International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 5 Sep-Oct 2022, pp: 631-638 www.ijprajournal.com ISSN: 2456-4494

Development And Validation Of Rp-Hplc Method For Nabumetone And It's Pharmaceutical Dosage Form

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Submitted: 20-09-2022 Accepted: 30-09-2022

ABSTRACT
For the determination of Nabumetone (Naphthyl acetic acid derivative), a simple, precise, and accurate isocratic reversed RP-HPLC stability indicating method was established and validated. The mobile phase consisted of Acetonitrile: Methanol: Water (50:10:40 v/v). On a bulk sample of Nabumetone, forced degradation investigations were conducted using an acid (0.1 MHydrochloric acid), a base (0.1M sodium hydroxide), oxidation (3.0% hydrogen peroxide), heat degradation (70°C). Degradation products correspond to good resolution between the peaks. With RP-HPLC detection, the Nabumetone calibration curves demonstrated high linearity in the concentration range of 1–5µg/ml (230 nm). The correlation coefficients were equal to 0.999.The approach possesses the required accuracy, selectivity, sensitivity, and precision to test degradation products originating from stress investigations, and hence it is stability-indicating.

Keywords: Analytical method development, method validation, NSAIDS, RP-HPLC.

I. INTRODUCTION

Nabumetone is a non-steroidal anti-inflammatory drug. Nabumetone provides symptomatic improvement in many patients with arthritis. Nabumetone is a nonsteroidal anti-inflammatory prodrug that works through the metabolite 6-methoxy-2-naphthylacetic acid to produce its effects (6-MNA). Nabumetone is non-acidic and undergoes significant first-pass metabolism after absorption to create the primary circulating active metabolite (6-MNA), which is a much more effective inhibitor of cyclo-oxygenase (COX)-2. O-demethylation, ketone reduction to alcohol, and oxidative breakage of the side-chain to generate acetic acid derivatives are the three key metabolic routes for nabumetone.[1,2]

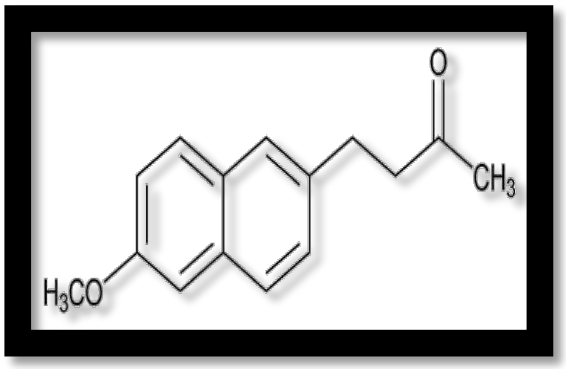

Fig. 1: Chemical Structure of Nabumetone

Nabumetone is primarily used to control pain and inflammation in patients with osteoarthritis (OA) or rheumatoid arthritis (RA). The efficacy of nabumetone in patients with ankylosing spondylitis, soft tissue injuries, and juvenile RA has also been evaluated.In clinical investigations, nabumetone has a dose-dependent effect on platelet aggregation through its metabolite 6-MNA, however no impact on bleeding time.[3]

Pain relievers, analgesics, and antipyretics have all been examples of NSAIDs.. Inhibitors of cyclooxygenases, COX1 and/or COX2, fall into this category. The majority of NSAIDs are nonselective COX1 and COX2 inhibitors. They have a carboxylic acid functional group or another less weakly acidic group and are over-the-counter medications. Ibuprofen, naproxen, ketoprofen, aspirin, acetaminophen, fenoprofen, flurbiprofen, oxaprozin, indomethacin, sulindac, tolmetin, diclofenac, lumiracoxib, nabumetone, piroxicam, meloxicam, mefenamic acid, and meclofenamic acid are some examples COX1 and COX2 are

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cyclooxygenase enzymes that convert arachidonic acid to prostaglandins, which are involved in pain, inflammation, and fever. The COX2 isoenzyme, which is found primarily in wounded tissues, is primarily responsible for pain and inflammation.[4]

HPLC Method Development[5]

The HPLC procedures are used to identify, separate, purify, and estimate the quantity of organic molecules in complex mixtures. Aside from that, it entails determining dosage form content homogeneity, monitoring dissolution profiles, and determining antioxidant and microbial preservative levels. To build a routine HPLC method, we must choose the ideal column, mobile phase, and detection wavelength. Determining the best combination of these criteria ensures that the desired results are delivered sooner.

Method Validation[6]

Validation of an analytical method is intended to demonstrate that it is suitable for its intended use

1. Specificity- The ability to assess the analyte definitively in the presence of components that may be present is known as specificity. Impurities, degradants, matrix, and other substances are examples of these.
2. Accuracy- The closeness of agreement between the value acknowledged as a conventional true value or an approved reference value and the value found is expressed by the accuracy of an analytical method.
3. Precision- The degree of deviation (closeness of agreement) between a set of measurements acquired from multiple sampling of the same homogeneous sample under the stipulated conditions is expressed by the precision of an analytical method.
4. Linearity- The capacity of an analytical process to produce test results that are directly proportional to the concentration (quantity) of analyte in the sample (within a specific range) is known as linearity.
5. Robustness- The robustness of an analytical procedure is a measure of its ability to remain unaffected by modest but deliberate changes in method parameters, and it indicates its reliability throughout regular application.
6. Assay- It is Analytical approach used to a synthetic combination of drug product components to which known quantities of the drug substance to be examined is compared to find purity of drug.

Stress testing on the drug substance is recommended by the International Conference on Harmonization (ICH) guideline Q1A (R2) for parent drug stability testing to establish stability characteristics and support the applicability of the proposed analytical method.[7,8]

II. MATERIALS AND METHODS

Material:

The reference standard of Nabumetone was obtained as gift sample from Emcure pharmaceuticals. All chemicals used were of HPLC grade of Merck. Acetonitrile , methanol as having HPLC grade of Merck Limited were used for chromatographic procedure. Gel formulation of Nabumetone was prepared as sample. 1gm of gel consisted 1% of Nabumetone.

Instrumentation:

A Jasco Extrema LC system-4000was used. The peaks were quantified by means of PC based ChromNav software

Chromatographic conditions:

The chromatographic separation was performed at 25^{o}C temperature on reverse phase ChromatopakPearless Basic C18 (250 × .6mm × 5µm)column. The mobile phase consisted of Acetonitrile : methanol: water (50: 10: 40 v/v). The separation was carried out at detector wavelength 230 nm and flow rate of 0.9 ml/minute.

Preparation of Standard and Sample Solution:

The standard stock solutions of Nabumetone (3µg/ml) was prepared by dissolving appropriate amounts of drug compounds in acetonitrile. Whereas in the preparation of sample solution, quantity of gel equivalent to 10 mg of Nabumetone was weighed and dissolved in acetonitrile. It was further diluted in order to get solution having concentration 3µg/ml of drug.

Method Validation:

1. Specificity- Specificity was carried as interference from placebo; first only placebo and then injecting synthetic mixture containing placebo and API's as gelsample.
2. Accuracy- Accuracy was determined by the standard addition method. Samples of Nabumetone were spiked with 80, 100 and 120% impurities standard and the mixtures were analyzed by the proposed method. The experiment was performed in triplicate. For

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each concentration, the recovery (%) and RSD (%) were calculated.

3. Precision- Precision was determined as both repeatability and intermediate precision, in accordance with ICH recommendations. Intra-day variation was used to determine sample absorbance repeatability, whereas inter-day variation was used to determine intermediate precision. For inter-day variation, six homogenous test solutions of Nabumetone were analysed.
4. Linearity-Standard stock drug solution of Nabumetone with concentration of 10µg/ml was prepared in diluent. For preparation of calibration curve of drug 1, 2, 3, 4, 5 ml of standard stock solution of Nabumetone were transferred to series of 10 ml volumetric flasks and with the diluent, the volume was increased up to the mark. Each solution was injected after filtration through 0.45µm membrane filter and chromatograms were recorded at 230 nm. The prepared dilutions were injected sequentially, the peak area for each dilution was calculated, and the concentration was plotted against the peak area..
5. Robustness-The robustness of the method was determined to assess the effect of small but deliberate variation of the chromatographic conditions on the determination of related substances of Nabumetone. Robustness was determined by in column oven temperature to 20°C and 30°C, changing the mobile phase flow rate to 0.8 and 1.0 ml/min and the wavelength to 228nm and 232 nm.
6. Assay of formulated dosage form-To determine the amount of Nabumetone in formulated gel formulation, amount of gel equivalent to 10mg drug was weighed. The weighed quantity of gel was dissolved in acetonitrile and further sonicated for 15 min to ensure complete dissolution of drug. The solution was filtered and volume was made up to 10ml using acetonitrile. further diluted to achieve final concentration of 3µg/ml of Nabumetone. The prepared dilution was injected in series, peak area was calculated for each dilution. Area of standard injected in series and was recorded to calculate % Assay.

Forced Degradation Studies

To determine whether the analytical method was stability-indicating, Nabumetoneactive pharmaceutical ingredient (API) powder were stressed under various conditions to conduct forced degradation studies. Intentional degradation was attempted to stress conditions of acidic hydrolysis (using 0.1M HCl), alkaline hydrolysis (using 0.1M NaOH), oxidative degradation (using 3.0% H_2O_2) and dry thermal treatment (heated at 70°C in crucible). After completion of the degradation processes, the solutions were neutralized and diluted with mobile phase. Stress degradation conditions were decided on the basis of tolerable pH range of the column. Resolution between drug and its degradants peak should be more than 1.5. Attempt was made to decompose the drug by exposing drug to stress conditions and then milder conditions were used. This was done to reduce the time of degradation. The tolerable pH range of column is 2.5-8.5 therefore higher alkaline stress conditions cannot be used.

Acidic degradation

Drug was exposed to acidic condition to achieve degradation. 10 mg drug was dissolved in 1ml of acetonitrile and 1 ml of 0.1M hydrochloric. Here reaction solution needs to be neutralized before injecting in to HPLC system to prevent damage to chromatographic column, dilution were carried out with acetonitrile to have final concentration of sample as 3 ppm. Solution of drug in alkaline medium was kept at ambient temperature. Sample was analyzed at different time interval.

Alkaline degradation

Drug was exposed to alkaline condition to achieve degradation. 10 mg of drug was dissolve in mixture of 10 ml of acetonitrile and 1ml 1 N sodium hydroxide. Here reaction solution needs to be neutralized before injecting in to HPLC system to prevent damage to chromatographic column, dilution were carried out with acetonitrile to have final concentration of sample as 3ppm. Solution of drug in alkaline medium was kept at ambient temperature. Sample was analyzed at different time interval.

Oxidative degradation

A hydrogen peroxide solution was used as an oxidising media for the drug. 10 mg of drug was dissolved in 1 ml of acetonitrile and 1 ml of 3% hydrogen peroxide were added. Here reaction solution needs to be neutralized before injecting in to HPLC system to prevent damage to chromatographic column,dilution were carried out with acetonitrile to have final concentration of sample as 3 ppm. Sample was analyzed at different time interval.

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Thermal stress studies

1 gm Drug was transferred to crucible and kept in oven at 70 ^{O}C and sample was subjected to analysisat different time interval. Dilution were carried out with acetonitrile to have final concentration of sample as 3 ppm. Sample was analyzed at different time interval.

III. RESULTS AND DISCUSSION

The developed and validated method of nabumetone was aimed to establish chromatographic conditions, capable of qualitative and quantitative determination of nabumetone in pharmaceutical preparations. Nabumetone was completely separated on C18 column by RP-HPLC using the isocratic elution of Acetonitrile, methanol and water as mobile phase. Flow rate was changes from 0.7ml/min to 1.2 ml/min, eventually proper resolution was achieved at flow rate of 0.9mL/min and retention time of 7.8 minutes.

Linearity- The method gave a linear response to Nabumetone drug within the concentration range of 1 - 5µg/ml. The correlation co-efficient and Y-intercept of the plot were also evaluated during the linear regression of the graph of peak area Vs Concentration. For Nabumetone, the linear regression equation was found to be y = 213441x - 9255.7andcorrelation coefficient as 0.9991, where 'y' is the peak area and 'x' is the drug solution concentration.

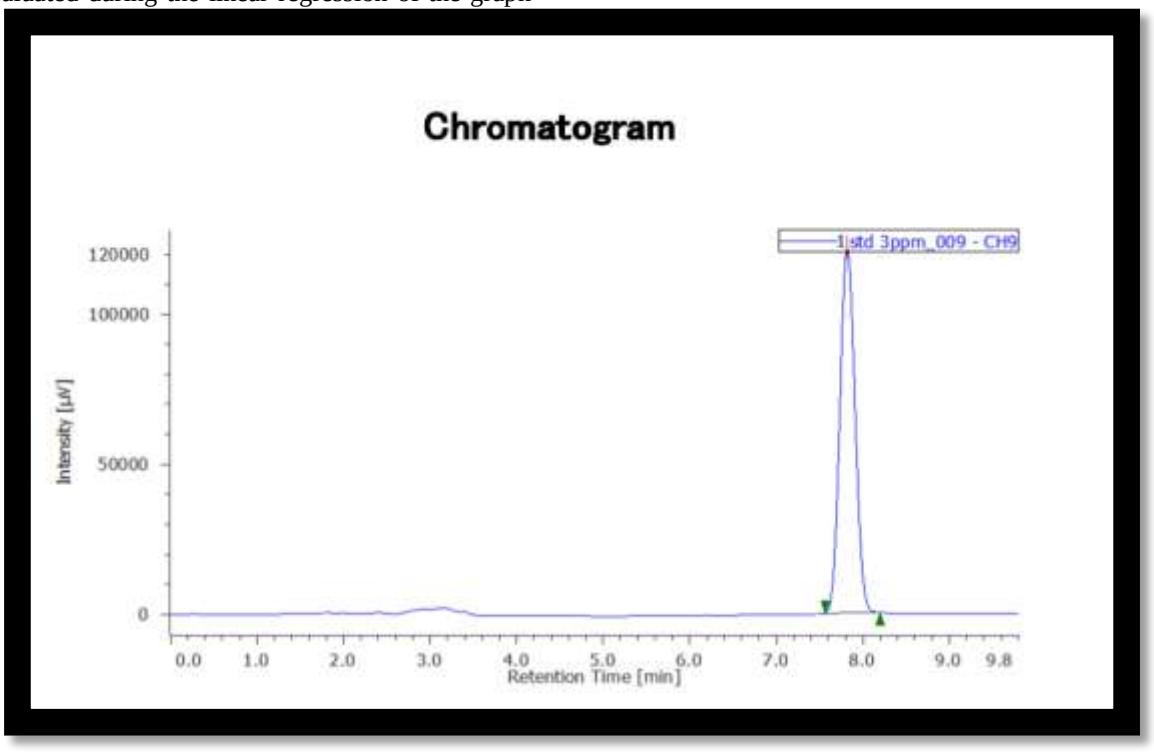
Accuracy-The Nabumetone was recovered in the range of 98.8 to 102.0 % for various concentrations as shown in table 2.

Precision- The repeatability, intra-day and inter-day precision results are found within range.The RSD values were below 2%, indicating a good precision.

Robustness–The robustness of the method gave the mean, standard deviation (SD) and RSD within the limits. Results are shown in table 3.

Assay of formulated dosage form- The labeled amount of Nabumetone1% gel was recovered 99.88%. The RSD value is below 2%. The retention time was found to be 7.8 min.

System suitability studies The system suitability parameters such as retention time, capacity factor, theoretical plate number, peak purity and resolution factor of optimized method were associated with confined values as shown in the table 4.



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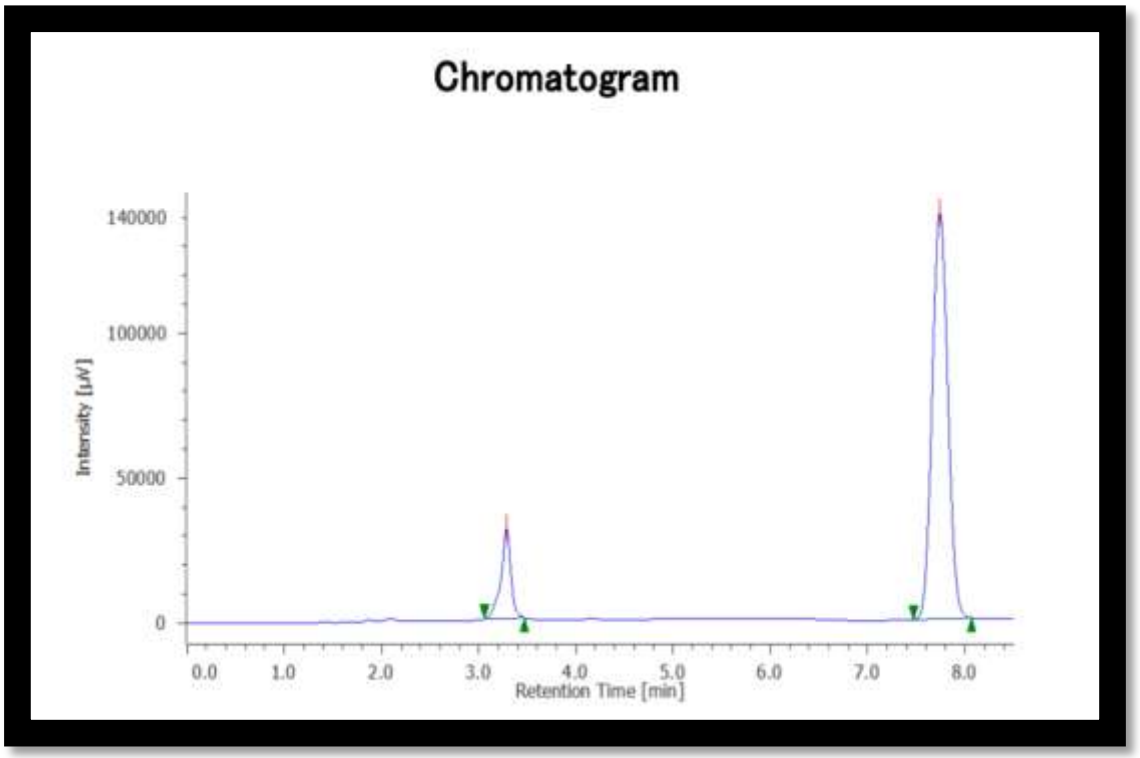

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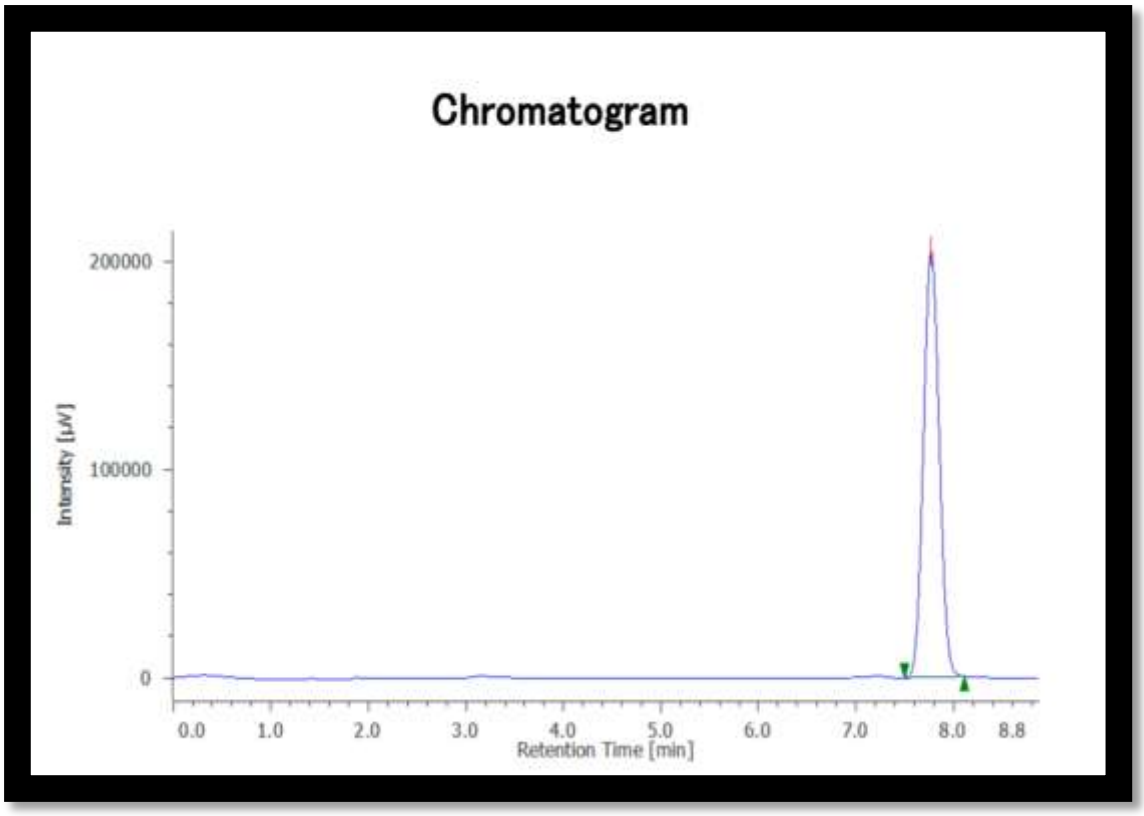

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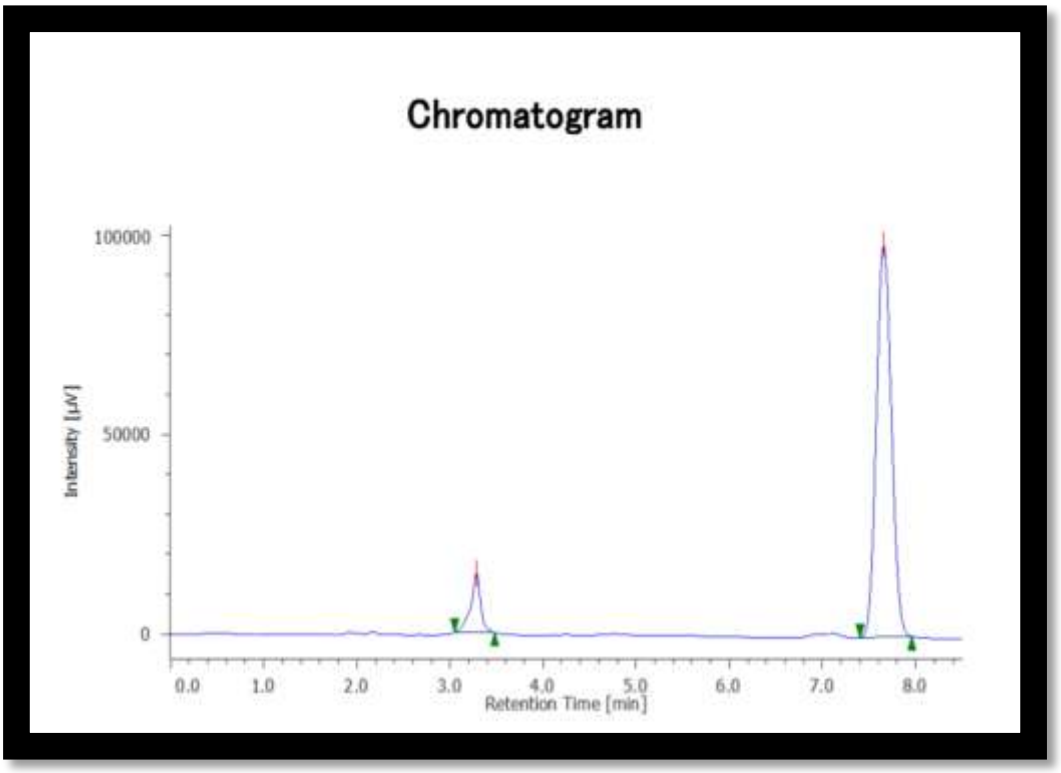

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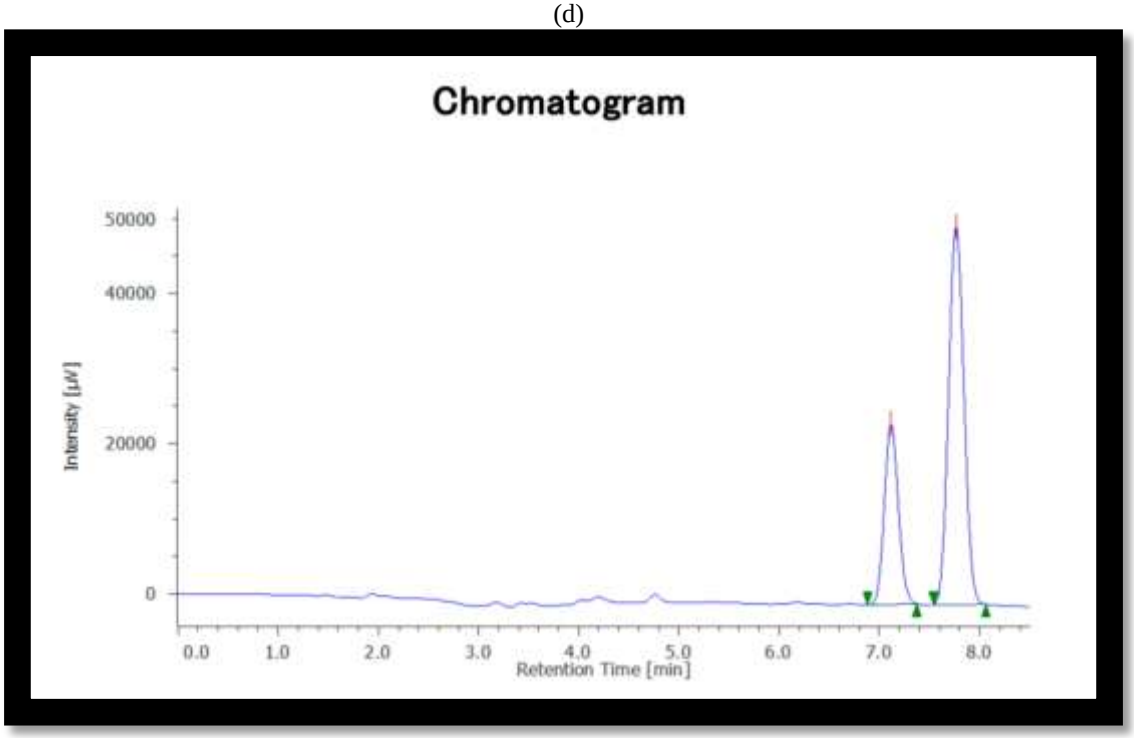

(e)

Fig. 2 Chromatogram showing: a) sharp peak at optimize condition (b) acidic hydrolysis at 60 mins (c) alkaline hydrolysis at 2 hrs (d) oxidative hydrolysis at 30mins(e) thermal heat degradation at 30 mins

Table 1: Degradation studies of Nabumetone

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Stress condition	Degradation time	Area of peak	% Degradation
Acidic	60 min	690967	2.32%
Alkaline	No degradation	707400	-
Oxidative	30 mins	623929	11.799%
Thermal	30 mins	568087	19.69%

Table 2: Recovery study data of Nabumetone

Conc.	Sample added(µg/ml)	Standard added (µg/ml)	Total concentration	Concentration found	% Mean recovery
80%	3	2.4	5.4	5.436	100.748
100%	3	3	6	6.0058	99.940
120%	3	3.6	6.6	6.6054	99.69

Table 3: Robustness study of Nabumetone

Chromatographic changes		% RSD (Peak area)	Symmetry factor
Flow rate	0.8 ml/min	0.9118	1.10
	0.9 ml/min	1.1210	1.04
	1.0 ml/min	0.8788	1.1
Wavelength	228 nm	0.6224	0.98
	230 nm	0.4211	1.21
	232 nm	0.6264	1.30
Temperature	20^0 C	0.4421	1.31
	25^0 C	0.7841	1.08
	30^0 C	0.0993	1.02

Table 4: Summary of Validation parameter

System suitability	Result
Theoretical plates	9129
Linearity range (µg/mL)	1 µg/ml - 5 µg/ml
Retention time	7.8 min
Co-relation co-efficient	0.9991

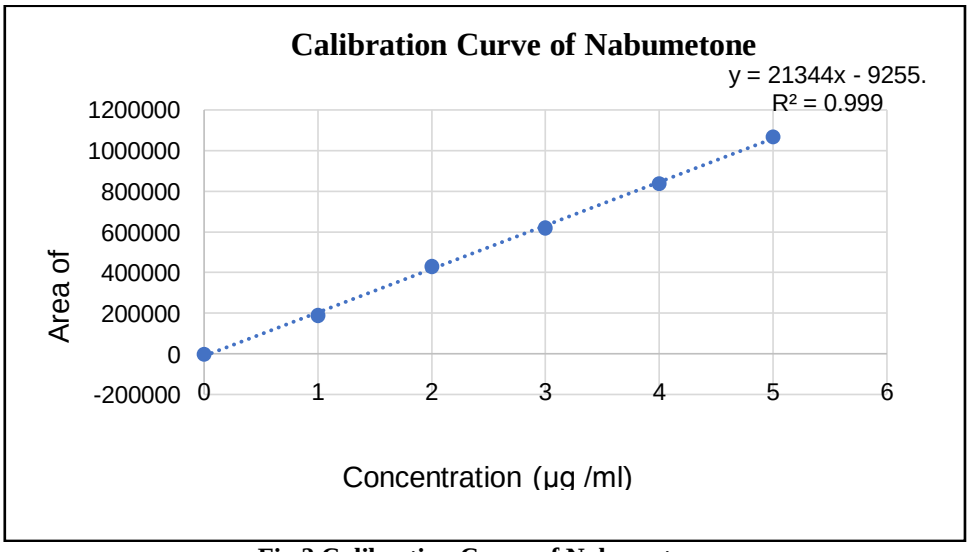

Fig.3 Calibration Curve of Nabumetone

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IV. CONCLUSION

The optimized reverse phase HPLC method for Nabumetone is linear, accurate, precise, robust, simple, rapid and selective. It can be adopted apparently for routine quality control analysis of raw materials, formulations and testing.

ACKNOWLEDGEMENT

The authors are grateful to EmcurePharmaceuticals for providing gift samples of Nabumetone.

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