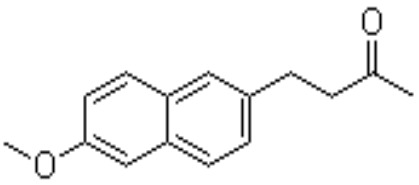


Fig: 1 chemical structure of Nabumetone

Nabumetone is a prodrug, undergoes extensive (8-10) first pass metabolism to 6-methoxy-2-naphthylacetic acid (6-MNA) which is responsible for the therapeutic activity.6-MNA, is responsible for the inhibition of cyclooxygenase enzyme which involves in the arachidonic acid conversion pathway .there by 6-MNA decreases prostaglandin synthesis. The literature survey reveals that several chromatographic methods have been used for the analysis of Nabumetone in biological fluids. Few papers were published reporting analytical methods for Nabumetone. Different methods employed RP-HPLC with UV detection,(11-12) photodiode array (PDA) detection, fluorescence detection and mass spectroscopic detection for the determination of Nabumetone. We here present a new method for the determination of Nabumetone in bulk and pharmaceutical dosage forms which utilizes a very cheap solvent on C_{18} analytical column .this method leads to a better retention, very sharp and symmetrical peak shapes and exhibits a very good selectivity for nabumetone. No reports were found for the estimation of NAB using methanol and phosphate buffer as solvents by Reverse phase high performance liquid chromatography (RP-HPLC).the method was optimized and validated as per (16) the international conference on harmonization (ICH) guidelines.

MATERIALS AND METHODS

Apparatus

The instrument used in the study was liquid chromatographic system of Younglin containing variable wavelength programmable UV detector and rheodyne injector with 20µl fixed loop was used. The chromatographic analysis was performed using Autochro3000 software. A waters C_{18} column with 4.6mm×150mm, 5µm particle size was used.

Chemicals and Reagents

All solvents were of HPLC grade Methanol obtained from SD fine chemicals (India) **(**SDFCL), Di sodium orthophosphate, all are HPLC grade reagents Di hydrogen Potassium Orthophosphate were obtained from fisher Scientifics, Water was purified with Milli-Q Plus, Millipore System (USA). All solutions and solvents were filtered through membrane filter (Millipore millex-HV filter units 4.5 µm pore size, nylon) and degassed before use.

Marketed formulation

Relafen tablets containing 500mg and 750 mg of Nabumetone were purchased from local market.

Preparation of Standard solution

The standard stock solution was prepared with methanol to a concentration of

1000µg/ml.The standard solutions from 20 to 120µg/ml (20, 40, 60, 80,100,120µg/ml) were serially diluted with methanol and sonicated for 10 minutes.

Sample preparation

Average tablet mass was calculated from the total mass of Nabumetone tablets(500mg).they were then finely grounded and homogenized(13) and a portion of powder was weighed accurately and transferred to a 100ml volumetric flask and made upto the mark with methanol. The mixture was sonicated for 15-20 minutes for uniform mixing and filtered through whatmann filter paper(No.1) .Appropriate volume was transferred to 25ml volumetric flask and volume was made upto the mark with mobile phase to obtain 1000µg/ml of Nabumetone. The solution was sonicated for 10 min and injected under the above chromatographic conditions, the chromatogram was recorded (fig: 2) and the peak area was measured.

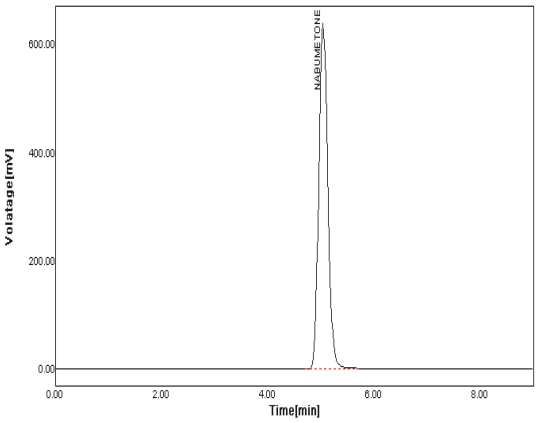


Fig: 2 Chromatogram of Nabumetone

For the method development of nabumetone the wavelength is determined using UV calibration method.

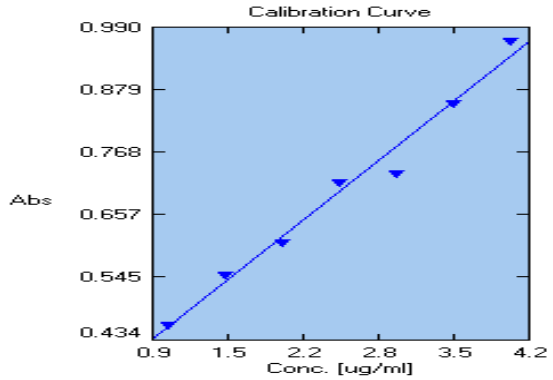
The maximum wavelength was found at 261 nm. The values were tabulated in the table 7 and the UV calibration graphs were shown in the Fig No. 3.

U.V. Linearity & calibration curve

Table-No: 7 showing the Absorbance values of U.V Calibration Curve

S.No	ID	Type	Conc [µg/ml]	Absorbance	Wavelength 261.00 nm
1	Nabumetone	Standard	1.0000	0.459	0.459
2	Nabumetone	Standard	1.5000	0.549	0.549
3	Nabumetone	Standard	2.0000	0.607	0.607
4	Nabumetone	Standard	2.5000	0.714	0.714
5	Nabumetone	Standard	3.0000	0.729	0.729
6	Nabumetone	Standard	3.5000	0.854	0.854
7	Nabumetone	Standard	4.0000	0.965	0.965

Fig. No. 3 U.V Calibration Curve of Nabumetone



Validation

Linearity

The calibration curve was obtained at six concentration levels of Nabumetone standard solutions ranging from 20-120µg/ml (20, 40, 60, 80,100,120µg/ml). Calibration curve was constructed by plotting concentration of compound versus peak area.10µl of solution were injected into liquid chromatographic system. The linearity was evaluated by least square regression method.

Accuracy

Accuracy Was Determined by Calculating Recoveries of Nabumetone by method of standard addition. Recovery studies were performed in triplet by standard addition method of 80%, 100% and 120% concentration levels known amount of drug were added to a pre-quantified sample solution and the amounts were estimated.

Precision

Precision is determined by two type of tests, intra-day and inter-day precision. The intra-day and inter-day precision study was carried out by estimating the corresponding responses 3 times on the same day and 3 different days (first, second, third day)for 3 different concentrations of Nabumetone which represents low ,medium and high concentrations in analytical range.

Specificity

The specificity was by spiking commonly used excipients into a pre-weighed quantity of drug. The chromatogram was taken by appropriate dilutions and the quantities of drug were determined.

Limit of detection

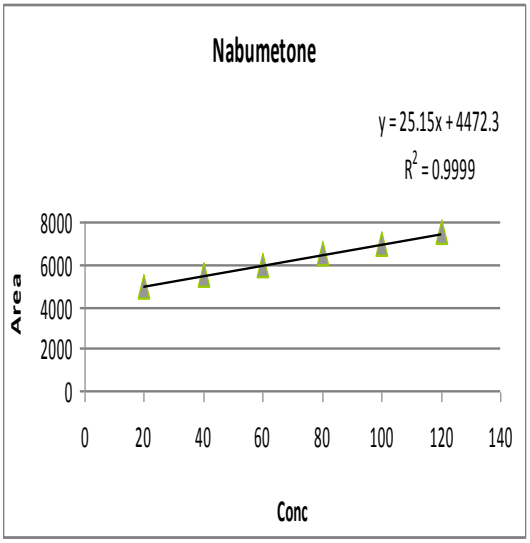
Limit of detection was the concentration that yielded signal to noise ratio(S/N) 3:1 and calculated by using the formula

$$LOD = 3.3\ \sigma/S$$

Limit of Quantification

Limit of quantification was the concentration that yielded signal to noise(S/N) 10:1 and calculated using the formula

$$LOQ = 10\ \sigma/S$$



Linearity of Nabumetone by RP-HPLC Method

Method	Range(µg/ml)	LR [a]	R^2	LOD(µg/ml)	LOQ(µg/ml)
RP-HPLC	20- 120	Y=25.15+4472.3	0.9999	0.0281	0.0938

Precision Data of Nabumetone

Parameter	Nabumetone
System precision (%RSD)	0.077278
Method precision (%RSD)	0.1249777

System suitability

Retention time	5.0333
Theoretical plates	3512.1
Tailing factor	1.1364
Flow rate	1.0ml/min

Recovery Studies

Concentration	Nabumetone
80%	99.94226
100%	100.0352
120%	100.1503

Robustness Data

Variation	Retention Time	Peak area	Tailing factor	Theoretical plates
pH (2.5)	4.8	5968	1.025	3512
pH (3.5)	5.001	6972	1.031	3523
Flow (0.9ml)	5.015	7516	1.1	3522
Flow (1.1ml)	4.99	4980	1.022	3499

Validation results for Nabumetone

S.no	Parameter	Acceptance criteria	Experimental values
1	Specificity	Non-Interference Of Placebo	Passed
2	System suitability	Number Of Theoretical Plates-NLT2000	3512.1
3	Precision	%RSD of system precision NMT2% %RSD of method precision NMT2%	0.077278 0.124977
4	Linearity	Correlation coefficient NLT 0.995	0.9999
5	Accuracy	Percentage Recovery (97-103%)	99.942-100.150
6	Ruggedness	%RSD of standard NMT2% %RSD of sample NMT2%	Passed
7	Robustness	%RSD of standard NMT2% %RSD of results between different mobile phases NMT2%	Passed
8	LOD& LOQ	Signal noise ratio should be more than 3:1 & 10:1	0.0281 &0.0938

CONCLUSION

A simple, sensitive method has been established for isocratic separation and simultaneous determination of Nabumetone bulk and Tablet Formulation as well as in this method proves high efficiency and good baseline factor when compared previous article. The linearity, precision, and accuracy of the method prove it is highly reproducible under quality-control conditions if the procedures described above are followed precisely.

ACKNOWLEDGEMENT

The authors are thankful to Mr. V. Ravi Kumar, Managing Director of Bright's Labs, Dilushkunagar, Hyderabad for providing necessary facilities and support during this work.

REFERENCES

1. Altun Yuksel, Dogan Burcu ,Ozkan .Sibel A and Uslu Bengi. *Acta Chim Slov.*, **2007**; 54: 287-294.
2. Prafulla Kumar Sahu, M. Mathrusri Annapurna. Analysis of Nabumetone in Bulk and Tablet Formulation by a New and Validated Reverse Phase High Performance Liquid Chromatography, *E-Journal of Chemistry*, **2009**; 6(S1): S59- S64.
3. Merck Index. 13th ed, Merck & CoInc, NJ.**2001**: 1136.
4. M. Nobilis, M. Hol Apek et.al. Journal of Chromatography A, 1031: 229-236.
5. The United States Pharmacopoeial Convention, the United States Pharmacopoeia, 29thed, Rockville, **2006**.
6. The Official Compendia of Standards, British Pharmacopoeia, vol.1, Her Majesty's Stationery Office: London, **1993**.
7. The Official Compendia of Standards, Indian Pharmacopoeia, vol.2, the Controller of Publication: New Delhi, **1996**.

8. A.J. Vyas, N.A. Aggarwal et.al. International journal of chem tech., 2010; 2(1): 412-418.
9. Current Index of Medical Specialties, Update Prescribes Handbook Published by CMP Medica India Pvt.Ltd. Bangalore, **2007**, 117-119,263.
10. Merck Index, Merck Research Laboratories, division of Merck and company NJ, USA, 13th ed., **2001**, 1088.
11. United States Pharmacopoeia 26, NF 21, **2003**, 1259.
12. A.H.Mohite et al. Development of UV-spectroscopic method for Nabumetone in tablet formulation, *Journal of pharmacy Research,* **2009**; 2(7):1221-1223.
13. Davies NM. Cli. Pharmakokinet, **1997**; 33: 403-416.
14. S.Mallik Basha, G.Devala Rao et.al. Biomed Chromatography, **2010**;2(4)
15. YS Rao, KPR Chowdary J.V.L.N seshagiri Rao. A colorimetric Assay Method for Nabumetone, *Indian journal of Pharmaceutical sciences*, **2003;** 65(2): 206-207.
16. AL-Momani, I.F.; *Anal.Lett.***, 1997**; 30: 2485.
17. ICH, International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use,Q2B(R1),Guideline on validation of analytical procedure: Text and Methodology,Geneve,**2005**.
18. Rao R Nageswara, Meena S, Nagaraju D and Rao A Raghu Ram. *Biomed chromatogr.,* **2004**; 19: 362-368.
19. Patel Bhavin N, Sharma Naveen, Sanyal Mallika, Prasad Arpana and Shrivastav Pranav S. *Biomed Chromatogr.,* **2008**; 22: 1213-1224.