Development and validation of stability-indicating RP-HPLC method for the determination of Nabumetone

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ABSTRACT

A simple, precise, and accurate, isocratic, reversed RP-HPLC stability indicating method was developed and validated for determination of Nabumetone (Naphthyl acetic acid derivative). Isocratic RP-HPLC separation was achieved on Inert Sil ODS 3V (5µ, 25 cm× 4.6 mm, i.d.) column using mobile phase consisted of 0.47 g of tetrabutyl ammonium hydroxide sulphate (TBAH) dissolved in 1000 ml of a mixture of acetonitrile, water and triethylamine (290:710:1, v/v) adjusted with phosphoric acid to pH 6.5. Forced degradation studies were performed on bulk sample of Nabumetone using acid (2.0 N hydrochloric acid), base (1 N sodium hydroxide), oxidation (3.0% hydrogen peroxide), Dry heat (105°C) and Wet heat degradation (70°C reflux). Good resolution between the peaks corresponds to degradation products. The calibration curves of Nabumetone showed good linearity in the concentration range 2 –100 µg/ml with UV detection (239 nm). The correlation coefficients were better than 0.999. With limit of detection and quantification 0.29 and 0.87 µg /ml, respectively. The method has the requisite accuracy, selectivity, sensitivity and precision to assay degradation products resulting from the stress studies did not interfere with assay is thus stability-indicating.

Keywords: Stability indicating method, Nabumetone, Recovery studies, Tetra butyl ammonium hydroxide sulphate (TBAH), stress studies.

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INTRODUCTION

Nabumetone 4-(6-methoxynaphthalen-2-yl) butan-2-one, is a nonsteroidal anti-inflammatory drug (NSAID) of naphtylalkanone class, structure as shown in figure 1. The drug has proved to be effective in the treatment of rheumatoid arthritis, osteoarthritis and acute soft tissue injuries. Nabumetone is a prodrug which undergoes extensive first pass metabolism to 6-methoxy-2-naphthylacetic acid (6-MNA), the major circulating metabolite; 6-MNA is largely responsible for the therapeutic efficacy of nabumetone [1, 2, 3].
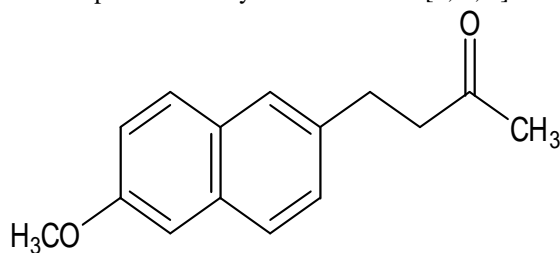

Figure 1: Structure of Nabumetone

A literature survey revealed different analytical methods for nabumetone in human plasma and determination of the active metabolite of nabumetone in biological fluids by heavy atom-induced room temperature phosphorescence [4], simultaneous determination of COX-2 inhibitors in pharmaceuticals dosage form [5], in human plasma by liquid

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chromatography-atmospheric pressure chemical ionization-tandem mass spectrometry [6], by micellar—stabilized room temperature phosphorescence [7], by high-performance liquid chromatography with photodiode array and mass spectrometric detection [8]. To our knowledge, no validated stability-indicating HPLC method for the determination of Nabumetone in bulk drug has been reported in the literature.

According to current good manufacturing practices, all drugs must be tested with a stability-indicating assay method before release. Stress testing of the drug substance can help identify the likely degradation products, which can in turn help establish the degradation pathways and the intrinsic stability of the molecule and validate the stability-indicating power of the analytical procedures used. The nature of the stress testing will depend on the individual drug substance and the type of drug product involved. Considering the susceptibility of Nabumetone under variety of conditions, it was felt that a HPLC method of analysis that separates the drug from the degradation products formed under ICH suggested conditions (hydrolysis, oxidation and thermal stress) would be of general interest. These studies provide valuable information on drug's inherent stability and help in the validation of analytical methods to be used in stability studies. Attempts were made to develop a suitable single stability indicating HP-LC method [9] that can be used to determine the related substances and also the assay of bulk samples of Nabumetone. The International Conference on Harmonization (ICH) guideline Q1A (R2) for parent drug stability testing suggests that stress testing on the drug substance should be performed to establish stability characteristics and to support the suitability of the proposed analytical method [10,11].

MATERIALS AND METHODS

Material
The reference standard of Nabumetone (Nabumetone) was obtained as gift sample from Ipca laboratory. All chemicals used were of HPLC grade of Merck. Potassium dihydrogen phosphate, Triethylamine and Ortho-phosphoric acid, Tetra butyl ammonium hydroxide sulphate (TBAH) as having HPLC grade of Merck Limited were used for chromatographic procedure. Mili-Q Water was used to prepare solutions. Tablet dosage form manufactured by Ipca laboratory; NILTIS was used. Each tablet containing 500 mg of Nabumetone.

Instrumentation:
A SHIMDZU AHT HPLC system consisting of PV-980 pump, a 975 UV-Visible detector was used. The peaks were quantified by means of PC based Class-VP software.

Chromatographic conditions:
The chromatographic separation was performed at 25 ℃ temperature on reverse phase Inert Sil ODS 3V (5µ, 25 cm× 4.6 mm, i.d.) column. The mobile phase consisted of 0.47 g of TBAH dissolved in 1000 ml of a mixture of acetonitrile, water and triethylamine (290:710:1, v/v) adjusted with phosphoric acid to pH 6.5. The separation was carried out at detector wavelength 239 nm, flow rate of 1.5 ml/minute with retention time of 7.00 min. The injection volume of standard & sample solutions was 5µl.

Preparation of Standard and Sample Solution
The standard stock solutions of Nabumetone (Nabumetone) (100 µg/ml) was prepared by dissolving appropriate amounts of drug compounds in acetonitrile. Whereas in the preparation of sample solution, quantity of powdered tablet equivalent to 10 mg of Nabumetone was weighed and dissolved in acetonitrile. It was further diluted in order to get solution having concentration 50 µg/ml of drug.

Forced degradation studies
To determine whether the analytical method was stability-indicating, Naphthyl acetic acid derivative (Nabumetone) active 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 2 N HCl), alkaline hydrolysis (using 1N NaOH), oxidative degradation (using 3.0% H_2O_2) and dry and wet thermal treatment (heated at 105 °C and 70°C with reflux condition respectively. 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 10-30% of 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 subjected to acidic condition to achieve degradation from 10 – 30%. 60 mg drug was dissolved in 60 ml of methanol and 60 ml of 4 N hydrochloric acid were added, so normality of acid become 2 N. 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 60 ppm. Initially solution of drug in acidic medium was kept at ambient temperature, but sufficient degradation was not achieved so more stress condition in form of reflux was given for different time interval and sample was analyzed.

Alkaline degradation

Drug was subjected to alkaline condition to achieve degradation from 10 – 30%. 60 mg of drug was dissolve in mixture of 60 ml of methanol and 60 ml 2 N sodium hydroxide, so normality of solution became 1 N. 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 60 ppm. Solution of drug in alkaline medium was kept at ambient temperature. Sample was analyzed at different time interval.

Oxidative degradation

Drug was exposed to oxidizing medium, hydrogen peroxide solution. Attempt was taken to achieve degradation between 10.0 – 30.0 %. 60 mg of drug was dissolved in 60 ml of methanol and 60 ml of 6% 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 60 ppm.

Thermal stress studies

Dry heat

1 gm Drug was transferred to crucible and kept in oven at 105 $^{\circ}$C for 8 hr and sample was prepared and subjected to analysis. It was found that degradation was less than 10 % so heating time was increased, dilution were carried out with acetonitrile to have final concentration of sample as 60 ppm.

Wet heat

60 mg drug was diluted with 60 ml of water and refluxed for 18 hr and sample were prepared at regular interval and subjected to HPLC, dilution were carried out with acetonitrile to have final concentration of sample as 60ppm.

RESULTS AND DISCUSSION

Optimization of analytical conditions:

Different columns containing octyl, octadecyl, phenyl and base deactivated silane stationary phase were tried for separation and resolution. The Inertsil base deactivated silane column became more advantageous over the other columns. Individual drug solution was injected into column, both elution pattern and resolution parameters studied as a function of pH, as a function of mobile phase component and their ratio. To develop a suitable LC method for estimation of nabumetone in formulations, different mobile phases were employed to achieve the best separation from degradant peaks. The selected and optimized mobile phase was ACN: Buffer: TEA (29:71:0.1) and conditions optimized were: flow rate (1.5 ml/minute), detector wavelength (239 nm). Run time was 7 min. Here the peaks were separated and showed better resolution, theoretical plate count and asymmetry was found as 1.09 for Nabumtone. The proposed chromatographic conditions were found appropriate for the quantitative determination of the drugs.

Results of forced degradation studies

Acidic degradation of Nabumetone was performed in 50% acetonitrile and 0. 1 N HCl at room temperature as it is insoluble in hydrochloric acid. Hydrolytic degradation of Nabumetone was observed to be very slow and less, thus higher forced condition were tried to accelerate degradation process. Then further degradation carried out in the mixture of acetonitrile and hydrochloric solution having strength of 2 N HCl with reflux at 70˚C. Drug got degraded into three degradants (RT of 2.85, 2.63, 1.30 min) and approximately 40% of drug was degraded in 18 hr. In case of alkaline degradation process was carried out for 52 hr at ambient temperature in 1 N NaoH and gave two major and one minor degradants peak (RT of 2.85, 2.00, 1.29 min). Approximately 45% of drug was degraded in 52 hr.Progress of degradation cannot be judged, here in oxidative degradation nabumetone is very labile to experimental condition and degradant peak further undergo degradation thus continuously degradant Rt changes. Initially (RT of 1.47 then change to 1.58, further 1.58 and 2.85 and finally 1.58 min) and approximately 30% of drug was degraded in 12 hr. Thermal degradation was carried out as dry heat and wet heat. Dry heat show minimum degradation among all forced condition, as 12 % of drug was degraded in 16 hr with one principle degradant peak as 2.85 min RT. While wet heat shows 25% degradation within 18 hr with one principle degradant peak as 2.85 min RT. Degradants concentrations increase as time passes and the degraded products were well resolved from the parent drug as shown in figure 2 for chromatogram and figure 3 for % degradation vs. time in hr.

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The order of stability for Nabumetone was found to be H_2O_2 < wet heat <alkali <acid <dry heat. Developed RP-HPLC method is able to separate all degradants, produced from all stress condition from drug peak by resolution of more than 1.5 min. Analytical data for periodic evaluation are given in Table 2.
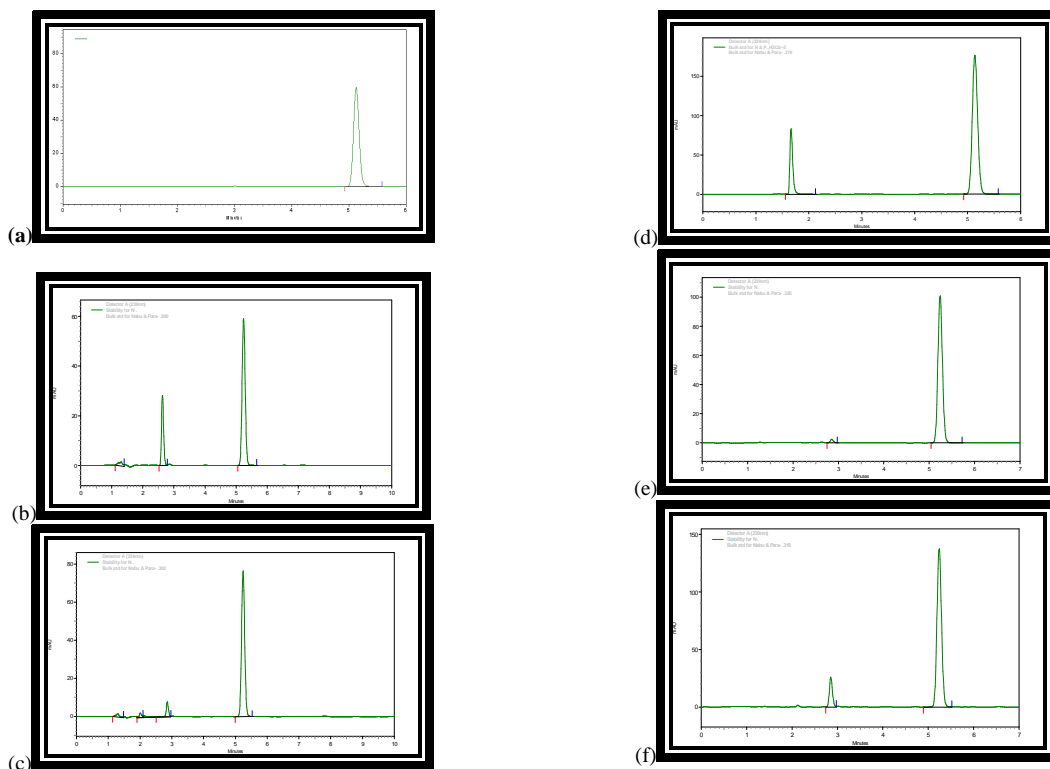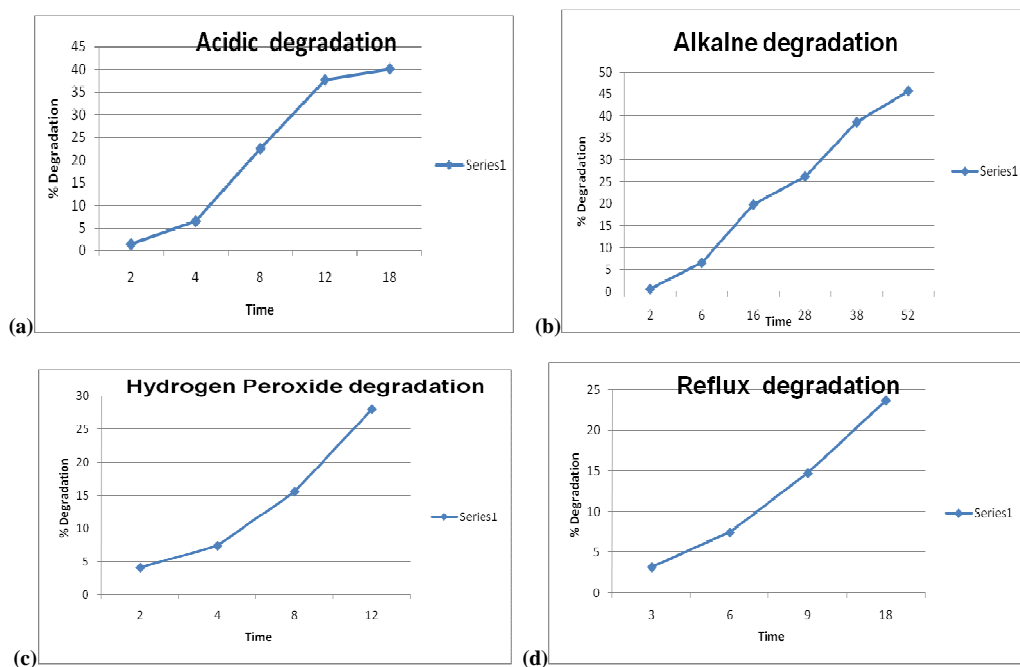

Figure 2.   Chromatogram showing: (a) sharp peak at optimize condition (b) acidic hydrolysis (2 N), at 18 hr reflux at 70 ˚C (c) alkaline hydrolysis (1N), at 52 hr (d) oxidative degradation, 3% H2O2 at 12 hr, (e) Thermal heat degradation after 16 hr in oven at 105 ˚C, (f) Wet heat degradation after 18 hr refluxed
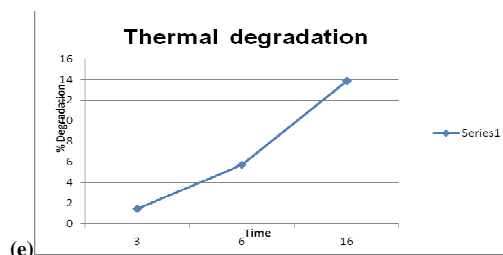
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(e)

Figure 3.   Graphs showing % degradation vs time: (a) acidic degradation (b) alkaline degradation (c) Oxidative degradation (d) Reflux degradation (e) Thermal degradation

Table 2 Analytical data for periodic evaluation of various stress degradation conditions

Acidic degradation, 2 N HCl (reflux at 70˚C)	18 hr reflux	40.08 %	**2.63, 1.30**
	12 hr reflux	37.62 %	**2.85, 2.63, 1.29**
	8 hr reflux	22.45 %	**2.85, 2.63, 1.30**
	4 hr reflux	6.44 %	**2.84, 2.63**
	2 hr reflux	1.39 %	**2.85, 2.63, 1.30**
Alkaline degradation, 1 N NaOH *At ambient temperature*	52	45.67 %	**2.85, 2.00, 1.30**
	38 hr	38.57 %	**2.85, 2.00, 1.30**
	28 hr	26.23 %	**2.00, 1.30**
	16 hr	19.85 %	**2.85, 2.63, 1.30**
	6 hr	6.57 %	**2.85, 1.29**
	2 hr	0.60 %	**2.85, 1.29**
Oxidative degradation 3% hydrogen peroxide *At ambient temperature*	12 hr	28.04 %	**1.58**
	8 hr	15.57 %	**1.58, 2.85**
	4 hr	7.43 %	**1.58**
	2 hr	4.10 %	**1.47**
Dry heat degradation (at 105 ºC)	16 hr	11.87 %	**2.85**
	6 hr	4.72 %	**2.85**
	3 hr	1.46 %	**2.85, 2.51**
Wet heat degradation	18 hr	23.67 %	**2.85**
	9 hr	14.72 %	**2.85**
	6 hr	7.43 %	**2.85**
	3 hr	**3.17 %**	**2.85**

Method validation [11]:
System suitability:
The system suitability of the method was studied to determine the reproducibility of the chromatographic system and column performance was acceptable for the intended analytical application. Four parameters i.e. precision of peak area of five replicate injections, retention time of eluted drugs, number of theoretical plates, asymmetry factor and resolution between two peak of analytes were evaluated. The results are shown in Table 1.

Table 1: Results of system suitability study

Retention time	Relative Retention time	Theoretical plates	Assymetry factor	Resolution
5.12 min	3.37	3759.43	1.27	15.72

Linearity:
The Linearity of analytical method is its ability to obtain test results, which are directly proportional to the concentration of analyte in the test sample. The linearity of the assay method was established by injecting test samples in the range of 2-100 µg/ml. Each solution was injected twice into HPLC and the average area at each concentration was calculated. The regression analysis was carried out from graph of peak area Vs Concentration; correlation co-efficient and Y- Intercept of plot was also evaluated. Linear regression equation and correlation coefficient was found to be y = 18388 X +51580 and r = 0.9992 for Nabumetone, where 'y' is area of peak and 'X' is the concentration of drug solution.

Accuracy:
The accuracy study was performed by spiking placebo with known quantity of API. The accuracy of test method was demonstrated by preparing recovery samples at the level of 80%, 100%, and 120% of target concentration. The recovery samples were prepared in triplicate at each level. The above samples were injected and the percentage recovery for amount added, were estimated. The precision of recovery at each level was determined by computing the relative standard deviation of triplicate recovery results. The result for accuracy is shown in Table 3, indicating

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good accuracy of the method for determination of drug.

Table 3: Results of Accuracy Study

Drug	Preanalysed conc. (g/ml)	Amount spiked* (mg)	Amount recovered* (mg)	% recovery*	% RSD
Nabumetone	40.02	40	79.84	99.80	
	40.02	50	89.86	99.84	0.85
	40.02	60	100.26	100.26	

*Average of three experiments

Precision:
Precision was determined by two ways; by System precision and Intermediate precision. System precision was demonstrated by making five replicate injections of standard solution. The peak area of analyte for replicate injections was recorded. The %RSD for the analyte peak area of these replicate injections was evaluated. The results of System precision is shown in Table 4, indicating that an acceptable precision was achieved for determination of drug, as revealed by RSD < 2.0%. The intermediate precision of test method was demonstrated by carrying out precision study at three concentration level as 80 %, 100%, 120% (i.e 40, 50, 60µg/ml). Intermediate precision study includes intra-day and inter-day analysis. The result summary of intermediate precision is shown in Table 5.

Table 4: Results of precision studyResults of system precision

Nabumetone	
Injection No.	Standard response
1	974264
2	986901
3	985347
4	978454
5	971262
Average	979245
SD	6800.97
% RSD	0.69

Table 5: Result of Intermediate Precision

Conc. (g/ml)	Intra-day (n=3)	% RSD	Inter-day (n=3)	% RSD
40	40.31 ± 0.475	1.17	40.82 ± 0.358	0.87
50	50.23 ± 0.271	0.54	50.28 ± 0.426	0.74
60	60.90 ± 0.427	0.70	60.98 ± 0.522	0.93

Robustness:
Robustness of the test method was demonstrated by carrying out system suitability under normal conditions and each of the altered conditions as follows.

Flow rate was changed by -10% and +10%; Organic phase ratio of mobile phase was changed by -5% and +5% absolute; Mobile phase pH was changed by -0.02 and +0.02, temperature changed by 5 °c. The result summary of robustness study are summarised in Table 6, result indicates that the method is robust for determination of drug.

Limit of detection and limit of quantification:
Limit of detection and limit of quantification was established based on the residual standard deviation method. LOD and LOQ were found to be 0.29 µg/ml and 0.89 µg/ml for drug.

Specificity:
Specificity was carried as interference from placebo; first only placebo and then injecting synthetic mixture containing placebo and API's as tablet ratio.

Table 6: Result Summary of Robustness Study

Condition	RSD of replicate injection	Tailing Factor	Theoretical plates
Normal	0.06	1.09	11057
Flow rate 1.3 ml/min	0.17	1.1	11326
Flow rate 1.7 ml/min	0.08	1.03	10865
Mobile phase (28:72:0.1)	0.09	1.02	10798
Mobile phase (32:68:0.1)	0.86	1.13	11162
Mobile phase pH -0.02	0.42	1.09	11236
Mobile phase pH +0.02	0.31	1.17	11023
Temp 20 °c	0.12	1.21	11204
Temp 30 °c	0.99	1.01	10962

CONCLUSION

The data demonstrate that the RP-HPLC method we have developed showed acceptable linearity, specificity, accuracy, precision and robustness in the concentration range of 2-100 µg/ml for Nabumetone as per the requirement of ICH guidelines. In this study, stability of drug was established according to ICH-recommended stress conditions. There was no interference of any degradants or excipients in the determination In conclusion, the proposed method could be routinely used for the analysis of drug in pharmaceutical dosage form.

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